RESEARCH ARTICLE

Overexpression of TRIM44 mediates the NF-κB pathway to promote the progression of ovarian cancer

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Abstract

Background Ovarian cancer (OC) is the second most commonly seen cancer in the US, and patients with OC are commonly diagnosed in the advanced stage. Research into the molecular mechanisms and potential therapeutic targets of OC is becoming increasingly urgent. In our study, we worked to discover the role of TRIM44 in OC development.

Objective This study explored whether the overexpression of TRIM44 mediates the NF-kB pathway to promote the progression of OC.

Methods A TRIM44 overexpression model was constructed in SKOV3 cells, and the proliferation ability of the cells was detected using the CCK-8 assay. The migration healing ability of cells was detected using cell scratch assay. Cell migration and invasion were detected using Transwell nesting. TUNEL was applied to detect apoptosis, and ELISA and western blot were used to detect the expression of NF-κB signaling pathway proteins. The pathological changes of the tumor tissues were observed using HE staining in a mouse ovarian cancer xenograft model. Immunofluorescence double staining, RT-PCR, and western blot were used to determine the expression of relevant factors in tumour tissues.

Results TRIM44 overexpression promoted the proliferation, migration, and invasion of SKOV3 cells in vitro and inhibited apoptosis while enhancing the growth of tumours in vivo. TRIM44 regulated the NF-κB signaling pathway.

Conclusions TRIM44 overexpression can regulate the NF-κB signaling pathway to promote the progression of OC, and TRIM44 may be a potential therapeutic target for OC.

Keywords Ovarian cancer · TRIM44 · NF-κB signaling pathway · Migration and invasion · Apoptosis

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Introduction

Ovarian cancer (OC) is one of the most common malignant tumors in women, second only to cervical cancer and endometrial cancer in incidence, showing a trend of increase and rejuvenation year by year (González-Martín et al. 2019). Globally, OC is the seventh most common cancer in women and the eighth most common cause of cancer death, with a five-year survival rate of less than 45% (Webb and Jordan 2017). Currently, the standard treatment regimen for OC is primary tumor cytoreductive surgery supplemented with systemic platinum-based chemotherapy as the standard treatment regimen for OC (Mirza et al. 2016). With the development of precision medicine, OC molecularly targeted therapy has also gained increasing attention. Therefore, in order to develop effective therapeutic and metastasis-related molecular targets, it is essential to study predictive biomarkers and understand the molecular mechanisms of OC development.

Tripartite motif protein 44 (TRIM44), an essential member of the TRIM family, is overexpressed in a variety of malignancies, such as non-small cell carcinoma (Wang et al. 2022), hepatocellular carcinoma (Dai et al. 2021), colorectal cancer (Li et al. 2019), breast cancer (Kawabata et al. 2017), and cervical cancer (Liu et al. 2019). The high expression of TRIM44 is significantly associated with the progression and prognosis of malignant tumors. In addition, it was shown that TRIM44 protein was highly expressed in both epithelial OC tissues and cells, and was significantly associated with clinicopathological factors and poor prognosis (Liu et al. 2018; Yu et al. 2021). It can be seen that also plays a role as a pro-oncogene in OC. However, the impact of the high TRIM44 expression phenotype on the malignant biological behaviour of OC cells and its potential mechanism of action is not clear.

The NF-κB signaling pathway plays a key role in many events of cellular life activities, such as natural immunity, adaptive immunity, inflammatory response, as well as cell migration and apoptosis (Li and Verma 2002; Karin and Greten 2005). The activation pathways of the NF-κB signaling pathway mainly include classical pathway, paracrine pathway, and atypical activation, among which the IKK/IκB/NF-κB signaling pathway belongs to the classical NF-κB signaling pathway, and is the key factor for various inflammatory factors, cellular proliferation, and cell death. The IKK/IκB/NF-κB signaling pathway is a classical NF-κB signaling pathway, which is an important pathway to produce various inflammatory factors, cell proliferation, migration, and apoptotic factors (Pahl 1999; Kutuk and Basaga 2003; Hayden and Ghosh 2004). This pathway primarily activates the IKK complex via the tumour necrosis factor receptor (TNFR), consisting primarily of of IKKα, IKKβ, and IKKγ, inducing phosphorylation and ubiquitination of IκBα and degradation of IκBs protein, resulting in the release of RelA/p50 dinners (Hayden and Ghosh 2012). Studies have demonstrated that the NF-κB signaling pathway exhibits an activated state in ovarian cancer, promotes cell proliferation, inhibits apoptosis, and facilitates tumorigenesis and development (Cen et al. 2013; Razani et al. 2011; Courtois and Smahi 2006; Courtois 2005).

In this study, the mechanism of overexpression of TRIM44 on OC progression was investigated. The results showed that TRIM44 promoted the proliferation of SKOV3 cells and the growth of tumours in xenografts, inhibited apoptosis, and enhanced cell migration and invasion. In addition, we found that TRIM44 overexpression may enhance the invasive, metastatic, and anti-apoptotic abilities of OC cells by promoting the activation of the NF-κB signaling pathway. Our data suggest that TRIM44 mediates the NF-kB pathway to promote OC progression, which may help to facilitate the development of new diagnostic or therapeutic biomarkers to improve the prognosis of OC patients.

