Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

TMCO1 regulates cell proliferation, metastasis and EMT signaling through CALR, promoting ovarian cancer progression and cisplatin resistance

Guangyu Sun[1,2], Shan Gong[1], Suwei Lan[1], Ying He[1], Yanhua Sun[3], Zhengmao Zhang[1,*]

[1] *Department of Gynecology, The Fourth Hospital of Hebei Medical University, Hebei Tumor Hospital, Shijiazhuang, Hebei, China*
[2] *Department of Gynecology, Cangzhou People's Hospital, Cangzhou, Hebei, China*
[3] *Department of Gastrointestinal Hernia Surgery, Cangzhou People's Hospital, Cangzhou, Hebei, China*

Article Info

Article history:

Received: November 29, 2023
Accepted: January 10, 2024
Published: January 31, 2024

Use your device to scan and read the article online

Abstract

This study aimed to explore the involvement of Transmembrane and coiled-coil domains 1 (TMCO1) in ovarian cancer progression and its regulatory mechanisms in cisplatin resistance. Using the GEPIA database, we analyzed TMCO1 expression in ovarian cancer and normal tissues. In a cohort of 99 ovarian cancer patients, immunohistochemistry and immunofluorescence were employed to assess TMCO1 expression in tumor and adjacent tissues, correlating findings with clinical and pathological characteristics. TMCO1 overexpression and knockout cell models were constructed, and their impact on non-cisplatin-resistant (SK-OV-3) and cisplatin-resistant (SK-OV-3-CDDP) ovarian cancer cells was investigated through cloning, wound healing, Fluo 4, and Transwell experiments. Knocking down CALR and VDAC1 was performed to examine their effects on TMCO1, cell proliferation, and malignant markers. Subcutaneous tumor models in nude mice elucidated the in vivo role of TMCO1 in tumor growth. Expression levels of CALR, VDAC1, angiogenesis indicators (CD34), and epithelial-mesenchymal transition (EMT) markers were evaluated. TMCO1 expression in ovarian cancer tissue significantly differed from normal tissue, correlating with survival rates. TMCO1 overexpression was associated with lymph node metastases, late FIGO stage, and larger tumors. TMCO1 promoted proliferation, calcium ion elevation, cytoskeletal remodeling, and metastasis in SK-OV-3 and SK-OV-3-CDDP cells, upregulating VDAC1, CALR, Vimentin, N-cadherin, β-catenin, and downregulating E-cadherin. Silencing TMCO1 inhibited cell growth, proliferation, and angiogenesis in vivo, suppressing the expression of CALR, VDAC1, Vimentin, N-cadherin, and β-catenin. Overall, this study highlighted TMCO1 as a crucial regulator in ovarian cancer progression, influencing VDAC1 through CALR and impacting diverse cellular processes, offering potential as a targeted therapeutic strategy for ovarian cancer.

Keywords: Ovarian cancer; TMCO1; Cisplatin resistance; Proliferation; Migration; Ca^{2+} homeostasis

1. Introduction

Ovarian carcinoma is the most lethal gynecologic malignancy, and high-grade serous carcinoma as the main histologic subtype [1]. In 2020, there were 13,940 fatalities and 21,750 newly diagnosed cases of ovarian cancer [2]. Ovarian cancer is known as the "invisible killer" of women's health because of its atypical early symptoms and inadequate early diagnostic methods [3]. Initial therapy includes cytoreductive surgery and platinum-based chemotherapy [4]. Patients are highly sensitive to platinum at the time of initial therapy, approximately 90% of stage IIIC patients relapse within 5 years and eventually have resistance to platinum-based chemotherapy [5]. Due to a high proportion of patients who acquire chemoresistance, novel treatment approaches are required to be developed.

Calcium ions (Ca2+) participate in a series of biological behaviors as important second messengers in cells. The largest Ca2+ reservoir in the cell is the endoplasmic reticulum (ER). and Calcium (Ca2+) is a crucial second messenger in the cell and is involved in a variety of biological processes [6]. To obtain the proper Ca2+ signal, the Ca2+ concentration in the ER must be maintained in a stable state, and ER dysfunction Numerous human cancers, including ovarian carcinoma, and Ca2+ homeostasis are related [7]. The most notable ovarian cancer pathogenesis is Ca2+ transfer between the endoplasmic reticulum and mitochondria, which starts the Krebs cycle and enhances ATP production and biosynthesis while also promoting the spread of the disease [7]. Because ER-mitochondrial Ca2+ signaling causes cisplatin-triggered cell death, alterations in Ca2+ homeostasis within ER and mitochondria play a key role in ovarian cancer chemoresistance, including cisplatin resistance [8]. Transmembrane and coiled-coil domains 1 (TMCO1) is a highly conserved 188 amino acid cation channel resided in the membrane of ER and mitochondria [9]. TMCO1 diffuses easily throughout the ER membrane [10] and acts as a Ca^{2+} leak channel in the ER [11-13].

When ER Ca(2+) overloaded, TMCO1 released Ca(2+) load-activated Ca(2+) (CLAC) channel to maintaining

* Corresponding author.
E-mail address: zhangzhengmao111@163.com (Z. Zhang).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.1.14

homeostasis of Ca(2+) (9). Female fertility and the development of ovarian follicles are dependent on TMCO1 because it maintains the ER Ca2+ homeostasis of granulosa cells, the loss of which results in ER stress-induced apoptosis and elevated cellular ROS levels in granulosa cells and impaired ovarian follicle development [14]. Some studies suggest that TMCO1 is a very important risk factor in the progression of colorectal cancer [15]. Nevertheless, the role of TMCO1 in ovarian carcinoma remains indistinct.

Calreticulin (CALR), a highly conserved chaperone protein involved in numerous physiological processes, including cell adhesion, is a regulator of endoplasmic reticulum calcium homeostasis [16]. Studies have also shown that the interaction of CALR with natural cytotoxic trigger receptor 1 (NCR1) activates NK cells and participates in tumor immune escape mechanism [17]. TMCO1, CALR, and VDAC1 are all involved in the creation of the intracellular calcium homeostasis mechanism in cellular calcium homeostasis. Endoplasmic reticulum calcium homeostasis is specifically controlled by TMCO1 and CALR, and the balance of endoplasmic reticulum calcium homeostasis can impact the mitochondrial calcium homeostasis process. VDAC1 is a component in the regulation of mitochondrial calcium homeostasis that is found in the outer membrane of mitochondria and is sensitive to calcium ion rise [15, 18, 19]. The tumor microenvironment and metabolic reorganization of cancer cells are mediated by mitochondrial malfunction through dysregulation of mitochondrial calcium homeostasis [20]. Some of the proteins that control apoptosis and the permeability of the mitochondrial outer membrane are assumed to be calcium-signaling regulatory proteins that come from the endoplasmic reticulum [21].

Here, we demonstrate for the first time the role of TMCO1 in the progression of ovarian cancer, indicating that TMCO1 can reverse the mechanism of cisplatin resistance. Experiments have shown that TMCO1 can regulate the proliferation, calcium ion levels, cytoskeleton, and metastasis of non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells, as well as the expression of malignant phenotype marker proteins. In vivo experiments, silencing TMCO1 can significantly inhibit the growth, proliferation, and angiogenesis of non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells, and reduce the expression of CALR, VDAC1, and EMT-related marker proteins. In summary, we speculate that TMCO1 can regulate the calcium homeostasis of ovarian cancer cells and the production of cisplatin resistance through CALR regulation of VDAC1. This study proposes that targeting TMCO1 may be an effective new approach for the treatment of ovarian cancer.

2. Materials and methods:

2.1. Public databases

The expression of TMCO1 was analyzed in ovarian carcinoma and normal tissue specimens in the integrated TCGA and GTEx data utilizing the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) web server (http://gepia2. carcinoma -pku.cn/) [22]. Kapan-Meier curves of ovarian carcinoma subjects possessing highly and lowly expressed TMCO1 were conducted in the GSE26712 (n = 195) [23] and GSE27651 (n = 49) [24] cohorts from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/gds/) using the Kaplan-Meir Plotter (https://kmplot.com/). Log-rank test was applied to evaluate the survival difference between groups.

2.2. Human specimens

All clinical specimens of 99 ovarian carcinoma patients were collected from The Fourth Hospital of Hebei Medical University. Paired ovarian carcinoma specimens and adjacent non-tumor specimens were harvested during operation, immediately immobilized with neutral formalin solution, and then routine pathological diagnosis was performed. All the patients had no prior history of chemotherapy or radiotherapy. This project gained approval by the Ethical Committee of The Fourth Hospital of Hebei Medical University (2020166). Each patient signed written informed consent.

