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TRPM2-AS promotes ovarian cancer cell proliferation and inhibits cell apoptosis by upregulating the nearby gene TRPM2 via miR-6764-5p

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Abstract

Ovarian cancer (OC) becomes a fatal gynecologic malignant cancer in females worldwide. Target therapy is a promising therapeutical choice for patients with OC, and identifying biomarkers and exploring molecular mechanisms are necessary. In this study, the functions and mechanism of long noncoding RNA transient receptor potential cation channel subfamily M member 2 antisense RNA (TRPM2-AS) in OC were explored. TRPM2-AS expression in OC cells was analyzed utilizing reverse transcription quantitative polymerase chain reaction (RT-qPCR). Cell counting kit-8 (CCK-8) and colony forming assays were carried out to explore the influence of TRPM2-AS on OC cell viability and proliferation. Cell apoptosis was detected using TdT-mediated dUTP Nick-End labeling (TUNEL) and flow cytometry analysis. Protein expression of apoptotic markers was subjected to western blotting. RNA pulldown or luciferase reporter assays were applied to explore the interaction between TRPM2-AS and miR-6764-5p or the binding of miR-6764-5p and TRPM2. The results showed that TRPM2-AS is highly expressed in OC cells and was mainly localized in cytoplasm. TRPM2-AS depletion suppressed OC cell viability and proliferation while increasing cell apoptotic rate. TRPM2 displayed a high level in OC cells and was positively regulated by TRPM2-AS. TRPM2-AS interacted with miR-6764-5p and thereby upregulated TRPM2 expression. In addition, TRPM2 overexpression reversed the repressive impact of TRPM2-AS depletion on malignant OC cellular process. In conclusion, TRPM2-AS promotes OC cell viability and proliferation while enhancing cell apoptosis through interaction with miR-6764-5p to regulate TRPM2 level.

Keywords TRPM2-AS, miR-6764-5p, TRPM2, Ovarian cancer, ceRNA network

Introduction

Ovarian cancer (OC) is a common gynecological tumor in women, serving as a main cause of cancer-associated death for female [2, 41]. In the United States, the estimated new OC cases in 2023 is 19,710, and approximately 13,270 women in the United States may die of ovarian malignancy according to cancer statistics recorded by American Cancer Society [30]. During the past years, achievements have been made in improving the outcome of patients though surgical treatment [34], intravenous or intraperitoneal chemotherapy [6, 23], or combined treatment. Nevertheless, limitations such as recurrence, high risk, and indefinite side effects in the present therapeutical options cannot be ignored [11]. Hence, identifying effective diagnostic biomarkers and exploring their molecular mechanisms are necessary to extend the understanding of OC and enrich therapeutical methods.

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Long noncoding RNAs (lncRNAs) refer to transcripts longer than 200 nucleotides without the ability to encode proteins [25]. LncRNAs act as major players in different types of cancer [26, 31, 33], promoting or inhibiting cellular processes including proliferation, metastasis, and invasion [3, 15, 43]. Recently, many lncRNAs have been revealed to affect OC progression, such as lncRNA KCNQ1OT1 [9], linc00665 [39], and lncRNA GAS5 [45]. Based on bioinformatics analysis, lncRNA transient receptor potential melastatin 2 antisense RNA (TRPM2-AS) is markedly upregulated in tissue samples of ovarian serous cystadenocarcinoma (OV) which is the most common histological subtype of OC [14]. TRPM2-AS is located in chr21q22.3 with 875 nucleotides in length, which has been confirmed to play roles in gastric [40], prostate [28], and colorectal cancer [8]. However, TRPM2-AS is limitedly reported in OC [20]. At present, only one report revealed that TRPM2-AS contributes to OC development and cisplatin resistance via the miR-138-5p/SDC3 axis [7]. TRPM2 is the nearby gene of TRPM2-AS. According to a previous study, TRPM2-AS stabilizes TRPM2 by recruiting TAF15 [21]. As to competing endogenous RNA (ceRNA) hypothesis, the regulatory relationship between TRPM2-AS and TRPM2 via miRNA in cancer cells has not been reported.