Materials and methods

Cellular experiments

Cell culture and grouping

SKOV3 human OC cells Zhongqiao Xinzhou Biotechnology Co., Ltd (Shanghai, China). Cells were cultured in RPMI 1640 (Hyclone, SV30010) containing 10% fetal bovine serum (FBS, CLARK, FB15015), 100 IU/ml penicillin, streptomycin (Hyclone, SV30010) and 4 µg/ml puromycin (Puro, BioFroxx, 1299MG025). All cells were maintained at 37 °C with 5% CO_2 saturated humidity. Then, the cells were divided into the h-NC group and the h-TRIM44 group.

Construction and cell transfection of PGMLV-PE3-TRIM44 vector

Human TRIM44 expression vector PGMLV-PE3-TRIM44 or empty vector was obtained from Zhongqiao Xinzhou Biotechnology Co. For in vitro transfection, SKOV3 human OC cells (3×10^5 cells/well) were transfected with TRIM44 or empty vector using Lipofectamine 2000 (Invitrogen, Victoria, Australia) according to the manufacturer's protocol. The shRNA sequences used were TRIM44: 5'-CCGGGGCTTGATTTGAGTACCTATTCTCGAGAA TAGGTACTCAAATCAAGCCTTTTTG-3' and shCon:5'-AATTCAAAAAGGCTTGATTTGAGTACCTATTCT CGAGAATAGGTACTCAAATCAAGCCAGCC-3'. To establish stable cell lines, 2 µg/mL puromycin was used for one week after transfection. RT-qPCR and Western blotting determined overexpression efficiency.

Cell counting Kit 8 (CCK-8) assay

The CCK-8 (Tong Ren, Japan, CK04) assay was applied to detect the change in cell proliferation ability. Cells at the logarithmic growth stage were collected and inoculated in 96-well culture plates with appropriate concentrations, cells were grouped, and 200ul of complete medium was added to each well and continued to be cultured for 24 h and 48 h. After the cells had completely adhered to the wall, 10ul of CCK-8 reagent was added to each well and incubated for 4 h. Then the absorbance value at 450 nm was detected by an enzyme marker. The different cells in the two groups were calculated according to the following formula Survival rate $= [(As-Ab) / (Ac-Ab)] \times 100\%$.

Cell scratching experiment

The migration healing ability of cells was detected using a cell scratching assay. After 24 h of cell culture, when the cells were fused to 70% ~ 80%, horizontal scratches were made by using a straightedge and 1 ml gun to ensure that the area of each group of scratches was of the same size. Each well was rinsed with PBS buffer, and 2 ml of complete medium was added to each well. Moreover, 48 h of cell healing and the cell migration healing rate were calculated according to the following formula = (width after healing/scratch width) × 100%.

Cell migration and invasion assay

Cell migration and invasion were detected using Transwell nests (Corning, 3470) with or without matrix gel (invasion assay). For cell migration assays, transfected SKOV3 cells were resuspended with serum-free medium to a concentration of 1×10^5 cells/ml, 200 ul of cell suspension was inoculated into the upper chamber of the Transwell, and 500 ul of medium with 20% FBS was added to the lower chamber. After 48 h of incubation, the unmigrated cells in the upper part of the filter were removed by wiping with a cotton swab, and the cells migrating to the lower surface were fixed with 4% paraformaldehyde fixative for 20 min. 600ul of 0.1% crystal violet staining solution was added and stained for 30 min. The observation was performed under a microscope 200 times larger, photographed, and counted, and the mean value was taken for statistical analysis. The procedure for the cell invasion experiment was the same as for the migration experiment.

Apoptosis assay

TUNEL was applied to detect the change in the apoptosis of cells. TRIM44-transfected SKOV3 cells were prepared and fixed in 4% paraformaldehyde for 30 min; Proteinase K working solution was added dropwise for 20 min, and 0.1% Triton X-100 permeabilized the nuclear membrane for 8 min; after breaking the membrane, 50 µl of TUNEL (Servicebio, G1501) reaction solution was added dropwise to the crawler. After breaking, 50 µl of TUNEL (Servicebio, G1501) reaction solution was added dropwise and incubated for 2 h at 37℃ in a thermostat; the crawling film was covered with buffer and incubated for 10 min at room temperature and then incubated with the appropriate amount of DAPI (Servicebio, G1012) for 10 min in a light-proof environment.

Enzyme-linked immunoassay

Cells of the logarithmic growth phase were collected and inoculated in 6-well culture plates at appropriate concentrations. The cells were grouped, and after 48 h of cell culture, the cell supernatant was collected. The TNF-α (PYRAM, PH096396), IL-1β (PYRAM, PH099095), and IL-8 (PYRAM, PH099084) were detected in each group of samples strictly according to the Elisa kit instructions, PH099084).

Western blotting

To extract proteins from tissues and cells for analysis, different groups of cells and nude mouse tissues were homogenized in a lysis buffer containing protease inhibitors. After centrifugation, the supernatant was boiled and mixed with an equal volume of sample buffer. Proteins were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, IPVH00010). The membranes were incubated overnight at 4 °C with the primary antibody in TBST, and 0.5% skim milk. The membranes were incubated with the corresponding secondary antibodies for 1 h at room temperature. Finally, immunoreactive protein bands were detected using a gel imaging system (BIO-RAD, USA), photographed, and stored.

