Knockdown of *TRIM44* inhibits the progression of ovarian cancer and is related to the *FOXM1-EZH2* signaling pathway

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Background: Tripartite motif-containing protein 44 (*TRIM44*) was recently identified as a novel oncogene that is overexpressed in several types of human cancers. However, the biological functions of *TRIM44* in epithelial ovarian cancer (EOC) remain unclear. Here, we aimed to investigate the role of *TRIM44* in EOC and its clinical implications.

Methods: *TRIM44* was knocked down using shRNA transfection. In vitro proliferation, invasion, migration and apoptosis of ovarian cancer (OC) cells were detected by CCK8, colony formation assay, Transwell inserts, and flow cytometry analysis. The growth ability of xenograft tumors was examined *in vivo* in a nude mouse metastatic tumor model. Finally, we performed gene chip analysis and ingenuity pathway analysis (IPA) to analyze the potential gene network.

Results: High expression of *TRIM44* was observed in EOC tissues. Knockdown of *TRIM44* expression substantially suppressed the proliferation, migration, invasion and colony-forming ability of EOC cells *in vitro* and attenuated tumor growth *in vivo*. Mechanistic studies revealed that silencing *TRIM44* dramatically downregulated the expression of *FOXM1*, *EZH2*, *CCNE2*, *CCND3* and *BIRC5* in EOC cells, at least in part through inactivation of the *FOXM1-EZH2* signaling pathway.

Conclusions: Collectively, these data suggest that downregulation of *TRIM44* inhibits the progression of EOC through suppression of the *FOXM1-EZH2* signaling pathway. These results provide novel insight into the role of *TRIM44* in tumorigenesis and suggest that it could be a potential therapeutic target for ovarian carcinoma.

Keywords: Tripartite motif-containing protein 44 (*TRIM44*); ovarian cancer (OC); invasion; metastasis

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Introduction

Ovarian cancer (OC) is one of the most common human malignancies in women, resulting in an estimated 239,000 new diagnoses worldwide each year (1). Epithelial ovarian cancer (EOC) is the most common histological subtype of OC, accounting for 80% to 90% of cases. Approximately 70% of EOC patients are diagnosed with advanced-stage disease, which has the highest mortality rate among all female malignancies. In China, there were approximately 52,100 new cases and 22,500 deaths attributed to EOC in 2015 (2). The prognosis for patients suffering from EOC remains poor due to limited therapeutic strategies and late diagnosis. Despite improved treatments for OC, including debulking surgery, platinum-based chemotherapy and targeted therapy, the 5-year survival rate of advanced OC is only approximately 30% (3). Therefore, investigating predictive biomarkers and understanding the molecular mechanisms underlying EOC development are important for developing effective therapeutic targets.

Tripartite motif-containing protein 44 (*TRIM44*), a member of the TRIM family, has been documented to be overexpressed in various types of cancer, such as osteosarcoma (4), lung cancer (5), breast cancer (6), papillary thyroid cancer (7), testicular germ cell tumor (8) and prostate cancer (9). In addition, high expression of *TRIM44* is significantly associated with poor prognosis in malignant tumors (10,11). Our previous study demonstrated that high expression of *TRIM44* protein in ovarian tissues is significantly correlated with clinicopathological factors and poor prognosis (12). It was all reported that *TRIM44* facilitated OC proliferation, migration, and invasion by inhibiting FRK (13). However, expression of *TRIM44* in ovarian cell lines and its mRNA levels in ovarian tissues still need to be explored. In addition, the biological functions and carcinogenic mechanisms of *TRIM44* in EOC remain unclear.

Here, we report that *TRIM44* protein and mRNA levels are frequently increased in EOC clinical tissues and cell lines. Using lentivirus-driven shRNA knockdown, cell proliferation, migration, invasion, colony formation, and apoptosis analyses and *in vivo* animal experiments, we demonstrated that *TRIM44* promotes EOC cell proliferation, migration, invasion, colony formation and xenograft tumor growth. Furthermore, we showed that *TRIM44* downregulation inhibits the carcinogenesis of EOC cells in part through suppression of the *FOXO1-EZH2* signaling pathway. Our data suggest that *TRIM44* may be a potential therapeutic target for the treatment of OC.

We present the following article in accordance with the ARRIVE reporting checklist (available at <https://tc.amegroups.com/article/view/10.21037/tcr-21-2915/rc>).