Under the ceRNA mechanism, lncRNA can act as a "sponge" by interacting with miRNA to alter the expression of cancer-related targets. During the process, the suppressive effects of miRNA on mRNA can be inhibited [4, 24]. The ceRNA role of lncRNAs in OC are extensively investigated. LINC00936 hampers OC progression by binding to miR-221-3p and finally upregulates LAMA3 expression [29]. LncRNA LOC646029 obstructs OC cell growth, metastasis, and invasiveness by elevating SPRED1 expression via interaction with miR-627-2p [42]. Moreover, the ceRNA networks mediated by TRPM2-AS in laryngeal squamous cell carcinoma [37], retinoblastoma [12], and gastric cancer [40] have also been reported.

In the present work, the functions of TRPM2-AS in OC cell growth were explored. In addition, the relation between TRPM2-AS and TRPM2 as well as the ceRNA network mediated by TRPM2-AS were also under exploration. The study may extend the understanding of TRPM2-AS in OC and provide a novel regulatory axis for targeted therapy.

Results

TRPM2-AS shows high expression in OC cells with main distribution in cytoplasm

TRPM2-AS expression levels in ovarian serous cystadenocarcinoma (OV) tissues and normal samples were predicted using the bioinformatics tool GEPIA [32]. TRPM2-AS expression in OV tissues (n = 426) was markedly increased in comparison to that in normal samples (Fig. 1A). The prognosis of patients with high or low TRPM2-AS expression was analyzed using Kaplan Meier plotter [18]. Figure 1B showed that high TRPM2-AS level is related to poor survival rate in patients with OC (p = 0.0056). TRPM2-AS level in OC cells and human ovarian surface epithelial cells (HOSEpiC) were subjected to qPCR, and high TRPM2-AS level in OC cells was observed relative to its expression in HOSEpiC cells (Fig. 1C). Cellular RNA fractionation assays were performed to determine the distribution of TRPM2-AS in SKOV3 and CAOV3 cells. As shown by Fig. 1D, TRPM2-AS expression in the cytoplasm is much higher than that in the nucleus, suggesting the main localization of TRPM2-AS in OC cells. The results indicate that TRPM2-AS may participate in OC cell process by regulating genes post-transcriptionally.

Silencing of TRPM2-AS inhibits OC cell proliferation and augments cell apoptosis

TRPM2-AS expression was successfully silenced after transfection of shRNA targeting TRPM2-AS in OC cells (Fig. 2A). Compared with the sh-NC group, TRPM2-AS depletion decreased the numbers of viable OC cells and cell colonies (Fig. 2B, C). The results suggested the inhibitory effect of TRPM2-AS on cell viability and proliferation. Additionally, knockdown of TRPM2-AS increased the proportion of TUNEL-positive cells (Fig. 2D). The promoting role of TRPM2-AS depletion in cell apoptosis was further confirmed by flow cytometry analysis (Fig. 2E). Moreover, TRPM2-AS knockdown decreased Bcl-2 protein levels and elevated cleaved caspase-3 and Bax protein levels in SKOV3 and CAOV3 cells (Fig. 2F). The findings validate that the anti-apoptotic factor was downregulated while pro-apoptotic factors were upregulated in the absence of TRPM2-AS. According to the above functional experiments, TRPM2-AS deficiency hampers OC cell proliferation while enhancing cell apoptosis.

Positive expression correlation between TRPM2-AS and TRPM2

GEPIA predicts that TRPM2 levels in OV tissues (n = 426) are at least two folds higher than those in normal samples (n = 88) (Fig. 3A). High TRPM2 expression correlates to a poor survival outcome in OC patients as shown by the Kaplan Meier plotter (Fig. 3B). Spearman correlation coefficient analysis showed that TRPM2-AS expression was positively corelated to TRPM2 expression in OV tissues (p = 3.2e-46, Fig. 3C). Results of PCR analysis validated that TRPM2 expression was elevated in OC cells relative to that in HOSEpiC cells (Fig. 3D). Furthermore, the mRNA and protein levels of TRPM2 were decreased in OC cells silencing TRPM2-AS (Fig. 3E, F).