Animal experiments

Establishment of tumorigenic model in nude mice

Twenty female SPF-grade Balb/C nude mice, 4–5 weeks old, all weighing 16 ± 2 g, were purchased from Beijing Viton Lihua Laboratory Animal Technology Co. Mice and housed in a pathogen-free animal facility randomly assigned to the control group (shNC group) or TRIM44 overexpression group (shTRIM44 group), with 10 mice in each group. 1×10^6 units/mL transfection of SKOV3 cells with empty vector, and inoculate them subcutaneously into the right armpit of each nude mouse in the shNC group. Similarly, inoculate the same concentration of TRIM44 transfection into SKOV3 cells subcutaneously into the nude mice in the shTRIM44 group Subsequently, the nude mice were observed daily for body weight, dietary water, mental status, and tumor formation after tumor formation. Tumor volume was calculated every 3 days using the formula: Tumor volume = $0.52 \times$ minimum diameter$^2 \times$ maximum diameter. The tumor growth curve was plotted.

HE staining

Paraffin-embedded tumor tissue Sects. (4 µm thick) were prepared, dewaxed, and hydrated, stained with hematoxylin and eosin (Biosharp, BL735A) at 37 °C for 1 h. After dehydration and drying, the sections were sealed with neutral gum.

RT-qPCR

Quantification of IKKβ, IκBα, and NF-κB p65 gene expression in tumor tissues using real-time RT-PCR. Total RNA was extracted from nude mouse tumor tissues using Trizol reagent (Biosharp, BS259A). cDNA was synthesized using total RNA (2 μg) with the SuperScript II first-strand synthesis system according to the manufacturer's protocol. All forward and reverse primers (Table 1) PCR thermal cycling conditions were as follows: initial denaturation at 95 °C for 1 min, 40 amplification cycles including denaturation at 95 °C for 30 s, primer annealing at 60 °C for 20 s, and final extension at 72 °C for 20 s. RT-qPCR measured gene expression levels. Relative mRNA expression levels were calculated using the 2 -$\Delta\Delta$CT method and then normalized using an internal control.

Immunofluorescence double staining

Paraffin-embedded tumor sections were prepared and dehydrated in preparation for further analysis. The sections were treated in 3% hydrogen peroxide solution for 25 min, washed 3 times with PBS, and the tissue sections were placed in a repair cassette for antigen repair, followed by the addition of 5% BSA for 30 min. Finally, the sections were incubated with mouse anti-human TRIM44 (proteintech, 66249–1-LG, dilution ratio: 1:500), rabbit anti-human NF-κB p65 (absin, abs119958, dilution ratio: 1:1000) monoclonal primary antibody was incubated overnight at 4 °C. All sections were washed 3 times with PBS and incubated with the appropriate fluorescence secondary antibody (dilution ratio: 1:1000) for 60 min at room temperature under light-proof conditions. DAPI (Servicebio, G1012) was treated for 60 min at 37 °C under light-proof conditions to re-stain the cell nuclei. Subsequently, an anti-fluorescence quenching blocker (Servicebio, G1401) was added dropwise for blocking, and finally, images were observed and acquired in a fluorescence microscope.

Statistical analysis

The results of the experiment were analyzed with a one-way ANOVA method using SPSS 23.0 software. Data were presented as mean ± SD. $p < 0.05$ was set as a significant difference between groups.
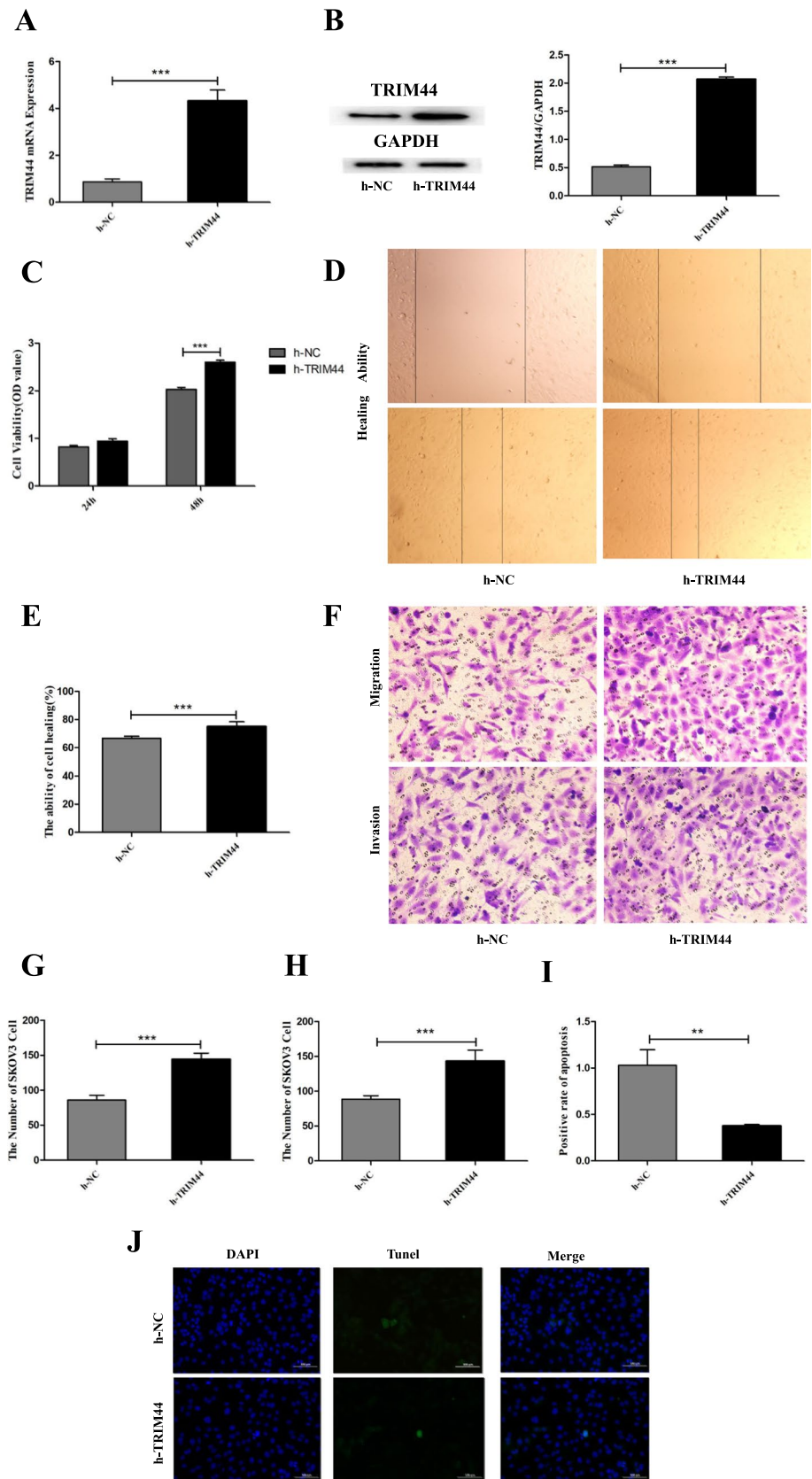
Results