2.3. Immunohistochemistry and immunofluorescence

Tumor tissues were fixed in 10% formalin, embedded in paraffin, and cut into 4 μm paraffin-embedded sections for immunohistochemistry and immunofluorescence assays. The sections were subjected to EDTA (PH8.0) antigen repair, and immunofluorescence was performed with 0.1 % Triton X-100 incubation for 15 min, followed by incubation with primary antibody against TMCO1 (1:100; 27757-1-AP; Proteintech, Rosemont, IL, USA), β-catenin (1:150; 51067-2-AP; Proteintech, Rosemont, IL, USA), Vimentin (1:200; 10366-1-AP; Proteintech, Rosemont, IL, USA), N-cadherin (1:100; 22018-1-AP; Proteintech, Rosemont, IL, USA), E-cadherin (1:100; 20874-1-AP; Proteintech, Rosemont, IL, USA), CD34 (1:200; ab81289; Abcam, Cambridge, MA, USA), VDAC1 (1:100; 55259-1-AP; Proteintech, Rosemont, IL, USA), and CALR (1:100; 27298-1-AP; Proteintech, Rosemont, IL, USA) at 4°C overnight. For immunohistochemistry, the sections were incubated by HRP-labeled secondary antibody (PV-6000; ZSGB-BIO, China) lasting 30 minutes. For immunofluorescence, the sections were incubated with Alexa Fluor® 488 secondary antibody (1:100; ZF-0511; ZSGB-BIO, China) lasting two hours.

2.4. Cell culture

Ovarian carcinoma SK-OV-3 cells (Serial: TCHu185, National Collection of Authenticated Cell Cultures. https://www.cellbank.org.cn/search-detail.php?id=76) and SK-OV-3/DDP Cisplatin-resistant cells (Serial: NYZQ0035, Shanghai Zhong Qiao Xin Zhou Biotechnology Co.,Ltd. https://www.zqxzbio.com/Web/Index/p_more/pid/3629.html) were grown in RPMI-1640 medium (Gibco, Rockville, MD, USA) plus 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin.

2.5. Transfection

Gene silencing was conducted in SK-OV-3 as well as SK-OV-3-CDDP cells in 6-well plates (1×10^5 cells / well). Plasmids carrying short hairpin RNAs (shRNAs) of TMCO1 (sh-TMCO1#1: TRCN0000062125: 5'-CCCTAATGGGAATGTTCAATT-3'; sh-TMCO1#2: TRCN0000062127:5'-CATCGAAATCTGCTGGGAGAT-3') or negative controls (sh-NC: TRCN0000072243:5' -CTTCGAAATGTCCGTTCGGTT-3') were purchased from company Shanghai Sangon (SangonBiotech, Shanghai, China, https://www.sangon.com/). Full-length TMCO1 was cloned into pcDNA3.1(+) ZB02427(Cloning site**:** NheI/BamHI), Empty vector was utilized as a control. Both were purchased from compa-

ny Shanghai Sangon (SangonBiotech, Shanghai, China, https://www.sangon.com/). The shRNAs were transfected into cells using Lipofectamine 8000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Cultured at 37℃ with 5% CO2 for 24 h, cells were collected for Western blotting assay.

2.7. Western blotting

Cells or tissues were washed with PBS, and lysed using ice-cold RIPA buffer plus protease inhibitor cocktail and phosphatase inhibitor cocktail. Afterwards, the protein concentration was quantified with BCA kit. The protein was separated through 8~10% SDS-PAGE, followed by transference to PVDF membranes. The membranes were sealed in 5% skim milk lasting one hour at room temperature, as well as incubated by primary antibody against TMCO1 (1:500; 27757-1-AP; Proteintech, Rosemont, IL, USA), β-catenin (1:2000; 51067-2-AP; Proteintech, Rosemont, IL, USA), VDAC1 (1:1000; 55259-1-AP; Proteintech, Rosemont, IL, USA), CALR (1:1000; 27298-1-AP; Proteintech, Rosemont, IL, USA), MMP2 (1:1000; 66366-1-Ig; Proteintech, Rosemont, IL, USA), MMP9 (1:1000; 10375-2-AP; Proteintech, Rosemont, IL, USA), N-cadherin (1:1000; 22018-1-AP; Proteintech, Rosemont, IL, USA), E-cadherin (1:1000; 20874-1-AP; Proteintech, Rosemont, IL, USA), Vimentin (1:2000; 10366-1-AP; Proteintech, Rosemont, IL, USA), Ki-67 (1:1000; ab16667; Abcam, Cambridge, MA, USA) or GAPDH (1:5000; 10494-1-AP; Proteintech, Rosemont, IL, USA) at 4 °C. Following being washed in TBST, incubation with secondary antibody (1:5000; ZB-2301 or ZB-2305; ZSGB-BIO, China) was presented at room temperature for 1 hour. The protein band was developed by chemiluminescence utilizing ECL reagent. GAPDH served as an internal control. Band intensity was quantified using ImageJ software.

2.8. Clonogenic assay

SK-OV-3 cells or SK-OV-3-CDDP cells were inoculated onto 6-well plates (1000 cells / well). All cells were cultivated until visible colonies were formed. Afterwards, the cells were fixed in 4% paraformaldehyde for 15 min, and the colonies were stained with 0.1% crystal violet for 20 min. After photographing, colonies were counted (one colony with over 50 cells).

2.9. Wound healing assay

SK-OV-3 cells or SK-OV-3-CDDP cells were inoculated onto 6-well plates. When the cells reached confluence, the monolayers were gently scratched utilizing pipette tips, and then carefully washed with PBS to remove debris. Images were captured 0 and 24 h after scratching.

2.10. Transwell experiment

Transwell Inserts (8 μm pore-size, Corning, Corning, NY, USA) was applied for measuring cellular invasion. 5×10^4 SK-OV-3 cells or SK-OV-3-CDDP cells were resuspended in serum-free medium and loaded onto uncoated inserts with polycarbonate membrane. Inserts were placed in a 24-well plate containing complete media (10% FBS) in the incubator. Following 24 hours, cells migrated to the underside of the insert were washed in PBS, fixed in paraformaldehyde lasting half hour, as well as stained by 0.1% crystal violet. Migrative cells were counted under 5 random regions.

2.11. Cellular immunofluorescence

SK-OV-3 or SK-OV-3-CDDP cells were inoculated onto coverslips in a 12-well plate, which were then fixed by 4% paraformaldehyde lasting 10 minutes, permeabilized by 0.1% triton X-100 lasting 15 minutes. Thereafter, the cells were sealed by 1% BSA for 1 hour at room temperature, as well as incubated by primary antibodies against TMCO1 (1:200; ab220729; Abcam, Cambridge, MA, USA), β-catenin (1:250; ab184919; Abcam, Cambridge, MA, USA), Vimentin (1:200; 10366-1-AP; Proteintech, Rosemont, IL, USA), N-cadherin (1:100; 22018-1-AP; Proteintech, Rosemont, IL, USA), VDAC1 (1:100; 55259-1-AP; Proteintech, Rosemont, IL, USA), CALR (1:200; 27298-1-AP; Proteintech, Rosemont, IL, USA) and E-cadherin (1:150; ab231303; Abcam, Cambridge, MA, USA) at 4°C overnight. After being washed 3 times in PBS, coverslips were incubated by Alexa Fluor® 488 secondary antibody (1:100; ZF-0511; ZSGB-BIO, China) lasting two hours. Under fluorescence microscopy, images were captured.

2.12. Animal experiments

The animal experiment was presented strictly in accordance with the Institutional Ethics Guidelines for Animal Experiments approved by the Animal Ethics Committee of The Fourth Hospital of Hebei Medical University (2020166). 4-week-old female nude mice (BALB/C; 18–20 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China, and fed following specific pathogen-free conditions. All nude mice were randomized into four groups (n = 5). Approximately 5×10^6 sh-TMCO1- or sh-NC-treated SK-OV-3 or SK-OV-3-CDDP cell lines were suspended by 0.1 mL sterile PBS, as well as injected subcutaneously into the axilla of mice, respectively. Tumor width (W) and length (L) were measured every week following the appearance of tumor, and the tumor volume (V) was calculated following the formula $V = (W^2 \times L)/2$. After 4 weeks, all mice were euthanized through intraperitoneal injection of pentobarbital sodium (200 mg/kg), and tumors were collected.

2.13. Detection of the intracellular calcium concentration

The cells were washed with HBSS 3 times and incubated with 1μM Fluo-4 AM (Thermo-Fisher, Waltham, MA, USA) dye at 37°C for 30 min. After incubation, the cells were washed 3 times with HBSS to fully remove the remaining working fluid. Finally, the cells were examined under a fluorescence microscope.

2.14. Actin cytoskeleton staining

According to the instruction of Actin-Tracker Green-488 (Beyotime, Shanghai, China), the cells were washed twice with PBS, fixed with 3.7% paraformaldehyde with PBS for 15 min, washed twice with 0.1% Triton-X100/PBS, Added Actin-Tracker Green and incubated for 30 minutes. Using fluorescence microscopy to observe changes in actin cytoskeleton and photograph.

2.15. Statistical analysis

All experiments were repeated at least thrice, and results were displayed as the mean ± standard deviation. All analysis was implemented utilizing the student's t-test or one-way analysis of variance. The chi-square tests were

utilized for evaluating the interactions of TMCO1 expression with clinical parameters. All tests were conducted utilizing Statistic Package for Social Science (SPSS) or GraphPad Prism 8.0 (GraphPad, La Jolla, CA, USA).