Methods

Patient samples

Fresh EOC samples and noncancerous ovarian tissue samples were collected from Harbin Medical University Cancer Hospital (Harbin, China). All patients underwent maximal cytoreduction followed by platinum-based combination chemotherapy. None of the patients received chemotherapy, radiotherapy or immunotherapy before surgery. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics board of Harbin Medical University Cancer Hospital (No. KY2021-48) and informed consent was taken from all the patients.

Cell lines and cell culture

The human EOC cell lines A2780 and SKOV3 were obtained from the Cell Resource Center of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (Gibco BRL, Grand Island, NY, USA) containing 10% fetal calf serum (FBS, HyClone, Australia). All cells were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C.

Lentiviral vector encoding shRNA targeting *TRIM44*

ShRNA was designed using Ambion Company's online design tool. The lentiviral vector encoding *TRIM44* shRNA was synthesized and packaged by Ruisai Biotechnology Co., Ltd. (Shanghai, China). Lentiviral vectors carrying three types of *TRIM44*-shRNA was used to screen for maximum silencing efficiency. We electrically transfected the *TRIM44*-shRNA vectors into SKOV3 and A2780 cells to construct a lentiviral vector encoding shRNA targeting *TRIM44*, and the empty vector was used as the control. Short hairpin oligonucleotides (*shTRIM44*: 5'-TGCTGTGAGTCATCAACCTATCCATGGTTTGGCCACTGACTGACCATGGATATTGATGACTCA-3' and *shCon*: 5'-CCTGTGAGTCATCAATATCCATGGTCAGTCAGTGGCCAAAACCATGGATAGGTTGATGACTCAC-3') were synthesized and cloned into the lentivirus-based vector pLenti6.3-MCS/V5 DEST (Thermo Electron Corporation, Waltham, MA, USA). Lentivirus production was performed

by following a standard protocol involving transfection of 293T cells with the recombinant shRNA vector, together with psPAX2 and pVSVG (Thermo Electron Corporation, Waltham, MA, USA).

Lentivirus infection

SKOV3 and A2780 cells were cultured in six-well plates, and lentiviral vectors with the TRIM44 shRNA sequence or NC shRNA sequence were added at a multiplicity of infection (MOI) of 100 to the plate when the cells reached 70% confluency. The medium was replaced after 48 h of infection, and transfection efficiency was evaluated using quantitative real-time polymerase chain reaction (qRT-PCR) and western blot assays.

Western blot analysis and antibodies

Protein was extracted from tissues and cells for analysis. The samples were boiled at 100 °C for 5 min. Equal amounts of protein were separated by SDS-PAGE followed by transfer to PVDF membranes (Millipore, USA). The membranes were incubated with primary antibodies overnight at 4 °C. The following primary antibodies: mouse anti-human TRIM44 (ab236422; 1:1,000 dilution; Abcam, Cambridge, UK), rabbit anti-human CCND3 (ab112034, 1:1,000 dilution; Abcam, Cambridge, UK), rabbit anti-human FOXM1 (ab245309, 1:1,000 dilution, Abcam, Cambridge, UK), rabbit anti-human EZH2 (#4905, 1:100 dilution, Cell Signaling Technology), rabbit anti-human CCNE2 (ab226388, 1:1,000 dilution; Abcam, Cambridge, UK), rabbit anti-human BIRC5 (PAB18224, 1:1,000 dilution; Abnova, China), and mouse anti-GADPH (AG019, 1:1,000 dilution, Beyotime) as internal controls. Immunoreactive protein bands were detected using an ECL detection system (Cell Signaling Technology, USA).

qRT-PCR

mRNA expression was quantified using real-time RT-PCR. Briefly, total RNA was extracted from the cell lines using TRIzol reagent (Ruisai Biotechnology Co., Ltd., Shanghai). After quantification, a total of 10 ng RNA was used for cDNA synthesis, followed by real-time PCR using the One Step SYBR® PrimeScript™ RT-PCR Kit II (Ruisai Biotechnology Co., Ltd., Shanghai) according to the manufacturer's instructions. ACTB was employed as an internal control. The primer sequences were as follows:

TRIM44-forward: 5'-GGCACTCTTCCAGCCTTCC-3'; TRIM44-reverse: 5'-GAGCCGCCGATCCACAC-3'; ACTB-forward: 5'-GCTGGTAACCTTGTGTGCGG-3'; ACTB-reverse: 5'-GAGCCGCCGATCCACAC-3'. PCR was carried out for 40 cycles (95 °C for 15 s, 60 °C for 20 s and 72 °C for 20 s) according to the instructions. Relative quantification of *TRIM44* mRNA was conducted using the $2^{-\Delta\Delta CT}$ method, followed by normalization using the internal control.