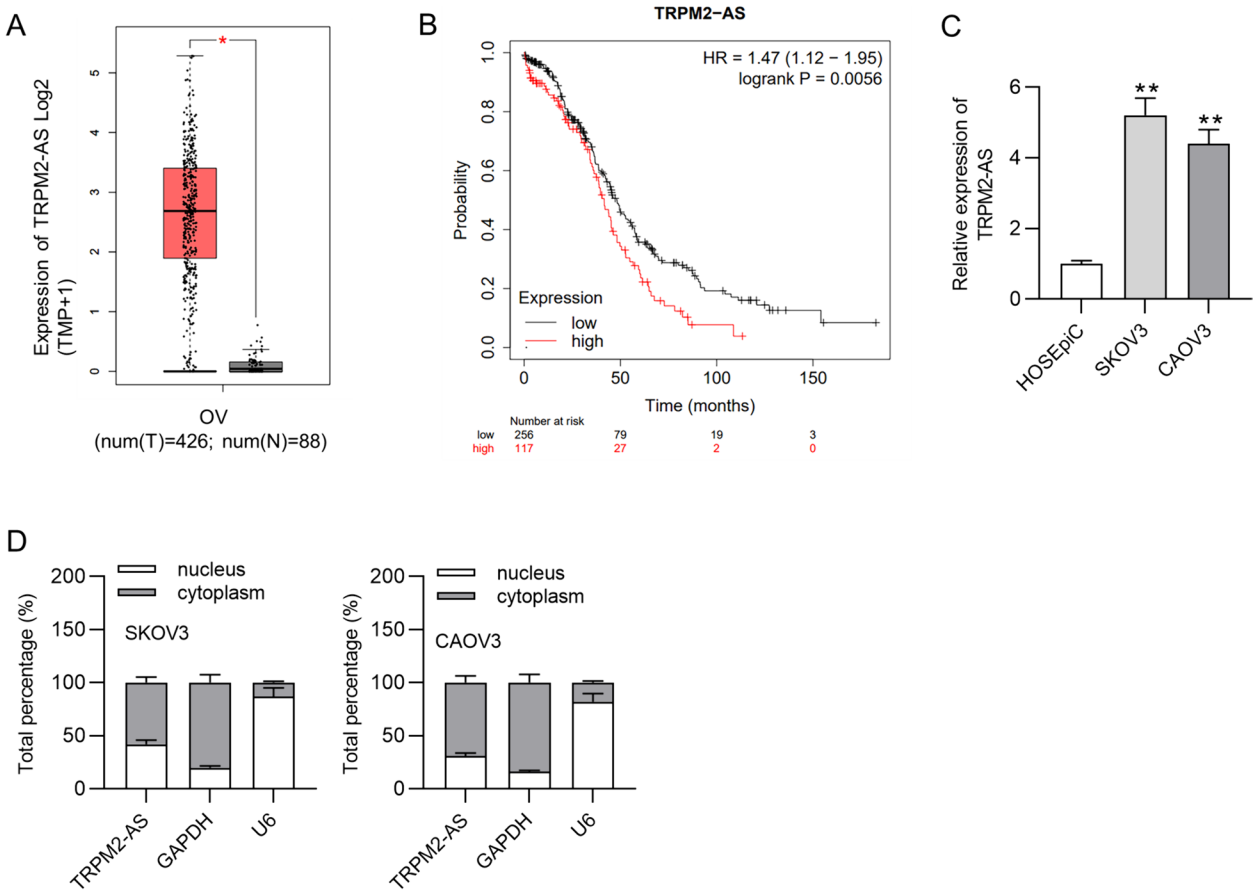

Fig. 1 High expression of TRPM2-AS in OC cells with main localization in the cytoplasm. **A** TRPM2-AS expression in 426 OV tissues and 88 normal tissues were analyzed using GEPIA. **B** Association of TRPM2-AS level and survival outcome of patients with OC was analyzed using Kaplan–Meier Plotter. **C** TRPM2-AS levels in OC cells and ovarian epithelial cells were measured by qPCR. **D** TRPM2-AS expression in nucleus and cytoplasm of OC cells were determined using cellular RNA fractionation assays and PCR. $^{**}p < 0.01$

The results confirmed the positive expression correlation between TRPM2-AS and TRPM2.