3. Results

3.1. TMCO1 is highly expressed in ovarian cancer and is associated with tumor size, late FIGO stage, lymph node metastasis, and overall survival

Evidences have indicated that TMCO1 dysfunction is linked with carcinogenic processes [25]. The analysis of comprehensive TCGA and GTEx profiles showed that compared to normal tissue (n=88), TMCO1 was significantly upregulated in ovarian cancer (n=426) (Figure 1A). In the GSE26712 and GSE27651 cohorts, ovarian cancer patients with high TMCO1 expression exhibited poorer overall survival compared to patients with low TMCO1 expression (Figure 1B, 1C).

We retrospectively collected tissue specimens and clinicopathological information from 99 patients with ovarian cancer. Immunohistochemistry confirmed that TMCO1 expression was significantly upregulated in ovarian cancer compared to adjacent non-tumor tissue specimens (Figure 1D, 1E). TMCO1 is mainly expressed in cytoplasm and cell membrane of tumor cells. The results of immunofluorescence were also consistent with those of immunohistochemistry (Figure 1F, 1G). Among the 99 patients, 61 were cisplatin-sensitive, 42 were positive for TMCO1 expression (68.85%), 38 were cisplatin-resistant, and 25 were positive for TMCO1 expression (65.79%). The expression level of TMCO1 in both cisplatin-sensitive and cisplatin-resistant patients was significantly correlated with tumor size, late FIGO stage, and lymph node metastasis (Table 1). These data suggest the role of TMCO1 in ovarian cancer progression and its clinical value for drug resistance and prognosis.

3.2. Effect of TMCO1 on proliferation of non-cisplatin-resistant/Cisplatin-resistant ovarian cancer cells

To evaluate the role of TMCO1 in ovarian cancer, TMCO1 was silenced and overexpressed in SK-OV-3 cells. The expression of sh-TMCO1#2 was more obvious, which was used as the follow-up experimental sequence

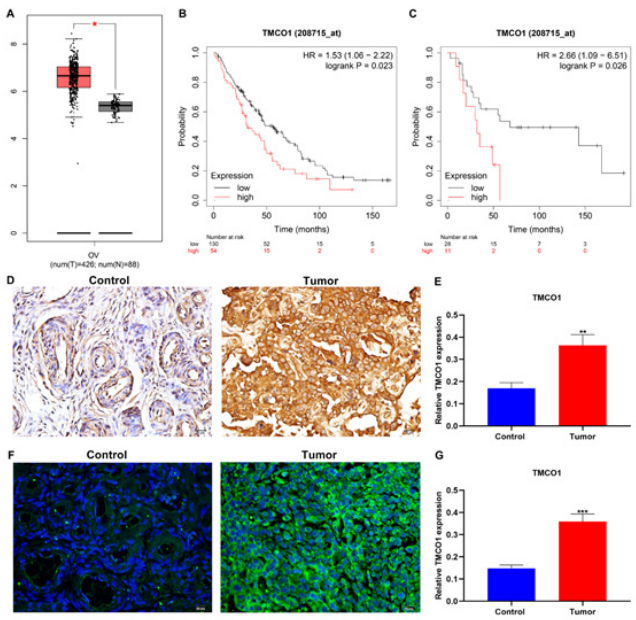

Fig. 1. TMCO1 presents high expression in ovarian carcinoma and is linked with undesirable clinical outcomes. (A) Box plot shows the RNA expression of TMCO1 in 426 ovarian carcinoma tissues (T) and 88 normal tissues (N) in the integrated TCGA and GTEx data. *P<0.05. (B, C) Kaplan-Meier analysis depicts the survival difference in ovarian carcinoma patients possessing high TMCO1 expression relative to those possessing low TMCO1 expression in the (B) GSE26712 and (C) GSE27651 cohorts. (D) Immunohistochemistry shows the expression and distribution of TMCO1 in adjacent normal tissues and ovarian carcinoma tissues. Scale bar, 20 μm. (E) A bar graph visualizes TMCO1 expression in two groups following immunohistochemistry results. **P<0.01. (F) Immunofluorescence examines the expression and distribution of TMCO1 in adjacent normal tissues and ovarian carcinoma tissues. Scale bar, 20 μm. (G) A bar graph shows TMCO1 expression in two groups in accordance with immunofluorescence results. ***P<0.001.

Table 1. Associations between TMCO1 expression and clinicopathological characteristics of ovarian carcinoma patients.

Clinical parameters	TMCO1 expressionn in Cisplatin sensitive patients			P-value	TMCO1 expressionn in Cisplatin-resistant patients			P-value
	Total (n=61)	Positive (n=42)	Negative (n=19)		Total (n=38)	Positive (n=25)	Negative (n=13)	
Age								
≤60	35	22	13	0.241	23	16	7	0.544
>60	26	20	6		15	9	6	
Tumor size (mm)								
≥30	31	25	6	0.043	23	18	5	0.045
<30	30	17	13		15	7	8	
FIGO stage								
I-II	20	8	12	0.001	13	5	8	0.010
III-IV	41	34	7		25	20	5	
Pathologic type								
Serous	42	31	11	0.214	20	14	6	0.564
Mucous and others	19	11	8		18	11	7	
Lymph node metastases								
Positive	46	36	10	0.005	18	16	2	0.004
Negative	15	6	9		20	9	11	
Distant metastases								
Positive	9	4	5	0.087	21	14	7	0.899
Negative	52	38	14		17	11	6	

(Figure 2A-D). In SK-OV-3 and SK-OV-3-CDDP cells, TMCO1 upregulation significantly increased the number of colonies (Figure 2E-G). In contrast, by knocking down TMCO1, SK-OV-3 and SK-OV-3-CDDP cell lines significantly reduced the number of colonies. Therefore, TMCO1 can regulate the proliferation ability of non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells.

3.3. TMCO1 regulated the migration of non-cisplatin-resistant/cisplatin-resistant ovarian cancer cells

In the wound healing experiment, overexpression of TMCO1 significantly shortened the wound distance of SK-OV-3 and SK-OV-3-CDDP cells (Figure 3A-D). In addition, silencing TMCO1 significantly widened the wound distance. Transwell results showed that overexpression of TMCO1 significantly increased the number of invasive cells (Figure 3E-H). On the contrary, silencing TMCO1 expression significantly reduced the number of invading cells. Comparing SK-OV-3 and SK-OV-3-CDDP cells in the same group, it was found that the migration and invasion abilities of SK-OV-3-CDDP cells were significantly enhanced. Therefore, TMCO1 can regulate the migration and invasion ability of SK-OV-3 and SK-OV-3-CDDP cells, indicating that TMCO1 plays a key role in the progression of ovarian cancer and the mechanism of cisplatin resistance.

3.4. TMCO1 regulated calcium levels and cytoskeletal remodeling in SK-OV-3 and SK-OV-3-CDDP cells

Overexpression of TMCO1 can upregulate the calcium ion levels in SK-OV-3 and SK-OV-3-CDDP cells,

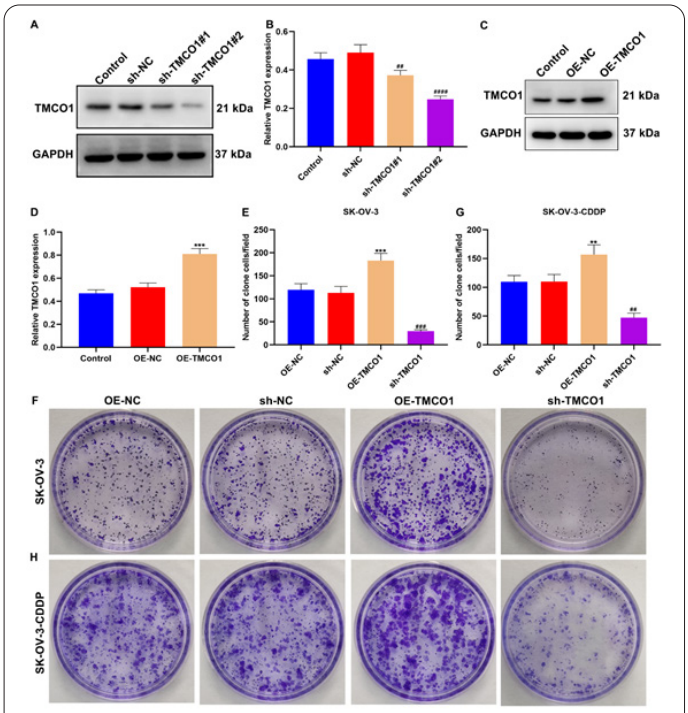

Fig. 2. TMCO1 up-regulation enhances proliferation of cisplatin-sensitive as well as resistant ovarian carcinoma cells. (A, B) Western blot verifies TMCO1 expression in SK-OV-3 cells administrated with specific shRNAs against TMCO1. (C, D) Western blot validates the overexpression of TMCO1 in SK-OV-3 cells. (E, F) The clonogenic assay shows the colonies of SK-OV-3 cell line with TMCO1-knockout or overexpressed plasmids. (G, H) Clonogenic assay displays the colonies of SK-OV-3-CDDP cell line with TMCO1-knockout or overexpressed plasmids. **P<0.01; ***P<0.001; ****P<0.0001.