Cell counting kit-8 (CCK-8) assay

An A CCK-8 assay (CK04, Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was performed to examine cell proliferation. After plating in 96-well plates at an initial density of 5×10^3 cells/well, SKOV3 and A2780 cells were transfected with *shTRIM44* or shNC (lentivirus-infected cells). At each time point, cells in each well were incubated with 10 µL of CCK-8 dye in 90 µL of culture medium for 2 h at 37 °C. Then, the OD values at 450 nm were measured using a VERS Amax Microplate Reader (Molecular Devices Corp, Sunnyvale, CA, USA).

Flow cytometry analysis of apoptosis

SKOV3 and A2780 cells were transfected with *TRIM44*-shRNA for 48 h. The cells were subsequently harvested by centrifugation at $10,000 \times g$ for 5 min. The cells were washed twice with cold PBS, centrifuged at $10,000 \times g$ for 5 min, and then resuspended in PBS. The cells were resuspended and stained with propidium iodide (PI, 1:100 dilution) and Annexin V-APC (Cell Signaling Technology, Danvers, USA) at 20 °C for 30 min in the dark. The stained cells were analyzed using a BD FACS Aria Cell Sorter.

Migration and invasion assays

The invasion and migration assays were performed using Transwell inserts (Corning Incorporated, Corning, NY, USA) with (invasion assay) or without Matrigel (migration assay). For the invasion assay, the Transwell chambers were pretreated with Matrigel (BD Bioscience, Mountain View, CA, USA). First, transfected ovarian cells were suspended in medium without FBS and seeded into the upper chamber. The lower chamber contained RPMI-1640 with 10% FBS. After culturing for 48 h at 37 °C, noninvasive cells on the upper chambers were removed using cotton swabs, while the invasive cells on the lower chamber were subsequently

fixed and stained with 0.1% crystal violet for 15 min. An inverted microscope (Olympus Corporation, Tokyo, Japan) was used to evaluate invasion and migration. The number of cells in five fields of view was counted under a microscope at $\times 100$ magnification. The cross divides the imaging area, and the number of cells in one area is used to replace the number of cells in a group under the premise that the number of cells is as uniform as possible and there is no excessive number of cells.

Colony formation assay

Following lentiviral infection, SKOV3 and A2780 cells were plated in 6-well plates at a density of 1.5×10^3 cells/well. Then, the cells were cultured in a humidified incubator at 37 °C with 5% CO₂. After 7 days, natural colonies formed, and the cells were washed three times with PBS, fixed in 1.5 mL of 4% paraformaldehyde and stained with crystal violet (Sigma-Aldrich) for 20 min. After the samples were washed twice with PBS, the staining solution was removed, and the cells were air dried. Colony formation was assessed using a light/fluorescence microscope to quantify the colony numbers.

Nude mouse in vivo xenograft assays

Experiments were performed under a project license (No. KY2021-48) granted by the ethics board of Harbin Medical University Cancer Hospital, in compliance with the “guidelines and suggestions laboratory animals” (Ministry of Science and Technology of the People’s Republic of China). A protocol was prepared before the study without registration. Female athymic BALB/c nude mice (aged six weeks, weighing 20–22 g) were obtained from Sippl B & K Laboratory Animal Co., Ltd. (Shanghai, China). Mice were housed in a pathogen-free animal facility and randomly assigned to the control or experimental group (six mice per group). To explore the effects of *TRIM44* on tumorigenesis *in vivo*, cells were intraperitoneal injected subcutaneously into the nude mice. Two groups of nude mice were injected with 50 μ L of 10^8 pfu/mL NC and KD virus on day 0, day 3, day 7, day 10, and day 14. A single mouse was considered an experimental unit. Tumor volume was estimated every three days using the following formula: $0.5 \times \text{length} \times \text{width}^2$. Tumor sizes were measured at least once or twice a week to monitor tumor growth. All mice were euthanized after 23 days, and confounding factors such as the order

of treatments and measurements or animal location were recorded. If the volume of the tumor decreased after inoculation and no longer increased, the sample was excluded.