TRPM2-AS interacts with miR-6764-5p, and miR-6764-5p targets TRPM2

The bioinformatics tool LncBase v.2 was employed to predict downstream miRNAs that can interact with TRPM2-AS [22]. The upstream miRNAs that can target TRPM2 were predicted with miRDB [5]. As shown by Fig. 4A, among 213 miRNAs from LncBase and 35 miRNAs from miRDB, there are 8 overlapping miRNAs. RNA levels of the eight miRNAs in SKOV3 and CAOV3 cells transfected with sh-TRPM2-AS#1 or control plasmids were measured using PCR. MiR-6764-5p was found to be markedly upregulated in OC cells silencing TRPM2-AS compared with its expression in the sh-NC group (Fig. 4B). The finding showed the negative expression correlation between TRPM2-AS and miR-6764-5p in OC cells. The expression of other miRNAs was not significantly altered by TRPM2-AS knockdown (Fig. 4B). Therefore, miR-6764-5p was identified for subsequent experiments. MiR-6764-5p level was amplified in OC cells via transfection of miR-6764-5p mimics, as shown by Fig. 4C. The binding sequences of TRPM2-AS and miR-6764-5p were predicted utilizing miRDB (function: Custom Prediction) (Fig. 4D). Compared with the relative luciferase activity of TRPM2-AS-Wt in control group, TRPM2-AS-Wt activity in miR-6764-5p mimics group was markedly reduced (Fig. 4E). Additionally, TRPM2-AS-Mut activity showed no significant alteration in the context of miR-6764-5p overexpression (Fig. 4E). Results of luciferase reporter assays validated the binding of TRPM2-AS and miR-6764-5p in OC cells. Afterwards, the binding of miR-6764-5p to TRPM2 was also explored. The binding sequences of miR-6764-5p and TRPM2 were predicted with the bioinformatics tool Targetscan [16]. Overexpressing miR-6764-5p weakened the relative activity of TRPM2-Wt instead of TRPM2-Mut reporters (Fig. 4G), which confirmed the binding of miR-6764-5p to TRPM2. RNA pulldown assays were carried out to