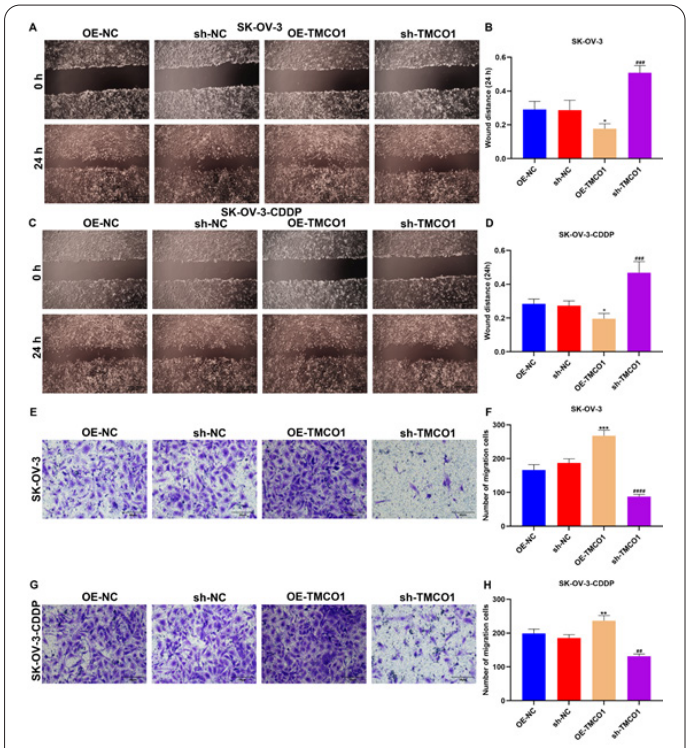

Fig. 3. High TMCO1 expression facilitates migration of cisplatin-sensitive as well as resistant ovarian carcinoma cells. (A, B) A wound-healing experiment was applied to examine the migration capacity of SK-OV-3 cells subjected to TMCO1-knockout or overexpressed plasmids. Scale bar, 200 μm. (C, D) A wound wound-healing experiment was applied to evaluate the migrative capacity of SK-OV-3-CDDP cells subjected to TMCO1-knockout or overexpressed plasmids. Scale bar, 200 μm. (E, F) A Transwell assay was conducted to investigate the number of migrative cells of SK-OV-3 cells subjected to TMCO1-knockout or overexpressed plasmids. Scale bar, 50 μm. (G, H) Transwell assay was presented for assessing the number of migrative SK-OV-3-CDDP cells with TMCO1-knockout or overexpressed plasmids. Scale bar, 50 μm. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

promote the remodeling of actin microfilaments (Figure 4A-C), and induce the increase and elongation of cell edge protrusions. After silencing TMCO1, this phenomenon weakens (Figure 4D-F). The results indicate that TMCO1 can affect cytoskeletal remodeling by regulating intracellular calcium levels, and participate in the mechanisms of ovarian cancer cell metastasis and drug resistance in this way.

3.5. The interaction of TMCO1 with CALR and VDAC1 and its influence on the expression of EMT markers

We found that overexpression of TMCO1 could up-regulate the expression of CALR, VDAC1 and EMT-related marker proteins β-catenin, Vimentin, N-cadherin and decrease the expression of E-cadherin in SK-OV-3 and SK-OV-3-CDDP cells. Silencing TMCO1 expression yielded opposite results (Figure 5A-P). CALR is involved in endoplasmic reticulum calcium homeostasis regulation. In this experiment, TMCO1 can regulate the expression of CALR and also affect the expression of VDAC1 in the mitochondrial outer membrane. Immunofluorescence experiments showed that TMCO1, CALR, and VDAC1 were mainly expressed in the cytoplasm of SK-OV-3 and SK-OV-3-CDDP cells. Overexpression and silencing of TMCO1 expression could regulate the expression of CALR and

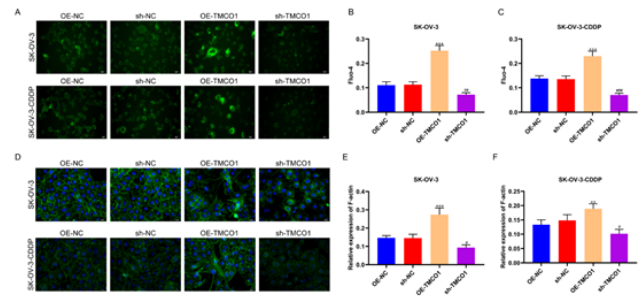

Fig. 4. TMCO1 regulated calcium levels and cytoskeletal remodeling in SK-OV-3 and SK-OV-3-CDDP cells. (A,B) Representative photographs of cytosolic Ca2+ level in SK-OV-3 cells and SK-OV-3-CDDP via Fluo-4 AM staining were displayed. Bar, 20 μm. (C,D) Phalloidin staining (Green) after TMCO1-knockout or overexpressed plasmids. Bar, 20 μm.

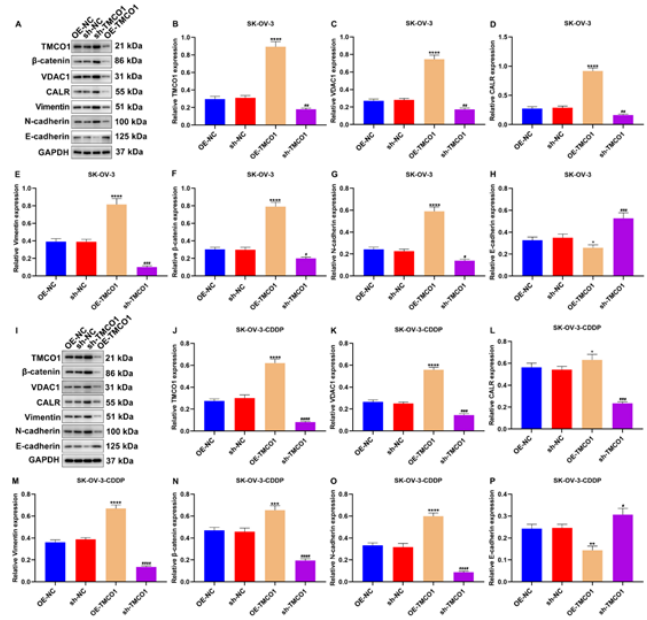

Fig. 5. The interaction of TMCO1 with CALR and VDAC1 and its influence on the expression of EMT markers. (A-H) Western blotting was conducted for examining (B) TMCO1, (C) VDAC1, (D) CALR, (E) Vimentin, (F) β-catenin, (G) N-cadherin, and (H) E-cadherin expression in SK-OV-3 cells subjected to TMCO1-knockout or overexpressed plasmids. (I-P) Western blotting was conducted to examine (J) TMCO1, (K) VDAC1, (L) CALR, (M) Vimentin, (N) β-catenin, (O) N-cadherin, and (P) E-cadherin expression in SK-OV-3-CDDP cell line subjected to TMCO1-knockout or overexpressed plasmids. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

VDAC1, accompanied by changes in N-cadherin and E-cadherin expressions, which were consistent with the above results. Further, confirm the role of TMCO1 in the progression of ovarian cancer. We speculate that TMCO1 may affect VDAC1 expression through CALR regulation of endoplasmic reticulum calcium levels, regulating ovarian cancer cell proliferation, cytoskeleton remodeling, and metastasis (Figure 6A-L).

To verify the interaction relationship between TMCO1 and CALR, VDAC1, we constructed CALR, VDAC1 siRNA sequences to transfect SK-OV-3 and SK-OV-3-CDDP cells. Si-CALR#3 and si-VDAC1 #3 were more significantly expressed as subsequent experimental sequences (Figure 7A-D). Tmco1-oe +CALR siRNA can block the effect of TMCO1 overexpression on up-regulation of CALR. Importantly, TMCo1-OE does not up-regulate the expression of VDAC1. However, the expression of CALR in the TMCO1-OE+VDAC1 siRNA group showed an up-regulated trend, indicating that the knockdown of