TRIM44 promotes OC cell proliferation, migration, and invasion, and inhibits apoptosis in vitro
To determine the potential cellular function of TRIM44 in OC, we overexpressed TRIM44 in SKOV3 cells. Western blotting and RT-qPCR assays showed a dramatic increase in TRIM44 expression in SKOV3 (Fig. 1A, B). First, we applied the CCK8 assay for cell viability analysis. We found that TRIM44 overexpression significantly promoted OC cell proliferation (Fig. 1C). Secondly, we used scratch assay to show that after 48 h of cell proliferation, the scratch healing distance of cells in the h-TRIM44 overexpression group was significantly narrower than that of the control group. Lateral migration ability of cells was accelerated, indicating that TRIM44 overexpression may be an improvement in the migration ability of SKOV3 cells (Fig. 1D, E). Moreover, the Transwell assay revealed that the number of cells migrating and invading cells through the membrane was significantly increased in the h-TRIM44 overexpression group compared with the control group, suggesting that TRIM44 overexpression could improve the migration and invasion ability of SKOV3 cells (Fig. 1F, G, H). Finally, TUNEL experiments showed that h-TRIM44 overexpression significantly reduced the number of apoptotic cells, and TRIM44 overexpression reduced the apoptotic capacity of SKOV3 cells (Fig. 1I, J). In conclusion, overexpression of TRIM44 promotes the malignant biological behaviour of OC cells, enhances proliferation, migration and invasion of SKOV3 cells and inhibits apoptosis.

Table 1 Primer sequences used in the study

Genes		Primer sequences	Product length
GAPDH	forward	5′-GAGCCCTTCCACAATGCCAAA-3′	240
	reverse	5′-GTCGTGGAGTCTACTGGTGTC-3′	
IκBα	forward	5′-CAGAGTCAGAGAGTTCACGGAGTTCAC-3′	111
	reverse	5′-AGTCCATGTTCTTTCAGCCCCTTTG-3′	
IKKβ	forward	5′-ACAGAGGAGGGACAGAAAGGGTATC-3′	80
	reverse	5′-CACGCTTGAGCAGGAATGGAGAC-3′	
NF-κB p65	forward	5′-GTGGGGACTACGACCTGAATG-3′	121
	reverse	5′-GGGGGCACGATTGTCAAAGATG-3′	

Fig. 1 Effect of TRIM44 on the malignant biological behavior of OC cells. (**A**) PCR assessment of TRIM44 expression changes. (**B**) Protein blotting to assess the change in TRIM44 expression. (**C**) Detection of the effect of TRIM44 on cell viability. (**D**, **E**) Representative images and quantification of the effect of TRIM44 on cell migration healing ability were examined. (**F**, **G**, **H**) To detect the effect of TRIM44 on cell migration and invasion. (**I**, **J**) Effect of TRIM44 on apoptosis (Apoptosis cell rate = TUNEL positive cell count ÷ total cell count × 100%). Data are presented as mean ± SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

TRIM44 regulates the growth of OC cells through NF-κB signaling pathway

To elucidate the mechanism of TRIM44-mediated OC progression, we investigated the NF-κB signaling pathway in SKOV3 cells. The results in Fig. 2A showed that TRIM44 overexpression significantly increased the expression levels of TNF-α, IL-1β, and IL-8 in the cell supernatant compared with the control. In contrast, further analysis applying Western blot experiments showed that TRIM44 overexpression significantly promoted IKKβ, p-IκBα and NF-κB p65 protein expression, while significantly inhibiting the expression of IκBα protein (Fig. 2B). In addition, TRIM44 overexpression significantly upregulated the expression of Bcl-2 and MMP-9 protein and downregulated the expression level of Caspase3 protein, the downstream target proteins of the pathway, compared with the control group (Fig. 2C, D, E). These results suggest that TRIM44 may be able to promote the anti-apoptotic and invasive ability of SKOV3 cells through activation of the NF-κB signaling pathway.