Statistical analysis

Data are expressed as the mean $\pm$ SD. Statistical analysis was performed using GraphPad Prism 7.0 software. Differences among groups were analyzed using ANOVA or Student’s *t* test. Data with $P < 0.05$ were considered statistically significant.

Results

TRIM44 expression is increased in EOC tissues and cell lines

We first examined the mRNA and protein levels of *TRIM44* in fresh EOC samples and noncancerous ovarian tissue samples. The results showed that both mRNA and protein expression of *TRIM44* were significantly upregulated in EOC samples compared to noncancerous tissue samples (Figure 1; $P < 0.05$), suggesting that *TRIM44* may represent a vital biomarker of EOC.

Lentiviral-mediated RNAi significantly inhibits TRIM44 expression in SKOV3 and A2780 cells

The individual shRNA oligos targeting *TRIM44* were validated. Western blot results indicated that lentiviral vectors with the *TRIM44* shRNA sequence effectively decreased the expression of *TRIM44* in SKOV3 and A2780 cells (Figure 2A,2B).

Knockdown of TRIM44 suppresses SKOV3 and A2780 cell proliferation in vitro

To determine whether *TRIM44* influenced the malignant properties of EOC cells, we first performed cell proliferation analysis using CCK-8 assays. We found that knockdown of *TRIM44* resulted in a significant reduction in cell proliferation (Figure 2C,2D). As shown in Figure 2, the growth curve of *shTRIM44*-treated cells began to drop on the third day compared to that of the nontreated and shNC-treated cells. The decrease reached 54.14% and 76.93% for *shTRIM44*-treated SKOV3 and A2780 cells, respectively, on the fifth day compared to shNC-treated cells ($P < 0.001$).

Figure 1 *TRIM44* expression is increased in epithelial ovarian cancer (EOC) cell lines and tissues. (A-C) mRNA and protein expression of *TRIM44* in 5 EOC tissue samples and 3 noncancerous ovarian tissues. *TRIM44* expression was elevated in EOC samples compared to N samples. *, P<0.05.

Figure 2 Effects of *TRIM44* on the proliferation, migration, invasion, apoptosis and colony formation of epithelial ovarian cancer (EOC) cells. (A,B) SKOV3 and A2780 cells were infected with a *TRIM44* shRNA-expressing lentivirus, and expression of *TRIM44* was evaluated by western blot. (C,D) The effect of *TRIM44* on cell viability was measured using CCK-8 (cell counting kit-8) assays. (E-H) Representative images and quantification of the Transwell migration and invasion assay data from the indicated cells (Crystal violet staining, 10×). (I,J) The effect of *TRIM44* on apoptosis was measured using apoptosis assays. (K,L) Representative images and quantification of colony formation data from *shTRIM44*- or *TRIM44*-infected EOC cells. Magnification: 10 times. *, P<0.05; **, P<0.01; ***, P<0.001.

Knockdown of TRIM44 suppresses the invasion and migration of SKOV3 and A2780 cells in vitro

In the Transwell invasion assay, we observed that invasion was inhibited in lenti-shTRIM44-transfected cells. The migration experiment showed that knockdown of TRIM44 reduced the migration of lenti-shTRIM44 virus-transfected cells (Figure 2E,2F,2H). The migration of SKOV3 cells transfected with TRIM44 shRNA was significantly inhibited compared to that of control cells (0.115 ± 0.002 versus 0.258 ± 0.002 , $P < 0.001$). The migration of A2780 cells transfected with TRIM44 shRNA was also significantly inhibited (0.030 ± 0.003 versus 0.086 ± 0.006 , $P < 0.001$). The invasion experiment revealed that knockdown of TRIM44 reduced the invasion of lenti-shTRIM44 virus-transfected cells (Figure 2E-2G). The invasion of SKOV3 cells transfected with TRIM44 shRNA was significantly inhibited compared to that of control cells (0.100 ± 0.004 versus 0.289 ± 0.005 , $P < 0.001$). Invasion of A2780 cells transfected with TRIM44 shRNA was significantly inhibited (0.308 ± 0.007 versus 0.497 ± 0.004 , $P < 0.001$). These results indicate that lentiviral vectors containing TRIM44 shRNA effectively inhibit migration and invasion induced by TRIM44.