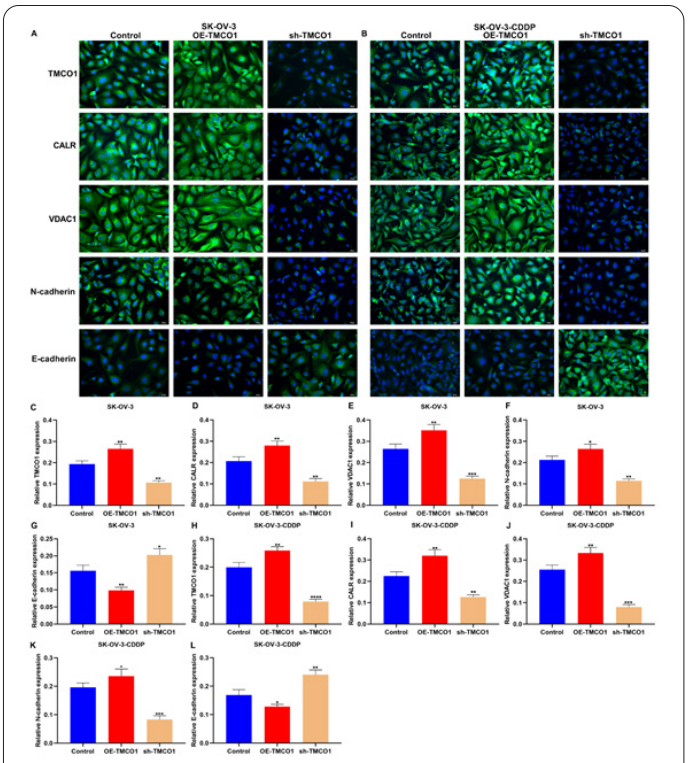

Fig. 6. The interaction of TMCO1 with CALR and VDAC1 and its influence on the expression of EMT markers. (A, B) Representative images of immunofluorescence for SK-OV-3 or SK-OV-3-CDDP cell lines. Bar scale, 20 μm. (C-G) The expression levels of (C) TMCO1, (D) CALR, (E) VDAC1, (F) N-cadherin, and (G) E-cadherin were quantified in SK-OV-3 cells subjected to TMCO1-knockout or overexpressed plasmids. (H-L) The expression levels of (H) TMCO1, (I) CALR, (J) VDAC1, (K) N-cadherin, or (L) E-cadherin were quantified for SK-OV-3-CDDP cell line with TMCO1-knockout or overexpressed plasmids. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

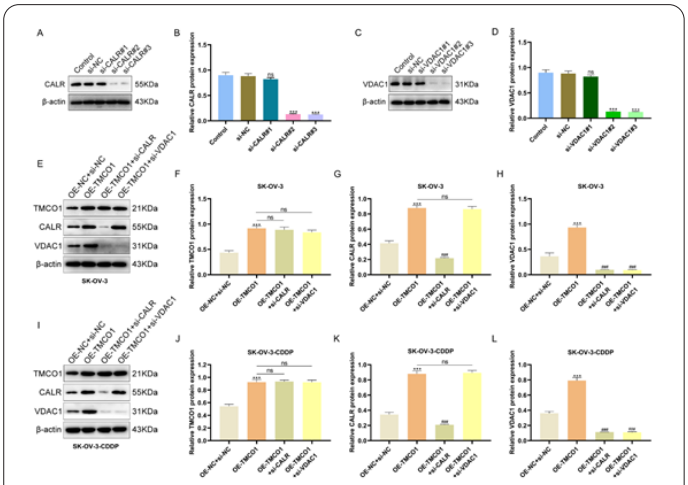

Fig. 7. The interaction of TMCO1 with CALR and VDAC1. (A-L) Western blotting was conducted to examine (B) TMCO1, (C)CALR and(D) VDAC1 expression with CALR-siRNA and VDAC1-siRNA transfections. (E-H) Western blotting was conducted for examining (F) TMCO1, (G) CALR, (H) VDAC1 in SK-OV-3 cells. (I-L) Western blotting was conducted for examining (J) TMCO1, (K) CALR, (L) VDAC1 With TMCO1 overexpression or CALR-siRNA and VDAC1-siRNA transfections in SK-OV-3-CDDP cells.

VDAC1 did not affect the expression of CALR, but the expression of VDAC1 was significantly reduced. (Figure E - L). CALR and VDAC1 knockdown had no significant effect on TMCO1 expression. Interestingly, the expression of VDAC1 can be down-regulated by lowering CALR. However, knocking down VDAC1 did not modulate CALR expression (Figure 8A-H).

3.6. Targeting TMCO1 inhibits the growth of SK-OV-3 and SK-OV-3-CDDP cells in xenograft models

To further confirm the role of TMCO1 in the progression and resistance mechanism of ovarian cancer, we divided the experimental group into four groups: non-cisplatin-resistant sh-NC (Control), cisplatin-resistant group (CDDP), non-cisplatin resistant+silenced TMCO1 group (sh-TMCO1), and cisplatin resistant+silenced TMCO1 group (CDDP+sh-TMCO1). Inject four groups of cells into the subcutaneous model of BALB/C mice. After 4 weeks, compared to the control group or CDDP group, silencing the expression of TMCO1 significantly inhibited tumor growth (Figure 9A-C). Immunohistochemistry (Figure 9D, E) and immunofluorescence (Figure 9F, G) were used to measure the expression of CD34 to evaluate angiogenesis. Silencing TMCO1 significantly reduced the microvessel density of tumor tissue in SK-OV-3 and SK-OV-3-CDDP cells. In summary, the above data demonstrate the anti-ovarian cancer progression and reversal of cisplatin resistance of TMCO1 in vivo.

3.7. Targeting TMCO1 inhibited the expression of CALR, VDAC1 and EMT markers in xenograft tumors

Western blot showed that silencing TMCO1 significantly decreased the expression of CALR, VDAC1 and EMT marker proteins (Vimentin, N-cadherin, β-catenin, MMP2, MMP9) in SK-OV-3 and SK-OV-3-CDDP tumor tissues. it also decreased the expression of cell proliferation index ki-67, indicating that the proliferation ability of tumor cells was impaired. In addition, silencing TMCO1 enhanced the expression of E-cadherin, indicating the inactivation of EMT signal in tumor cells (Figure 10A-K).

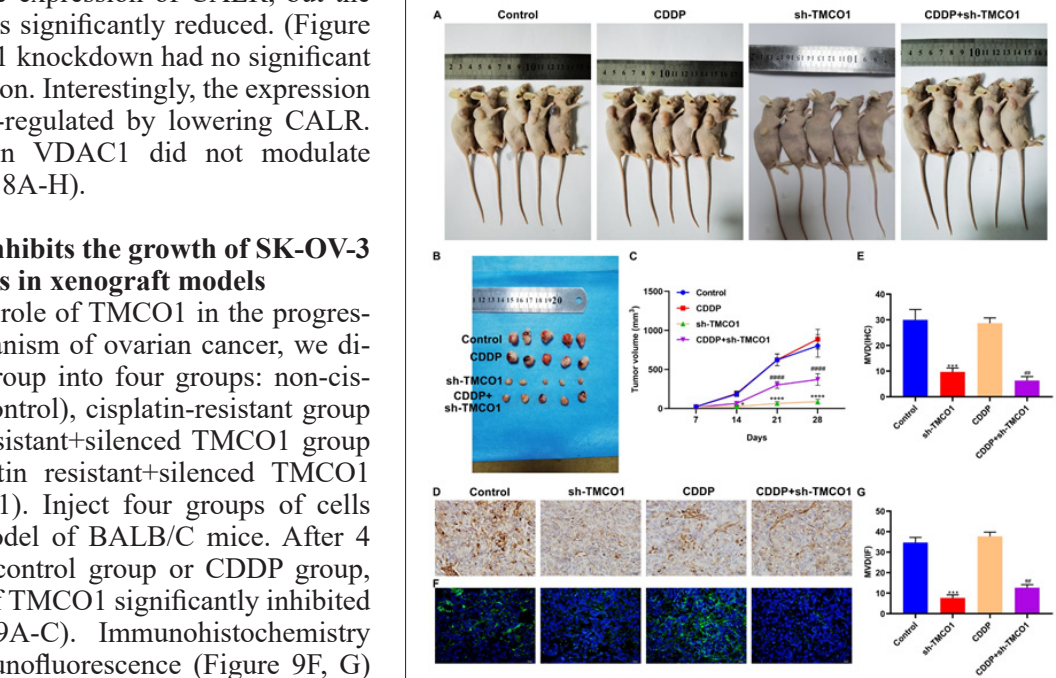

Fig. 9. Targeting TMCO1 inhibits the growth of SK-OV-3 and SK-OV-3-CDDP cells in xenograft models. (A) Photographs of nude mouse models with sh-NC-transfected SK-OV-3 cell line (control group), mice injected with sh-NC-transfected SK-OV-3-CDDP cell line (CDDP group), mice injected with sh-TMCO1-transfected SK-OV-3 cells (sh-TMCO1 group), mice injected with sh-TMCO1-transfected SK-OV-3-CDDP cells (CDDP + sh-TMCO1 group). (B) Photographs of tumors from above four groups. (C) Tumor growth curves in each group. Compared with control group, *p<0.05; ****P<0.0001. Compared with CDDP group, ####P<0.0001. (D, E) Immunohistochemistry examining the expression of CD34 in tumors from four groups. Bar scale, 20 μm. (F, G) Immunofluorescence detecting CD34 expression in tumors from four groups. Bar scale, 20 μm. *P<0.05; ***P<0.001; ****P<0.0001.