TRIM44 promotes OC growth in vivo

We established a xenograft tumour model in nude mice to further investigate the effect and mechanism of TRIM44 on OC growth in vivo. As shown in Fig. 3A, compared with the control group, the tumors of TRIM44 overexpressing mice gradually increased in size with increasing inoculation time. The pathological results of HE staining showed (Fig. 3B) that the tumor tissues of the TRIM44 overexpression group showed solid growth with punctate necrosis of individual tumor cells (blue arrows), the high nucleoplasmic ratio of tumor cells, diverse karyotypes, sparse nuclei, and more visible. The tumor cells showed a high nucleoplasmic ratio, diverse karyotype, loose nucleoplasm, and more nuclear fission phase (black arrow). The positive regulatory effect of TRIM44 on NF-κB p65 was directly confirmed by further application of immunofluorescence double staining assay, which showed that upregulation of TRIM44 expression could promote the expression of TRIM44, NF-κB p65 protein (Fig. 3C,D). Moreover, the analysis of RT-qPCR experiments suggested that TRIM44 overexpression could increase IKKβ mRNA and NF-κB p65 mRNA expression and decrease IκBα mRNA in tumor tissues (Fig. 3E). In addition, TRIM44 overexpression significantly promoted TRIM44, IKKβ, p-IκBα, and NF-κB p65 protein expression and significantly inhibited IκBα protein expression in tumor tissues compared with controls (Fig. 3F, G). Overexpression of TRIM44 increased the expression levels of Bcl-2 and MMP-9 proteins and decreased the expression levels of caspase-3 proteins (Fig. 3H). These results suggest that TRIM44 may promote OC tumor growth in vivo by mediating the NF-κB signaling pathway, indicating that TRIM44 may be a potential OC pro-oncogene.
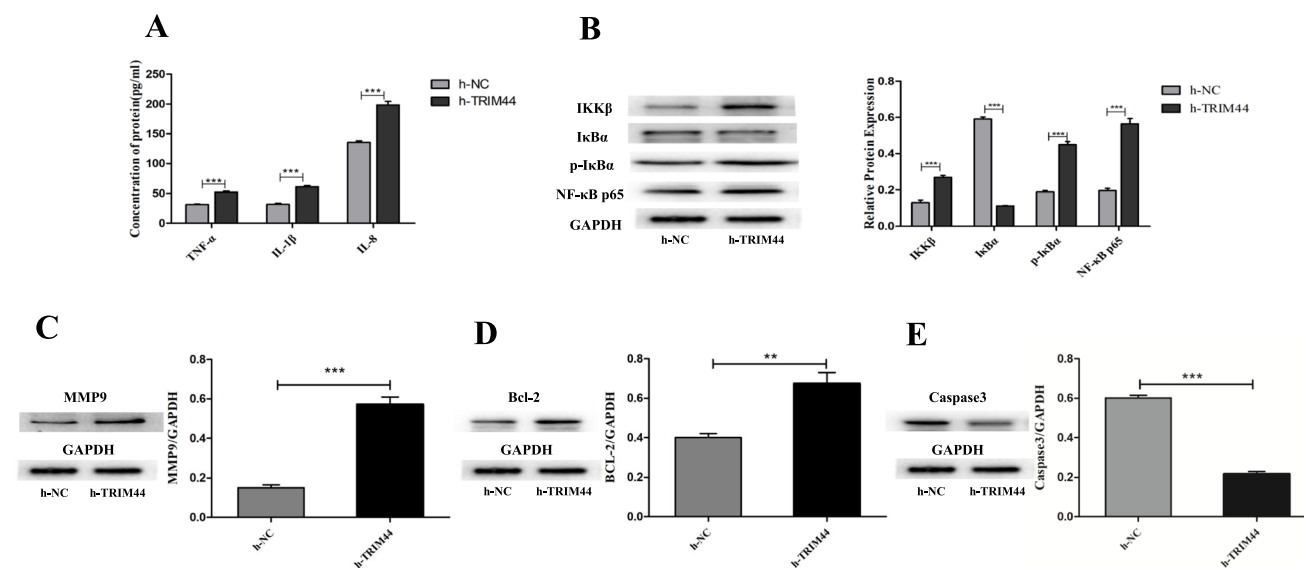