Knockdown of TRIM44 induces apoptosis of SKOV3 and A2780 cells in vitro

We next investigated potential cellular apoptosis events during cell death caused by TRIM44 knockdown in the SKOV3 and A2780OC cell lines. The results revealed that apoptosis levels were increased in both the SKOV3 and A2780 cell lines in response to transfection with lenti-shTRIM44 (Figure 2I,2J). Depletion of TRIM44 caused drastic morphological changes and a substantial decrease in cell number. Taken together, these results clearly indicate that TRIM44 depletion leads to apoptotic cell death in SKOV3 and A2780 cancer cells.

Knockdown of TRIM44 significantly inhibits colony-forming ability in SKOV3 and A2780 cells

The long-term effect of TRIM44 silencing on the colony-forming ability of SKOV3 and A2780 cells was assessed using colony formation assays. The size of the independent colonies was much smaller in shTRIM44-treated cells than in the nontreated and shNC-treated cells. Moreover, the number of colonies formed by SKOV3 and A2780 cells

was significantly decreased following TRIM44 knockdown ($P < 0.05$) (Figure 2K,2L). These data indicate that TRIM44 knockdown also significantly inhibits colony formation in SKOV3 and A2780OC cells.

Knockdown of TRIM44 inhibits in vivo xenograft tumor growth in nude mice

To evaluate the effect of TRIM44 on ovarian carcinogenesis *in vivo*, we established xenograft tumor models in nude mice using A2780 cells transfected with lenti-shTRIM44 treatment. Xenograft tumors were observed in the nude mice at the injection site, and tumors were harvested 30 days after injection. The tumor volumes were measured once a week. The volume of xenograft tumors is a common criterion for judging the growth of OC cells *in vivo*. As shown in Figure 3, tumor volumes in the lenti-shTRIM44-transfected cell treatment group were significantly decreased compared to those in the control group (Figure 4, $P < 0.05$). These results indicate that lentiviral vectors with the TRIM44 shRNA sequence inhibit xenograft tumor growth, indicating that downregulation of TRIM44 may be beneficial for the treatment of OC.

Global gene expression analysis following TRIM44 knockdown revealed modulation of key pathways in EOC cells

To elucidate the molecular mechanisms by which TRIM44 contributes to the carcinogenesis of OC cells, we performed gene chip analysis (gene expression profiling, using the Human Gene Expression Array PathArray™ platform) comparing the gene expression of SKOV3 NS cells versus SKOV3 shTRIM44 cells. Differentially expressed genes with at least a 1.2-fold change were identified. Common differentially expressed genes were analyzed using ingenuity pathway analysis (IPA). We identified 1,172 genes with significant expression changes (> 1.2 -fold with adjusted $P < 0.05$) (data not shown in this paper). The bioinformatics analysis showed that expression of 436 genes was significantly increased ($P < 0.05$) while that of 736 genes was significantly decreased ($P < 0.05$). The most markedly altered genes were AKT1S1 (AKT1 substrate 1, decreased 1.96-fold), USP14 (ubiquitin specific peptidase 14, decreased 1.85-fold), STK38L (serine/threonine kinase 38 like, decreased 1.83-fold), GLIPR1 (GLI pathogenesis-related 1, increased 1.84-fold), SEC23A (Sec23 homolog A, COPII coat complex component, decreased 1.76-fold),

Figure 3 *TRIM44* promotes epithelial ovarian cancer growth *in vivo*. (A) SKOV3 cells expressing *shTRIM44* were subcutaneously injected into nude mice, and the mice were monitored for 23 d. (B) A representative image of the tumors is shown. (C) A growth curve of the tumor volumes was constructed. *, $P < 0.05$; **, $P < 0.01$.