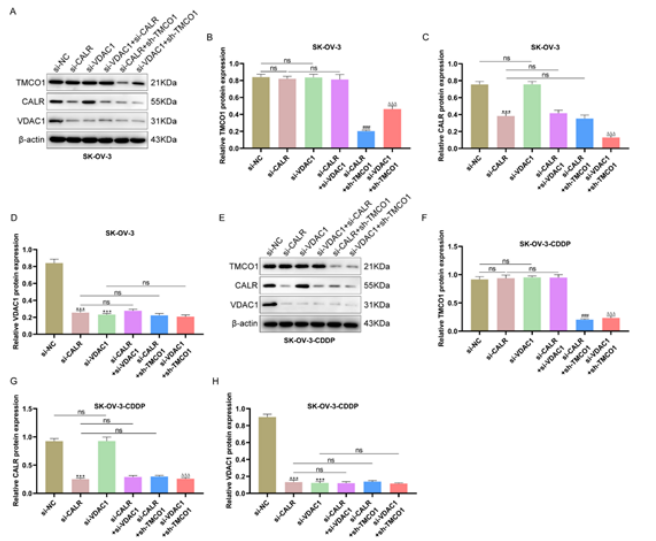

Fig. 8. The interaction of TMCO1 with CALR and VDAC1. (A-D) Western blotting was conducted for examining (B) TMCO1, (C) CALR and (D) VDAC1 expression with CALR-siRNA +VDAC1-siRNA or sh-TMCO1 with CALR-siRNA and VDAC1-siRN in SK-OV-3 cells. (E-H) Western blotting was conducted for examining (F) TMCO1, (G) CALR and (H)VDAC1 expression with CALR-siRNA +VDAC1-siRNA or sh-TMCO1 with CALR-siRNA and VDAC1-siRNA in SK-OV-3-CDDP cells.

In addition, we utilized immunohistochemistry and immunohistochemistry fluorescence staining to validate the expression of TMCO1, CALR, VDAC1, and EMT marker proteins. It was found that the expression of TMCO1, CALR, and VDAC1 in the cisplatin-resistant group (CDDP) was significantly higher than that of Control, while there was no significant difference in the expression of other indicators. These results further demonstrate the key role of TMCO1, CALR, and VDAC1 in the mechanism of cisplatin resistance (Figure 11A-P). Importantly, silencing TMCO1 can significantly reduce CALR, VDAC1, and EMT-related markers (Vimentin, N-cadherin β- Catenin expression is accompanied by an increase in E-cadherin expression. In summary, we elucidated the role of TMCO1 in ovarian cancer progression and cisplatin resistance mechanisms from in vitro to in vivo using non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells.

4. Discussion

The relationship between the endoplasmic reticulum and the mitochondria is becoming increasingly clear as a factor in the development of cancer cell metastasis [26-

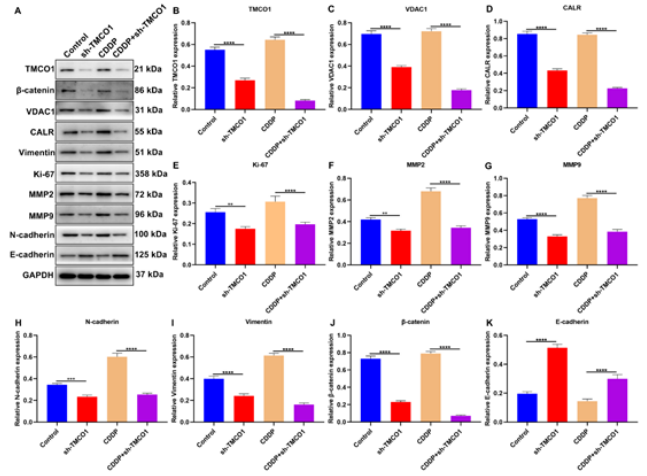

Fig. 10. Targeting TMCO1 inhibited the expression of CALR, VDAC1 and EMT markers in xenograft tumors. (A-K) Western blot was applied for examining (B) TMCO1, (C) VDAC1, (D) CALR, (E) Ki-67, (F) MMP2, (G) MMP9, (H) N-cadherin, (I) Vimentin, (J) β-catenin, or (K) E-cadherin expression within xenograft tumors developed by sh-NC or sh-TMCO1-transfected SK-OV-3 or SK-OV-3-CDDP cell line. **P<0.05; ***P<0.001; ****P<0.0001.

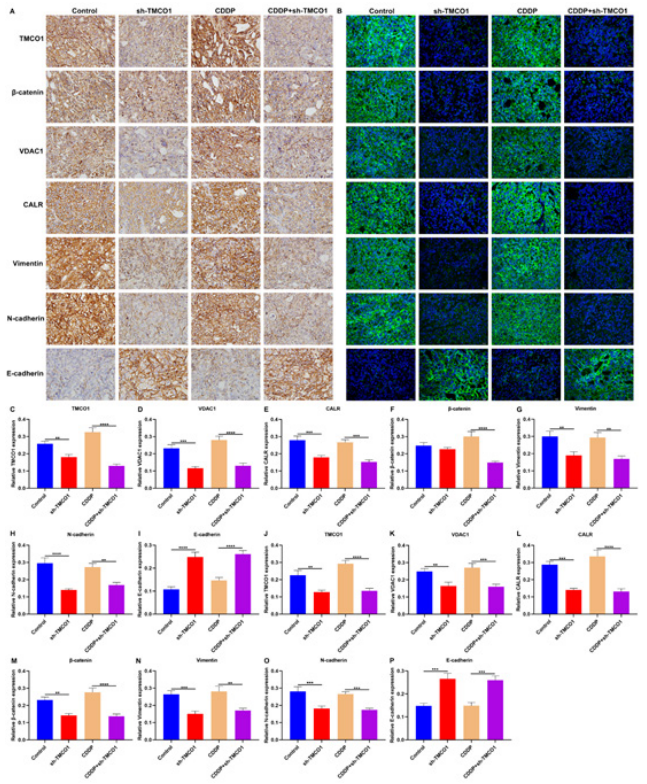

Fig. 11. The role of TMCO1, CALR, and VDAC1 in xenograft tumors. (A, B) Representative images of (A) immunohistochemistry as well as (B) immunofluorescence in xenograft tumors. Bar scale, 20 μm. (C-I) Expression of (C) TMCO1, (D) VDAC1, (E) CALR, (F) β-catenin, (G) Vimentin, (H) N-cadherin, or (I) E-cadherin was quantified in xenograft tumors developed by sh-NC or sh-TMCO1-transfected SK-OV-3 or SK-OV-3-CDDP cell line according to immunohistochemistry. (J-P) Expression of (J) TMCO1, (K) VDAC1, (L) CALR, (M) β-catenin, (N) Vimentin, (O) N-cadherin, or (P) E-cadherin was quantified within xenograft tumors developed by sh-NC or sh-TMCO1-transfected SK-OV-3 or SK-OV-3-CDDP cell line according to immunofluorescence. **P<0.05; ***P<0.001; ****P<0.0001.

28]. Ca2+ can go from the endoplasmic reticulum to the mitochondria, taking involvement in lipid production and metastasis, protein homeostasis, and mitochondrial transport. These processes change as tumors develop, including ovarian cancer [29]. The endoplasmic reticulum calcium homeostasis mechanism contains a calmodulin called TMCO1 that regulates Ca2+ homeostasis in the endoplasmic reticulum via CLAC channels (30). Tandem repeat assay research revealed that TMCO1 carries a greater risk of colorectal cancer [15].

This work is the first to describe the role of TMCO1 in the course of ovarian cancer, and it shows that tumor size, advanced FIGO, and lymph node metastases were substantially linked with TMCO1 expression levels in both cisplatin-sensitive and resistant individuals. TMCO1 might therefore be a biomarker for ovarian cancer prognosis. In vitro tests revealed that TMCO1 can regulate the growth and metastasis of ovarian cancer cells that aren't resistant to cisplatin as well as those that are. It can also regulate VDAC1 through CALR to affect intracellular calcium ion levels and cytoskeletal remodeling while suppressing the expression of EMT-related markers to disrupt the mechanism by which cisplatin-resistant ovarian cancer cells spread. The involvement of TMCO1 in cell proliferation and angiogenesis of non-cisplatin-resistant and cisplatin-resistant ovarian cancer tissues was further supported by in vivo research. Similar to in vitro studies, TMCO1 silencing also had an anti-tumor impact. As a result, TMCO1 is an important factor in the development of ovarian cancer and the mechanism of cisplatin resistance, offering a new guideline for the treatment of ovarian cancer.