Fig. 2 TRIM44 regulates apoptosis and invasion of OC cells through the NF-κB signaling pathway. (A) Levels of TNF-α, IL-1β, and IL-8 in each group. (B) Protein expression levels of IKKβ, IκBα, p-IκBα, and NF-κB p65. (C) Expression levels of protein expression levels of MMP-9. (D) Protein expression levels of Bcl-2. (E) Protein expression levels of Caspase3. Data are presented as mean ± SD (n = 3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$
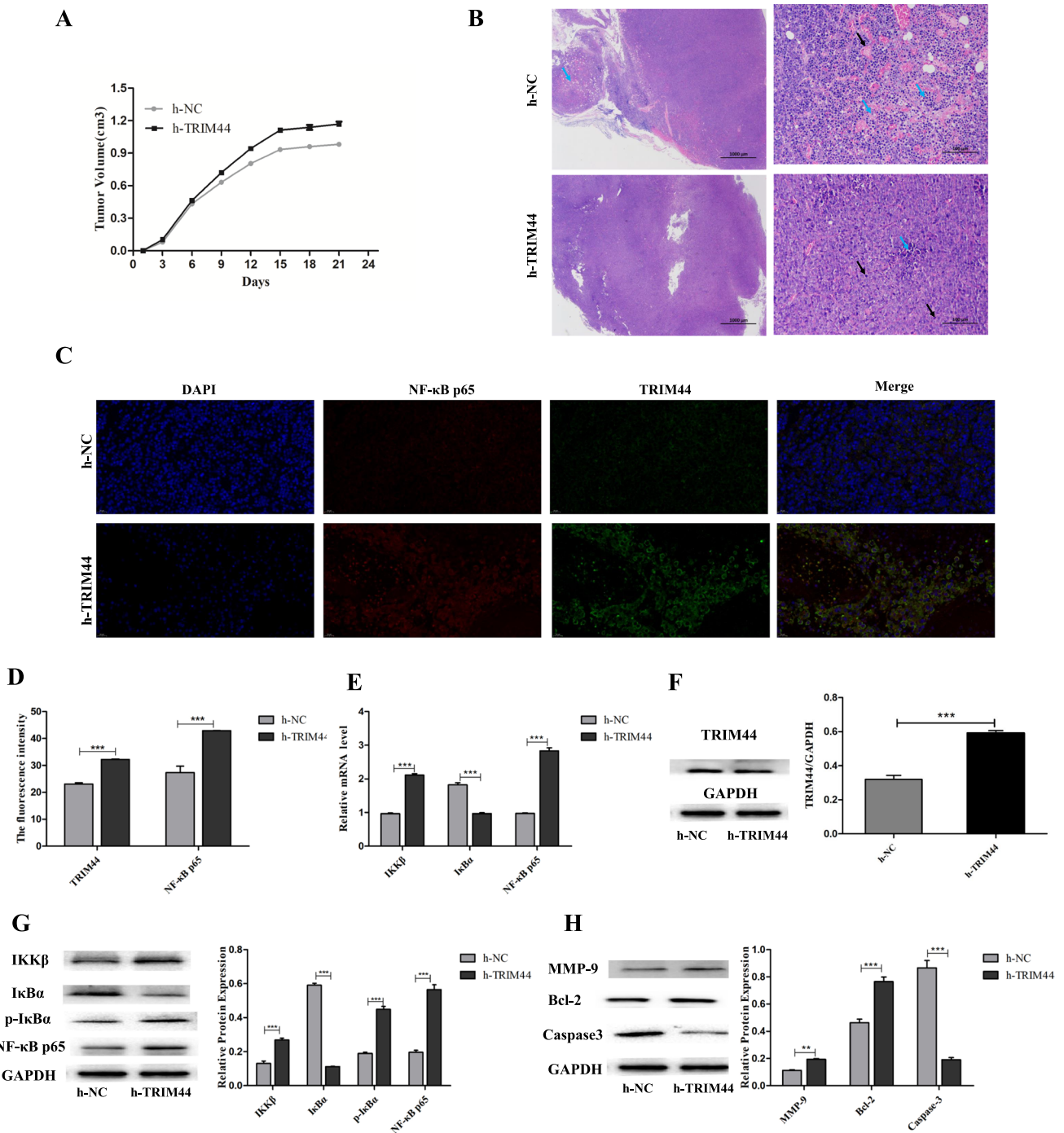
Fig. 3 TRIM44 contributes to the growth of OC tumors in vivo. (**A**) SKOV3 cells overexpressing TRIM44 were inoculated subcutaneously in nude mice and monitored for 21 days. (**B**) Pathological changes in tumor tissues were observed. (**C**, **D**) Representative images and quantitative data for detecting the expression levels of TRIM44, NF-κB p65 in tumor tissues. (**E**) Detection of gene expression levels of IKKβ, IκBα, and NF-κB p65 in tumor tissues. (**F**) Detection of TRIM44 protein expression levels in tumor tissues. (**G**) Detection of IKKβ, IκBα, p-IκBα, NF-κB p65 protein expression levels in tumor tissues. (**H**) Detection of IKKβ, IκBα, p-IκBα, NF-κB p65 protein expression levels of Bcl-2, Caspase3, MMP-9 in tumor tissues. Data are presented as mean ± SD ($n = 8$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Discussion

In this study, we propose that TRIM44 acts as a progression and metastasis marker in OC cells and OC-bearing xenograft mice. TRIM44 overexpression promotes SKOV3 cell proliferation and xenograft tumor growth, inhibits apoptosis, and enhances migration and invasion of OC cells. Moreover, a different mechanism of action is shown in Fig. 4 that TRIM44 overexpression stimulated the secretion of IKKβ factor by upregulating the levels of inflammatory cytokines in the tumor microenvironment (TNF-α, IL-1β, IL-8), which further promoted the degradation and phosphorylation of IκBα, initiated the entry of NF-κB p65 into the nucleus, and promoted the activation of NF-κB signaling pathway, leading to the activation of Metallomatrix protease (MMP-9), inflammatory chemokine (IL-8), and the expression of genes encoding apoptotic genes (Bcl-2, Caspase-3) were upregulated or downregulated, thus promoting the invasion, metastasis, and anti-apoptotic ability of OC cells.