and *SPC24* (*NDC80* kinetochore complex component, decreased 1.72-fold). Many of these *TRIM44*-regulated differentially expressed genes in EOC cells are involved in regulating cell proliferation, wound healing, the cell cycle, DNA damage, and cellular assembly and organization. In addition, functional analysis of the genes using IPA revealed that differentially expressed genes were primarily enriched in the superpathway of cholesterol biosynthesis (*ACAT2*, *FDPS*, *IDII*, *LBR*, *LSS* and *MVD*, etc.), the superpathway of serine and glycine biosynthesis I (*PHGDH*, *PSAT1*, *PSPH*, and *SHMT2*), cyclins and cell cycle regulation (*CCNA1*, *CCNA2*, *CCNB2*, *CCND3*, *CCNE2*, *CDK1*, *E2F2*, *E2F8*, etc.), estrogen-mediated S-phase entry (*CCNA1*, *CCNA2*, *CCNE2*, *CDK1*, *E2F2*, etc.) the superpathway of geranylgeranyl diphosphate biosynthesis I signaling (*ACAT2*, *FDPS*, *IDII*, *MVD*, and *MVK*), the NER pathway (*ACAT2*, *FDPS*, *IDII*, *MVD*, and *MVK*), cell cycle control of chromosomal replication (*CDC6*, *CDK1*, *CDK10*, *LIG1*, and *MCM3*, etc.), and oxidative phosphorylation (*COX7A1*, *NDUFAB1*, *NDUFB11*, etc.). Taken together, these data indicate that *TRIM44* may be a key regulator of cell proliferation and migration that controls cancer progression. Most of these pathways are implicated in tumorigenesis or chemotherapy resistance (all $P < 0.05$; Figure 5).

TRIM44 knockdown suppresses the *FOXM1-EZH2* signaling pathway in EOC cells

The potential gene network interacting with *TRIM44* was also analyzed using IPA and revealed that *FOXM1-EZH2* signaling was a downstream signaling pathway regulated by *TRIM44* (Figure 6). Subsequent western blot assays demonstrated that silencing *TRIM44* dramatically downregulated the expression of several key proteins in the *FOXM1-EZH2* signaling pathway, including *FOXM1*, *EZH2*, *CCNE2*, *CCND3* and *BIRC5* (Figure 6, $P < 0.05$). These data further suggest that *TRIM44* promotes human EOC progression via the *FOXM1-EZH2* signaling pathway.

Discussion

In this study, we propose that *TRIM44* acts as a metastasis-associated marker in the context of EOC cells. Knockdown of *TRIM44* inhibited the invasion of EOC cells, whereas ectopic expression of *TRIM44* induced this activity. Gene chip analysis further demonstrated that *FOXM1-EZH2* signaling is involved in the EOC cell invasion and metastasis induced by *TRIM44*. Overexpression and knockdown of *TRIM44* further confirmed that *TRIM44* regulates invasion/metastasis by modulating the *FOXM1-EZH2* signaling pathway.

Figure 4 The canonical pathways of the differentially expressed genes in SKOV3 cells following *TRIM44* knockdown. Several canonical pathways ($P < 0.05$) are shown in this figure. The line represents $P = 0.05$. The ratio indicates the proportion of genes involved in these pathways among all the genes involved in this pathway.

The TRIM family is composed of genes that encode proteins containing TRIM. The integrated module comprises three different types of domains: a RING domain (R), a B-box domain (B), and a coiled-coil (CC) region (RBCC). The TRIM protein family is involved in a wide range of biological processes, such as cell growth, development, and cellular differentiation (14,15). Increasing evidence has shown that many TRIM family members are involved in the oncogenesis and progression of OC. For instance, *TRIM52* plays an oncogenic role in the development of OC associated with the *NF- κ B* signaling pathway (16). Furthermore, it was demonstrated that knockdown of *TRIM11* not only affects the expression of apoptosis-related and invasion-related proteins but also reduces the phosphorylation levels of *ERK* and *AKT* in OC cells (17). In addition, *TRIM16* inhibits migration and invasion by suppressing the Sonic hedgehog signaling pathway in OC cells (18). Given the correlation between the expression levels of these TRIMs and the tumorigenesis of OC, TRIMs represent potential novel therapeutic targets in patients with OC.