The endoplasmic reticulum protein CALR is a calcium-binding protein that has been linked to the development of cancer [31]. When secreted proteins and membrane proteins are being synthesized, maturing, and being transported, CALR is involved in quality control. The primary roles of CALR are calcium homeostasis regulation and protein chaperoning. Tumor development may result from aberrant cell proliferation triggered by improper calcium ion homeostasis regulation [32]. By interacting with other improperly folded proteins, CALR promotes myeloproliferative tumors [33]. The response to paclitaxel therapy for ovarian cancer is associated with CALR expression [34]. Our research demonstrates that TMCO1 regulated the expression of CALR, and this impact is consistent with variations in intracellular calcium ion concentrations. It has been reported that TMCO1 and CALR control cell proliferation and death, respectively, in order to maintain calcium homeostasis in the endoplasmic reticulum [35]. However, our results showed that TMCO1 silencing also inhibited cell proliferation and cytoskeletal reorganization in addition to lowering CALR expression. Mitochondrial outer membrane protein is also involved in the control mechanism of intracellular calcium homeostasis. The mitochondrial outer membrane protein VDAC1 reacts preferentially to variations in intracellular calcium levels [36]. Additionally, VDAC1 has a role in cell division and apoptosis [37]. Overexpression of VDAC1 has been shown in breast cancer research to facilitate the growth, migration, and invasion of breast cancer cells [38]. Regulation of VDAC1 causes mitochondrial dysfunction and significant alterations in the signaling pathways that promote malignancy in prostate cancer [39]. One of the primary regulatory mechanisms for cancer cells to overcome

chemotherapy resistance is mitochondrial outer membrane proteins [40]. According to experimental data, PGC1α can inhibit apoptosis via the HSP70/HK2/VDAC1 signaling pathway, which leads to ovarian cancer developing cisplatin resistance [41]. Our in vivo and in vitro data demonstrated that overexpressing TMCO1 increased the expression of CALR and VDAC1 in non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells as well as xenograft tumor tissues, whereas silencing TMCO1 produced the opposite results. These findings clearly demonstrated the role of TMCO1 in the progression of ovarian cancer as well as the mechanism of ovarian cancer resistance. Most critically, we knocked down CALR and VDAC1 expression and discovered that both could prevent the expression of malignant phenotypic markers. According to the experimental findings, reducing CALR could regulate the expression of VDAC1 while could not regulate the expression of CALR and TMCO1. In light of the aforementioned findings, TMCO1 may regulate the expression of CALR and VDAC1. We hypothesize that TMCO1 may regulate VDAC1 via CALR to alter the development of ovarian cancer and the mechanism underlying cisplatin resistance.

Early on in cancer development, the tumor may infect nearby organs or spread to the peritoneal cavity [42]. According to the ovarian cancer staging system, stage II metastases to extraovarian/fallopian tube and pelvic organs, stage III metastases to retroperitoneal lymph nodes, and stage IV metastases to distant organs are all diagnosed [43]. EMT plays a significant role in tumor growth and drug resistance mechanisms, however, EMT is a reversible process in which epithelial cells lose polarity and intercellular adhesion to a spindle shape, split from the basal layer, and gain mobility [44]. This process is a precursor to tumor invasion and metastasis, which suggests that focusing on certain genes can slow tumor growth and overcome medication resistance [45]. Breast cancer-related carcinogenesis is greatly aided by dysregulated ion channels, which are linked to EMT-related marker genes [46]. In this work, TMCO1 up-regulation encouraged the expression of EMT-related markers and improved the ability of non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells to metastasize. We detected the migration of Cisplatin-resistant ovarian cancer cells in cytoskeleton and immune cell fluorescence assays, and the marginal protrusions lengthened and expanded. We could see small clusters of CALR fluorescence expression, particularly in the TMCO1 overexpression group of SK-OV-3-CDDP cells, and TMCO1 silencing may prevent the development of this phenomena. We hypothesize that this is most likely connected to endoplasmic reticulum oxidative stress, and we will look into the mechanism in more detail in the future. The distinction is that a prior study discovered that bladder urothelial carcinoma exhibits low levels of TMCO1 expression, which inhibits the growth of cancer [25]. This is probably related to where the tumor tissue came from because varied tissue sources and growing settings will impact how different ionic proteins perform their various activities. Using animal studies, we further confirmed the involvement of TMCO1 in the evolution of non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells. We showed in vivo that inhibiting TMCO1 can greatly decrease tumor growth and angiogenesis.

On the basis of silencing TMCO1, we created and applied CALR and VDAC1 siRNA to elaborate the regulatory link between the three. Surprisingly, TMCO1 silencing can drastically lower CALR and VDAC1 expression. We think that CALR and VDAC1 may be the TMCO1 effect factors.

As the experiment progressed and it was shown that CALR regulates VDAC1 and that VDAC1 cannot reverse-regulate CALR. We hypothesized that TMCO1 and CALR, as regulatory proteins of calcium homeostasis in endoplasmic reticulum, might affect the expression of VDAC1 by maintaining calcium homeostasis through the release of ions (primarily calcium ions; other incidental ions could not be excluded). We did not perform the detection of markers for mitochondrial dysfunction due to the constraints of the investigation.

5. Conclusion

In summary, clinical profiling, in vitro cell models, and in vivo xenograft models provide conclusive evidence that TMCO1 is a novel oncogene in ovarian cancer progression and ovarian cancer resistance mechanisms. In this study, TMCO1 regulates the expression of VDAC1 through CALR, affecting the proliferation, metastasis, calcium ion levels, cytoskeleton and EMT-related markers of non-cisplatin-resistant and cisplatin-resistant ovarian cancer cells. In addition, targeting TMCO1 significantly inhibits tumor growth, proliferation, and angiogenesis in vivo. Therefore, TMCO1 is a promising target for the treatment of ovarian cancer.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This project gained approval by the Ethical Committee of The Fourth Hospital of Hebei Medical University (2020166).

The animal experiment was presented strictly in accordance with the Institutional Ethics Guidelines for Animal Experiments approved by the Animal Ethics Committee of The Fourth Hospital of Hebei Medical University (2020166).

Informed Consent

Each patient signed written informed consent.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

GS substantially contributed to the conception and the design of the study, SG contributed to data analysis; SL contributed to manuscript drafting; YH YS and ZZ contributed to critical revisions. All authors read and approved the final manuscript.

Funding

This work was supported by Natural Science Foundation

of Hebei Province (grant number H2020206223).