The TRIM protein family has diverse physiological and pathological roles in the regulation of cell cycle progression, apoptosis, autophagy, gene expression, chromatin remodelling, signal transduction and immune response. (Koepke et al. 2021; Venuto and Merla 2019; Petrera and Meroni 2012; Kimura et al. 2015, 2016). Currently, more than 80 members of this family have been identified, which have the typical structural features of "RBCC," i.e., one or two B-box domains in the RING-finger domain, the Coil-Coil region, and the RING-finger domain, in order from the N-terminal to the triple C-terminal regions (Crawford et al. 2018). The results show that many of these domains have been identified in the RING-finger region. Increasing evidence suggests that many members of the TRIM family control the malignant biological behaviour of OC cells and are involved in the progression and metastatic spread of OC. For example, TRIM11 promotes the activation of the AKT/ERK signaling pathway, upregulates Bcl-2, downregulates Bax and other related apoptotic factors, and inhibits the apoptosis of OC cells (Chen et al. 2017). In contrast, TRIM21 acts as an oncogene in OC by promoting the expression of p21, inhibiting the expression of Caspase-3, Caspase-7, and total PARP, thereby inhibiting the proliferation of OC cells

Fig. 4 Mechanism of action of TRIM44 overexpression to promote OC progression
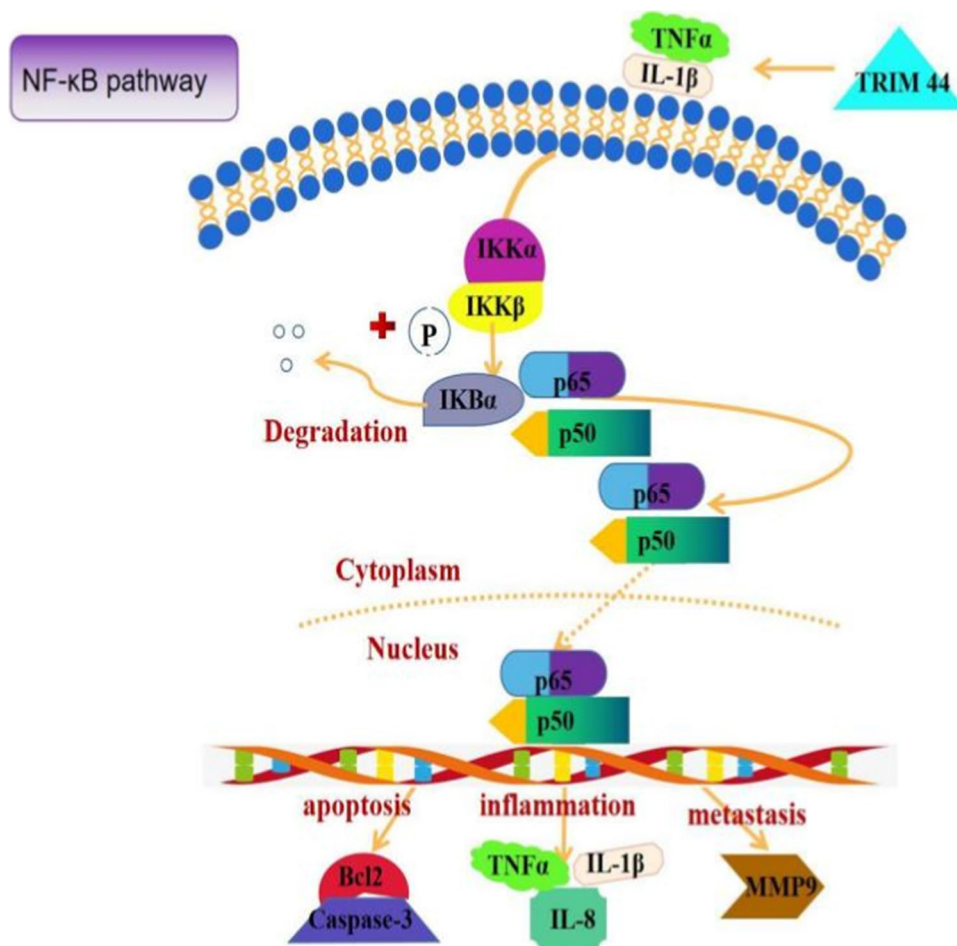
and promoting apoptosis (Sun et al. 2022). In addition, silencing the expression of TRIM28 significantly reduced the migration and invasion ability of OC cells by inhibiting the activation of the Wnt/β-catenin signaling pathway and EMT process (Deng et al. 2017). In view of the importance of TRIM in the development of OC in family members, it is suggested that TRIM may be a potential therapeutic target for OC patients.