TRIM44 is one of the members of the TRIM family, which has been demonstrated to have multifaceted roles in cancer progression. Multiple reports have confirmed that *TRIM44* is overexpressed in several human cancers and may promote tumor growth, proliferation and invasion (19). Kashimoto *et al.* showed that knockdown of *TRIM44* expression inhibited the proliferation, migration, and invasion of *TRIM44*-overexpressing gastric cancer cells (10). Another study identified and validated that *TRIM44* is independently associated with poor outcome and provides additional prognostic information in esophageal adenocarcinoma (20). Yamada *et al.* also found that *TRIM44* promotes cell proliferation and migration and inhibits apoptosis in testicular germ cell tumors (8). Our previous study found that high *TRIM44* protein expression was strongly correlated with advanced histological grade, high FIGO stage and lymph node metastasis in patients with EOC (12). Consistent with our previous data, the results of the present study indicated that *TRIM44* is significantly expressed in OC tissue samples. Furthermore, to explore the role of *TRIM44* in OC tumorigenesis, we knocked down *TRIM44* in SKOV3 and A2780 cells. We successfully constructed an shRNA lentiviral vector that targeted *TRIM44* and efficiently silenced the *TRIM44* gene. In the present study, we demonstrated that depletion of *TRIM44* in SKOV3 and A2780 cells suppressed invasion, migration, proliferation, and colony formation while

Figure 5 The potential gene network interacting with *TRIM44* was also analyzed using IPA (International Profession Certification Association), and *FOXM1-EZH2* signaling is a downstream pathway regulated by *TRIM44*.

inducing apoptosis. Metabolically, knockdown of *TRIM44* significantly inhibited tumor growth *in vivo*. Thus, our study is consistent with the finding that *TRIM44* is essential for malignant transformation of tumor cells. These findings suggest that *TRIM44* may act as an oncogene in EOC.

It has been reported that *TRIM44* is involved in cancer development and progression through various mechanisms, such as promoting *TNF α* -dependent phosphorylation of the *p65* subunit of *NF- κ B* and *I κ B α* in breast cancer cells (6), inducing *EMT* via *MAPK* signaling in intrahepatic cholangiocarcinoma or activating the *AKT/mTOR* signal transduction pathway in human esophageal cancer (20). A previous study in human esophageal cancer demonstrated that *TRIM44* promotes quiescent multiple myeloma cell occupancy and survival in the osteoblastic niche via *HIF-1 α* stabilization (21). In addition, a recent study found

that knockdown of *TRIM44* inhibits the proliferation and invasion of papillary thyroid cancer cells by suppressing the *Wnt/ β -catenin* signaling pathway. However, the underlying mechanisms of *TRIM44* in OC cell invasion and metastasis remain to be fully elucidated. In our study, gene chip data were analyzed using IPA. Gene expression profiling using the Affymetrix Human Gene 1.0 ST platform identified 1172 transcripts that were significantly differentially expressed based on a $P < 0.05$ threshold in SKOV3 EOC cells with *TRIM44* knockdown compared to the control cells. Functional analysis of the genes using IPA revealed that *TRIM44* knockdown modulates key pathways typically activated in cancer.

Moreover, the potential gene network interacting with *TRIM44* was analyzed using IPA and revealed that *FOXM1-EZH2* signaling was a downstream pathway regulated by

Figure 6 Western blotting of key proteins in the *FOXM1-EZH2* pathway in *TRIM44*-silenced SKOV3 cells. Proteins from cells treated with negative control (NC) shRNAs or *TRIM44* were used to investigate the protein expression of *FOXM1*, *EZH2*, *CCNE2*, *CCND3* and *BIRC5*. Total *GAPDH* was used as a loading control.

TRIM44. The *FOXM1-EZH2* signaling pathway is a well-characterized signaling pathway related to the development of various cancer types (22-24). However, it is still unknown whether the key proteins of the *FOXM1-EZH2* signaling pathway are functionally different or functionally redundant with respect to the carcinogenic mechanism of *TRIM44*. Subsequent western blot experiments confirmed that *FOXM1-EZH2* signaling was repressed by *TRIM44* knockdown, suggesting that *TRIM44* positively regulates the carcinogenesis of OC cells through *FOXM1-EZH2* signaling.

In conclusion, we revealed reduced expression of *TRIM44* transcript expression in human ovarian tumor tissues relative to noncancerous ovarian tissues and inhibition of human EOC cell invasion, migration, and

growth by *TRIM44*, at least in part through suppression of *FOXM1-EZH2* signaling. Our findings identify a potential role for the *TRIM44* gene in human EOC pathogenesis by activating the *FOXM1-EZH2* signaling pathway. These conclusions need to be further validated in larger experiments.