References

1. Liu C, Su H (2022) Evaluation of risk factors and effective factors in determining the prognosis of ovarian cancer. Cell Mol Biol 68:28-34. doi: 10.14715/cmb/2022.68.9.4
2. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. Ca-Cancer J Clin 70:7-30. doi: 10.3322/caac.21590
3. Liang H, Zhao X, Wang C, Sun J, Chen Y, Wang G et al (2018) Systematic analyses reveal long non-coding RNA (PTAF)-mediated promotion of EMT and invasion-metastasis in serous ovarian cancer. Mol Cancer 17:96. doi: 10.1186/s12943-018-0844-7
4. Lin M, Wang J, Wang S, Huang Y (2022) Expression Level of Keratin 7 in Epithelial Ovarian Cancer and Malignant Metastasis of Benign Epithelial Ovarian Tumors. Cell Mol Biol 68:153-161. doi: 10.14715/cmb/2022.68.2.22
5. Da CA, Baiocchi G (2021) Genomic profiling of platinum-resistant ovarian cancer: The road into druggable targets. Semin Cancer Biol 77:29-41. doi: 10.1016/j.semcancer.2020.10.016
6. Kouba S, Ouldamer L, Garcia C, Fontaine D, Chantome A, Vandier C et al (2019) Lipid metabolism and Calcium signaling in epithelial ovarian cancer. Cell Calcium 81:38-50. doi: 10.1016/j.ceca.2019.06.002
7. Ke Y, Zhuang X, You L (2022) Identification of Core Genes Shared by Endometrial Cancer and Ovarian Cancer Using an Integrated Approach. Cell Mol Biol 68:140-145. doi: 10.14715/cmb/2022.68.9.22
8. Xu Y, Wang C, Su J, Xie Q, Ma L, Zeng L et al (2015) Tolerance to endoplasmic reticulum stress mediates cisplatin resistance in human ovarian cancer cells by maintaining endoplasmic reticulum and mitochondrial homeostasis. Oncol Rep 34:3051-3060. doi: 10.3892/or.2015.4283
9. Wang QC, Zheng Q, Tan H, Zhang B, Li X, Yang Y et al (2016) TMCO1 Is an ER Ca(2+) Load-Activated Ca(2+) Channel. Cell 165:1454-1466. doi: 10.1016/j.cell.2016.04.051
10. Batchelor-Regan H, Xin B, Zhou A, Wang H (2021) From Disease Description and Gene Discovery to Functional Cell Pathway: A Decade-Long Journey for TMCO1. Front Genet 12:652400. doi: 10.3389/fgene.2021.652400
11. McGilvray PT, Anghel SA, Sundaram A, Zhong F, Trnka MJ, Fuller JR et al (2020) An ER translocon for multi-pass membrane protein biogenesis. Elife 9:e56889. doi: **10.7554/eLife.56889**
12. Wang X, Wang QC, Sun Z, Li T, Yang K, An C et al (2019) ER stress mediated degradation of diacylglycerol acyltransferase impairs mitochondrial functions in TMCO1 deficient cells. Biochem Bioph Res Co 512:914-920. doi: 10.1016/j.bbrc.2019.03.115
13. Zhang N, Tang M, Wen M, Cao Y, OuYang B (2021) Expression, purification and characterization of TMCO1 for structural studies. Protein Expres Purif 179:105803. doi: 10.1016/j.pep.2020.105803
14. Sun Z, Zhang H, Wang X, Wang QC, Zhang C, Wang JQ et al (2018) TMCO1 is essential for ovarian follicle development by regulating ER Ca(2+) store of granulosa cells. Cell Death Differ 25:1686-1701. doi: 10.1038/s41418-018-0067-x
15. Mukamel RE, Handsaker RE, Sherman MA, Barton AR, Hujoel M, McCarroll SA et al (2023) Repeat polymorphisms underlie top genetic risk loci for glaucoma and colorectal cancer. Cell 186:3659-3673. doi: 10.1016/j.cell.2023.07.002
16. Kjaer L, Skov V, Larsen MK, Boklund TI, Andersen M, Kefala M et al (2023) Case Report: First longitudinal study of a patient with CALR positive clonal hematopoiesis of indeterminate potential developing into pre-fibrotic myelofibrosis. Front Oncol 13:1176173. doi: 10.3389/fonc.2023.1176173
17. Sen SS, Lee DJ, Crespo A, Hu JJ, Walker C, Ma X et al (2023) The NK cell receptor NKp46 recognizes ecto-calreticulin on ER-stressed cells. Nature 616:348-356. doi: 10.1038/s41586-023-05912-0
18. Magalingam KB, Somanath SD, Ramdas P, Haleagrahara N, Radhakrishnan AK (2022) 6-Hydroxydopamine Induces Neurodegeneration in Terminally Differentiated SH-SY5Y Neuroblastoma Cells via Enrichment of the Nucleosomal Degradation Pathway: a Global Proteomics Approach. J Mol Neurosci 72:1026-1046. doi: 10.1007/s12031-021-01962-z
19. Liu X, Chen Y, Fu Y, Jiang D, Gao F, Tang Z et al (2023) Breaking Spatiotemporal Barriers of Immunogenic Chemotherapy via an Endoplasmic Reticulum Membrane-Assisted Liposomal Drug Delivery. Acs Nano 17:10521-10534. doi: 10.1021/acsnano.3c01446
20. Wang SF, Tseng LM, Lee HC (2023) Role of mitochondrial alterations in human cancer progression and cancer immunity. J Biomed Sci 30:61. doi: 10.1186/s12929-023-00956-w
21. Rosa N, Speelman-Rooms F, Parys JB, Bultynck G (2022) Modulation of Ca(2+) signaling by antiapoptotic Bcl-2 versus Bcl-xL: From molecular mechanisms to relevance for cancer cell survival. Bba-Rev Cancer 1877:188791. doi: 10.1016/j.bbcan.2022.188791
22. Tang Z, Kang B, Li C, Chen T, Zhang Z (2019) GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 47:W556-W560. doi: 10.1093/nar/gkz430
23. Vathipadiekal V, Wang V, Wei W, Waldron L, Drapkin R, Gillette M et al (2015) Creation of a Human Secretome: A Novel Composite Library of Human Secreted Proteins: Validation Using Ovarian Cancer Gene Expression Data and a Virtual Secretome Array. Clin Cancer Res 21:4960-4969. doi: 10.1158/1078-0432.CCR-14-3173
24. King ER, Tung CS, Tsang YT, Zu Z, Lok GT, Deavers MT et al (2011) The anterior gradient homolog 3 (AGR3) gene is associated with differentiation and survival in ovarian cancer. Am J Surg Pathol 35:904-912. doi: 10.1097/PAS.0b013e318212ae22
25. Li CF, Wu WR, Chan TC, Wang YH, Chen LR, Wu WJ et al (2017) Transmembrane and Coiled-Coil Domain 1 Impairs the AKT Signaling Pathway in Urinary Bladder Urothelial Carcinoma: A Characterization of a Tumor Suppressor. Clin Cancer Res 23:7650-7663. doi: 10.1158/1078-0432.CCR-17-0002
26. Bosc C, Broin N, Fanjul M, Saland E, Farge T, Courdy C et al (2020) Autophagy regulates fatty acid availability for oxidative phosphorylation through mitochondria-endoplasmic reticulum contact sites. Nat Commun 11:4056. doi: 10.1038/s41467-020-17882-2
27. Daw CC, Ramachandran K, Enslow BT, Maity S, Bursic B, Novello MJ et al (2020) Lactate Elicits ER-Mitochondrial Mg(2+) Dynamics to Integrate Cellular Metabolism. Cell 183:474-489. doi: 10.1016/j.cell.2020.08.049
28. Pandey S, Patil S, Ballav N, Basu S (2020) Spatial targeting of Bcl-2 on endoplasmic reticulum and mitochondria in cancer cells by lipid nanoparticles. J Mater Chem B 8:4259-4266. doi: 10.1039/d0tb00408a
29. Song M, Sandoval TA, Chae CS, Chopra S, Tan C, Rutkowski MR et al (2018) IRE1alpha-XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. Nature 562:423-428. doi: 10.1038/s41586-018-0597-x
30. Li J, Liu C, Li Y, Zheng Q, Xu Y, Liu B et al (2019) TMCO1-mediated Ca(2+) leak underlies osteoblast functions via CaMKII signaling. Nat Commun 10:1589. doi: 10.1038/s41467-019-09653-5
31. Li Y, Liu X, Chen H, Xie P, Ma R, He J et al (2021) Bioinformatics analysis for the role of CALR in human cancers. Plos One 16:e0261254. doi: 10.1371/journal.pone.0261254

32. Eggleton P, Bremer E, Dudek E, Michalak M (2016) Calreticulin, a therapeutic target? Expert Opin Ther Tar 20:1137-1147. doi: 10.1517/14728222.2016.1164695
33. Pecquet C, Papadopoulos N, Balligand T, Chachoua I, Tisserand A, Vertenoeil G et al (2023) Secreted mutant calreticulins as rogue cytokines in myeloproliferative neoplasms. Blood 141:917-929. doi: 10.1182/blood.2022016846
34. Lau TS, Chan L, Man G, Wong CH, Lee J, Yim SF et al (2020) Paclitaxel Induces Immunogenic Cell Death in Ovarian Cancer via TLR4/IKK2/SNARE-Dependent Exocytosis. Cancer Immunol Res 8:1099-1111. doi: 10.1158/2326-6066.CIR-19-0616
35. Zhao S, Feng H, Jiang D, Yang K, Wang ST, Zhang YX et al (2023) ER Ca(2+) overload activates the IRE1alpha signaling and promotes cell survival. Cell Biosci 13:123. doi: 10.1186/s13578-023-01062-y
36. Yue C, Qian Y, Wang C, Chen J, Wang J, Wang Z et al (2023) TRIM29 acts as a potential senescence suppressor with epigenetic activation in nasopharyngeal carcinoma. Cancer Sci 114:3176-3189. doi: 10.1111/cas.15852
37. Yang HL, Chiu LW, Lin YA, Pandey S, Vadivalagan C, Liao JW et al (2023) In vitro and in vivo anti-tumor activity of Coenzyme Q(0) against TWIST1-overexpressing HNSCC cells: ROS-mediated inhibition of EMT/metastasis and autophagy/apoptosis induction. Toxicol Appl Pharm 465:116453. doi: 10.1016/j.taap.2023.116453
38. Yang H, Wang Z, Hu S, Chen L, Li W, Yang Z (2023) miRNA-874-3p inhibits the migration, invasion and proliferation of breast cancer cells by targeting VDAC1. Aging (Albany Ny) 15:705-717. doi: 10.18632/aging.204474
39. Mahmoud AM, Kostrzewa M, Marolda V, Cerasuolo M, Maccarinelli F, Coltrini D et al (2023) Cannabidiol alters mitochondrial bioenergetics via VDAC1 and triggers cell death in hormone-refractory prostate cancer. Pharmacol Res 189:106683. doi: 10.1016/j.phrs.2023.106683
40. Rodrigues T, Ferraz LS (2020) Therapeutic potential of targeting mitochondrial dynamics in cancer. Biochem Pharmacol 182:114282. doi: 10.1016/j.bcp.2020.114282
41. Li Y, Kang J, Fu J, Luo H, Liu Y, Li Y et al (2021) PGC1alpha Promotes Cisplatin Resistance in Ovarian Cancer by Regulating the HSP70/HK2/VDAC1 Signaling Pathway. Int J Mol Sci 22doi: 10.3390/ijms22052537
42. Yousefi M, Dehghani S, Nosrati R, Ghanei M, Salmaninejad A, Rajaie S et al (2020) Current insights into the metastasis of epithelial ovarian cancer - hopes and hurdles. Cell Oncol 43:515-538. doi: 10.1007/s13402-020-00513-9
43. Huo Q, Xu C, Shao Y, Yu Q, Huang L, Liu Y et al (2021) Free CA125 promotes ovarian cancer cell migration and tumor metastasis by binding Mesothelin to reduce DKK1 expression and activate the SGK3/FOXO3 pathway. Int J Biol Sci 17:574-588. doi: 10.7150/ijbs.52097
44. Liang H, Yu T, Han Y, Jiang H, Wang C, You T et al (2018) LncRNA PTAR promotes EMT and invasion-metastasis in serous ovarian cancer by competitively binding miR-101-3p to regulate ZEB1 expression. Mol Cancer 17:119. doi: 10.1186/s12943-018-0870-5
45. Fan L, Lei H, Zhang S, Peng Y, Fu C, Shu G et al (2020) Non-canonical signaling pathway of SNAI2 induces EMT in ovarian cancer cells by suppressing miR-222-3p transcription and upregulating PDCD10. Theranostics 10:5895-5913. doi: 10.7150/thno.43198
46. Parthasarathi K, Mandal S, Singh S, Gundimeda S, Jolly MK, Pandey A et al (2022) In Silico Analysis of Ion Channels and Their Correlation with Epithelial to Mesenchymal Transition in Breast Cancer. Cancers 14doi: 10.3390/cancers14061444