TRIM44 is an essential member of the TRIM family. Several studies have shown that TRIM44 is over-expressed in a variety of tumour cells and tissues and is involved in the promotion of malignant biological behaviour such as proliferation, growth, migration, invasion and anti-apoptotic ability of tumour cells (Luo et al. 2015). For example, TRIM44 overexpression accelerates the G1/S phase transition of hepatocellular carcinoma cells, promotes the growth of hepatocellular carcinoma cells, and enhances the invasion and migration ability of cells (Zhu et al. 2016). Knockdown of TRIM44 expression inhibited the invasion and migration of intrahepatic cholangiocarcinoma cells and promoted apoptosis (Peng et al. 2018). Moreover, in the colorectum, TRIM44 overexpression significantly promoted the proliferation, migration, and invasion of colorectal cancer cells (Li et al. 2019). There was also evidence that TRIM44 was highly expressed in both epithelial ovarian cancer tissues and cells and had a significant correlation with FIGO stage and lymph node metastasis (Liu et al. 2018; Meng et al. 2022). TRIM44 was highly expressed in both epithelial OC tissues and cells and was significantly associated with the FIGO stage and lymph node metastasis. In order to further explore the impact of TRIM44 high expression phenotype on the malignant biological behavior of OC cells, in this study, we successfully constructed TRIM44 overexpressed SKOV3 cells and confirmed that TRIM44 promotes proliferation, inhibits apoptosis, and enhances cell migration and invasion of SKOV3 cells in vitro. In in vivo animal experiments, TRIM44 overexpression significantly promoted the growth of tumors in vivo. Thus, our study is consistent with the effect of TRIM44 on the malignant biological behavior of tumor cells. These data suggest that TRIM44 may have a role as an oncogene in the progression of OC.

As early as 1999, it was found that Rev-T avian retrovirus encodes the v-rel oncogene, and v-rel is an essential member of the Rel/NF-kappaB transcription factor family. Since then, the role of NF-κB in malignancy has received much attention (Gilmore 1999). Subsequent studies have confirmed that abnormal activation of the NF-κB signaling pathway regulates many tumor cells' proliferation, migration, invasion, and apoptosis and is closely related to tumorigenesis, development, and poor prognosis (Yenmis et al. 2021; Yi et al. 2021). For example, NF-κB activation in tumor-associated leukocytes, especially macrophages, promotes tumorigenesis by upregulating pro-tumor proinflammatory proteins, while NF-κB activation in precancerous cells promotes cell proliferation and metastasis (Li et al. 2005). Aberrant activation of the NF-κB signaling pathway in breast cancer cells promotes tumor cell invasion and metastasis (Yenmis et al. 2021). Zhu X et al. showed that overexpression of TRIM44 promoted the proliferation and growth of hepatocellular carcinoma by promoting NF-κB activation (Zhu et al. 2016). Kawabata H et al. found that knockdown of TRIM44 expression significantly inhibited TNFα-stimulated NF-κB-mediated transcriptional activity, which suppressed the ability of breast cancer cells to proliferate and migrate (Kawabata et al. 2017). In addition, numerous studies have shown that OC cell lines have high NF-κB background activity and that sustained activation of the NF-κB signaling pathway promotes OC cell proliferation and inhibits their apoptosis (Liu and Chen 2011). However, studies on the association between TRIM44 and NF-κB signaling pathways in OC are still scarce. In this study, we found that TRIM44 overexpression could promote the activation of the NF-κB signaling pathway, upregulate IKKβ protein in OC cells and tumor tissues, promote the degradation and phosphorylation of IκBα, and increase the nucleation of NF-κB p65 protein. These experimental data suggest that TRIM44 has a regulatory effect on the activation of the NF-κB signaling pathway.

Previous studies have confirmed that Bcl-2 and MMP-9 are overexpressed in OC cells, while Caspase-3 shows a low expression status, and they are associated with apoptosis, growth, invasion, and metastasis of OC (Beale et al. 2000; Alsafadi et al. 2016; Li et al. 2021). Other important inflammatory factors associated with the tumour microenvironment include TNF-α, IL-1β, and IL-8. Numerous studies have pointed out that factors associated with the tumor inflammatory microenvironment can affect the formation of tumor neovascularization by mediating the activation of NF-κB signaling, thus promoting tumor cell invasion and metastasis (Mu et al. 2020; Strozyk et al. 2014; Lai et al. 2011; Inoue et al. 2000). In the present study, we found that TRIM44 overexpression may promote OC cell proliferation and invasion and inhibit apoptosis by promoting the activation of the NF-κB signaling pathway, upregulating the expression of TNF-α, IL-1β, IL-8 inflammatory factors, Bcl-2 anti-apoptotic protein, MMP-9 metalloplasmic protease, and downregulating the expression of Caspase-3 apoptotic protein.

This study supports TRIM44 as an oncogene involved in OC. TRIM44 may promote migration, invasion, and apoptosis resistance of OC cells by activating the NF-κB signaling pathway. It suggests that TRIM44 may be a potential therapeutic target for OC treatment. These findings provide new clues for the treatment of OC.

Declarations

Ethical approval Not applicable.

Informed consent All authors contributed to the article and approved the submitted version.

Conflict of interest The authors declare no conficts of interest in this study.

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