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Footnote

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References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;65:5-29.
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017;67:7-30.
4. Wang H, Fang ZL, Zhang GH, et al. TRIM44, a crucial target of miR-410, functions as a potential oncogene in osteosarcoma. *Onco Targets Ther* 2018;11:3637-47.
5. Luo Q, Lin H, Ye X, et al. Trim44 facilitates the migration and invasion of human lung cancer cells via the NF- κ B signaling pathway. *Int J Clin Oncol* 2015;20:508-17.
6. Kawabata H, Azuma K, Ikeda K, et al. TRIM44 Is a Poor Prognostic Factor for Breast Cancer Patients as a Modulator of NF- κ B Signaling. *Int J Mol Sci* 2017;18:1931.
7. Zhou Z, Liu Y, Ma M, et al. Knockdown of TRIM44 inhibits the proliferation and invasion in papillary thyroid cancer cells through suppressing the Wnt/ β -catenin signaling pathway. *Biomed Pharmacother* 2017;96:98-103.
8. Yamada Y, Takayama KI, Fujimura T, et al. A novel prognostic factor TRIM44 promotes cell proliferation and migration, and inhibits apoptosis in testicular germ cell tumor. *Cancer Sci* 2017;108:32-41.
9. Tan Y, Yao H, Hu J, et al. Knockdown of TRIM44 Inhibits the Proliferation and Invasion in Prostate Cancer Cells. *Oncol Res* 2017;25:1253-9.
10. Kashimoto K, Komatsu S, Ichikawa D, et al. Overexpression of TRIM44 contributes to malignant outcome in gastric carcinoma. *Cancer Sci* 2012;103:2021-6.
11. Ong CA, Shannon NB, Ross-Innes CS, et al. Amplification of TRIM44: pairing a prognostic target with potential therapeutic strategy. *J Natl Cancer Inst* 2014;106:dju050.
12. Liu S, Yin H, Ji H, et al. Overexpression of TRIM44 is an independent marker for predicting poor prognosis in epithelial ovarian cancer. *Exp Ther Med* 2018;16:3034-40.
13. Yu XZ, Yuan JL, Ye H, et al. TRIM44 facilitates ovarian cancer proliferation, migration, and invasion by inhibiting FRK. *Neoplasia* 2021;68:751-9.
14. Hatakeyama S. TRIM Family Proteins: Roles in Autophagy, Immunity, and Carcinogenesis. *Trends Biochem Sci* 2017;42:297-311.
15. Cambiaghi V, Giuliani V, Lombardi S, et al. TRIM proteins in cancer. *Adv Exp Med Biol* 2012;770:77-91.
16. Yang W, Liu L, Li C, et al. TRIM52 plays an oncogenic role in ovarian cancer associated with NF- κ B pathway. *Cell Death Dis* 2018;9:908.
17. Chen Y, Sun J, Ma J. Proliferation and invasion of ovarian cancer cells are suppressed by knockdown of TRIM11. *Oncol Lett* 2017;14:2125-30.
18. Tan H, Qi J, Chu G, et al. Tripartite Motif 16 Inhibits the Migration and Invasion in Ovarian Cancer Cells. *Oncol Res* 2017;25:551-8.
19. Peng R, Zhang PF, Zhang C, et al. Elevated TRIM44 promotes intrahepatic cholangiocarcinoma progression by inducing cell EMT via MAPK signaling. *Cancer Med* 2018;7:796-808.
20. Xiong D, Jin C, Ye X, et al. TRIM44 promotes human esophageal cancer progression via the AKT/mTOR pathway. *Cancer Sci* 2018;109:3080-92.
21. Chen Z, Lin TC, Bi X, et al. TRIM44 promotes quiescent multiple myeloma cell occupancy and survival in the osteoblastic niche via HIF-1 α stabilization. *Leukemia* 2019;33:469-86.
22. Kim SH, Joshi K, Ezhilarasan R, et al. EZH2 protects glioma stem cells from radiation-induced cell death in a

- MELK/FOXM1-dependent manner. Stem Cell Reports 2015;4:226-38.
23. Harzer K, Yildiz Y. High β -glucosidase (GBA) activity not attributable to GBA1 and GBA2 in live normal and enzyme-deficient fibroblasts may emphasise the role of additional GBAs. Biol Chem 2015;396:1241-6.
 24. Perotti V, Baldassari P, Molla A, et al. An actionable axis linking NFATc2 to EZH2 controls the EMT-like program of melanoma cells. Oncogene 2019;38:4384-96.

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