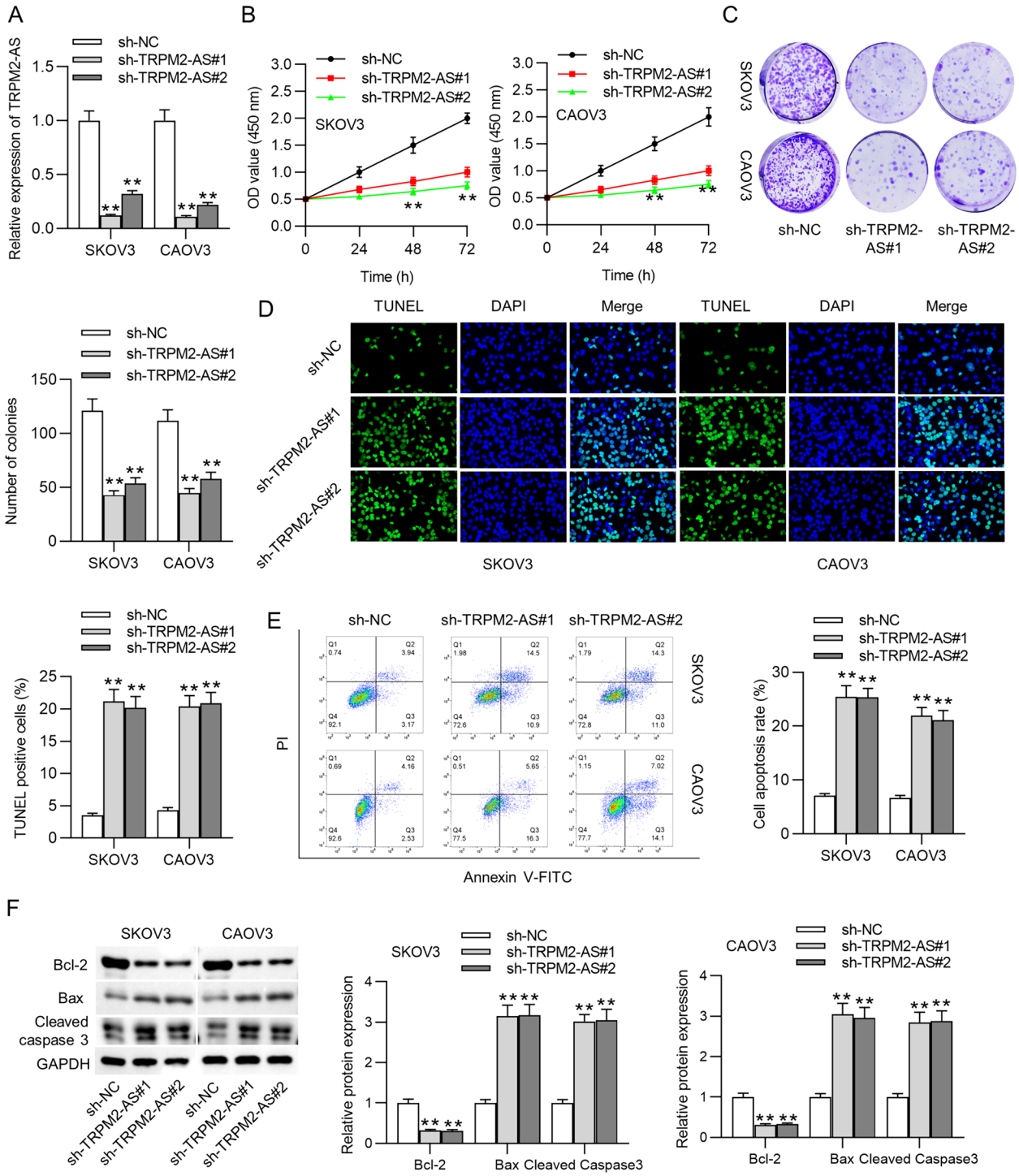

Fig. 2 Silencing of TRPM2-AS inhibits OC cell proliferation and promotes cell apoptosis. **A** PCR analysis was employed to evaluate knockdown efficacy of TRPM2-AS in OC cells. **B**–**E** In response to TRPM2-AS depletion, OC cell viability (**B**), proliferation (**C**), and apoptosis (**D, E**) were measured using CCK-8 assays (**B**), colony forming assays (**C**), TUNEL assays (**D**), and flow cytometry analyses (**E**). **F** Protein expression of apoptotic markers was quantified utilizing western blotting with normalization to GAPDH. $^{**}p < 0.01$

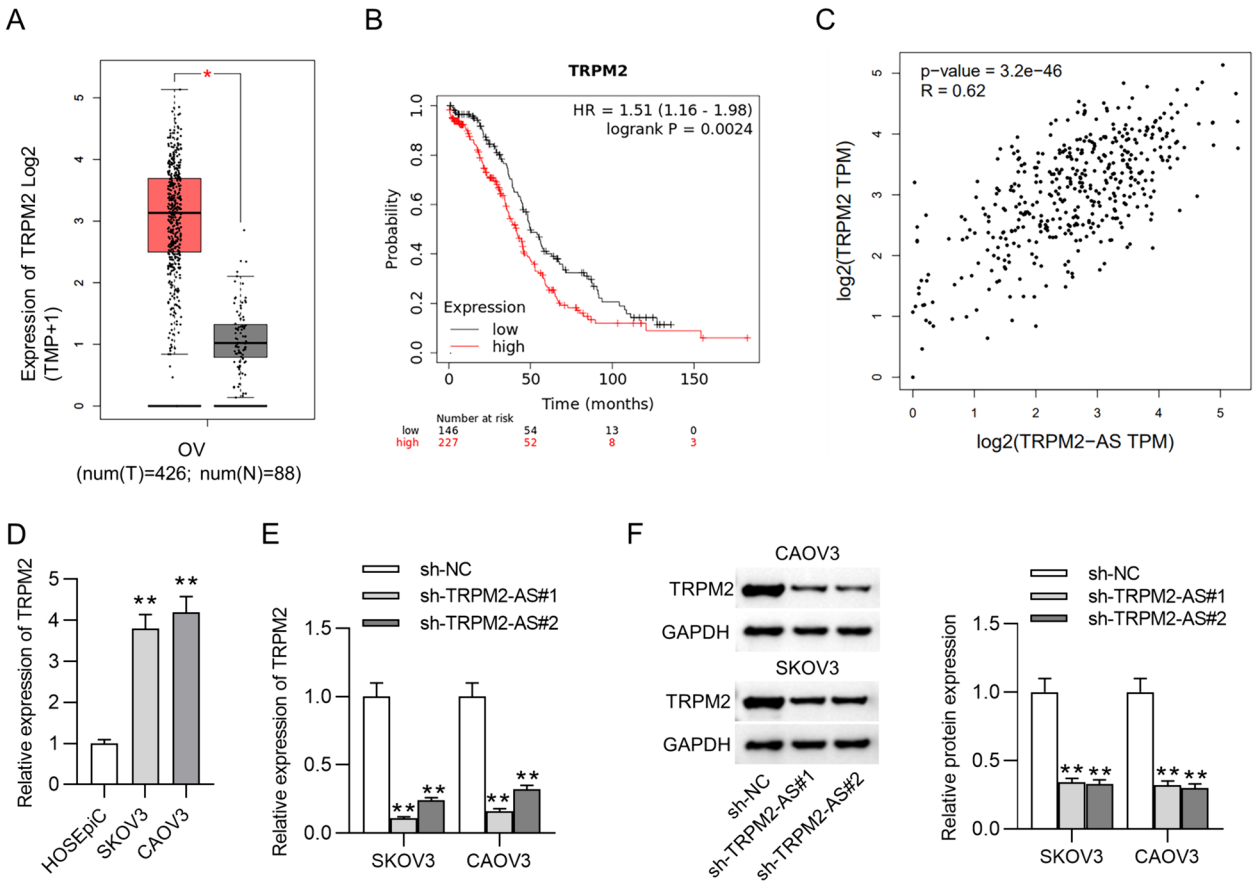

Fig. 3 Positive expression correlation between TRPM2-AS and TRPM2 in OC cells. **A** TRPM2 levels in 426 OV samples and 88 normal samples were analyzed using GEPIA. **B** Kaplan–Meier Plotter was used to illustrate the relationship of TRPM2 expression and the outcome of patients with OC. **C** Expression correlation between TRPM2-AS and TRPM2 in OV samples was assessed using Spearman expression correlation (on the GEPIA website). **D** TRPM2 expression in OC cells and ovarian epithelial cells were quantified utilizing PCR. **E**, **F** The impacts of TRPM2-AS knockdown on TRPM2 mRNA and protein levels in SKOV3 and CAOV3 cells were evaluated using PCR and western blotting. $^{**}p < 0.01$

further verify the relation among TRPM2-AS, miR-6764-5p, and TRPM2. As displayed in Fig. 4H, TRPM2 and TRPM2-AS were abundantly enriched in biotinylated miR-6764-5p-Wt group, implying that TRPM2-AS and TRPM2 were significantly pulled down by miR-6764-5p probe in OC cells. The enrichment of three players in the biotinylated miR-6764-5p-Mut group was not significant compared with that in the bio-NC group (Fig. 4H).

MiR-6764-5p inversely regulates TRPM2 expression

As illustrated by Kaplan Meier plotter, low miR-6764-5p level is related to low survival rate in OC patients ($p = 0.023$, Fig. 5A), which suggests that miR-6764-5p might be a tumor-suppressor in OC. The low miR-6764-5p level in OC cells was confirmed using PCR analysis (Fig. 5B). Then, the influence of miR-6764-5p interference on TRPM2 expression in OC cells was explored using qPCR and western blotting. Figure 5C, D illustrated that TRPM2 mRNA and protein expression were reduced in response to miR-6764-5p upregulation. The above findings indicate that miR-6764-5p targets TRPM2 3'UTR and inversely regulates TRPM2 expression.

Overexpressed TRPM2 rescues the repressive effect of TRPM2-AS depletion on OC cell growth

It was shown that overexpressed TRPM2 rescued the decrease in cell viability and proliferation induced by sh-TRPM2-AS (Fig. 6A, B). Additionally, high apoptotic rate of OC cells induced by TRPM2-AS deficiency was reduced again in the context of TRPM2 expression amplification (Fig. 6C, D). Moreover, TRPM2-AS deficiency led to Bcl-2 downregulation and upregulation of Bax and cleaved caspase 3 protein levels, and the changes were counteracted by TRPM2 overexpression (Fig. 6E).

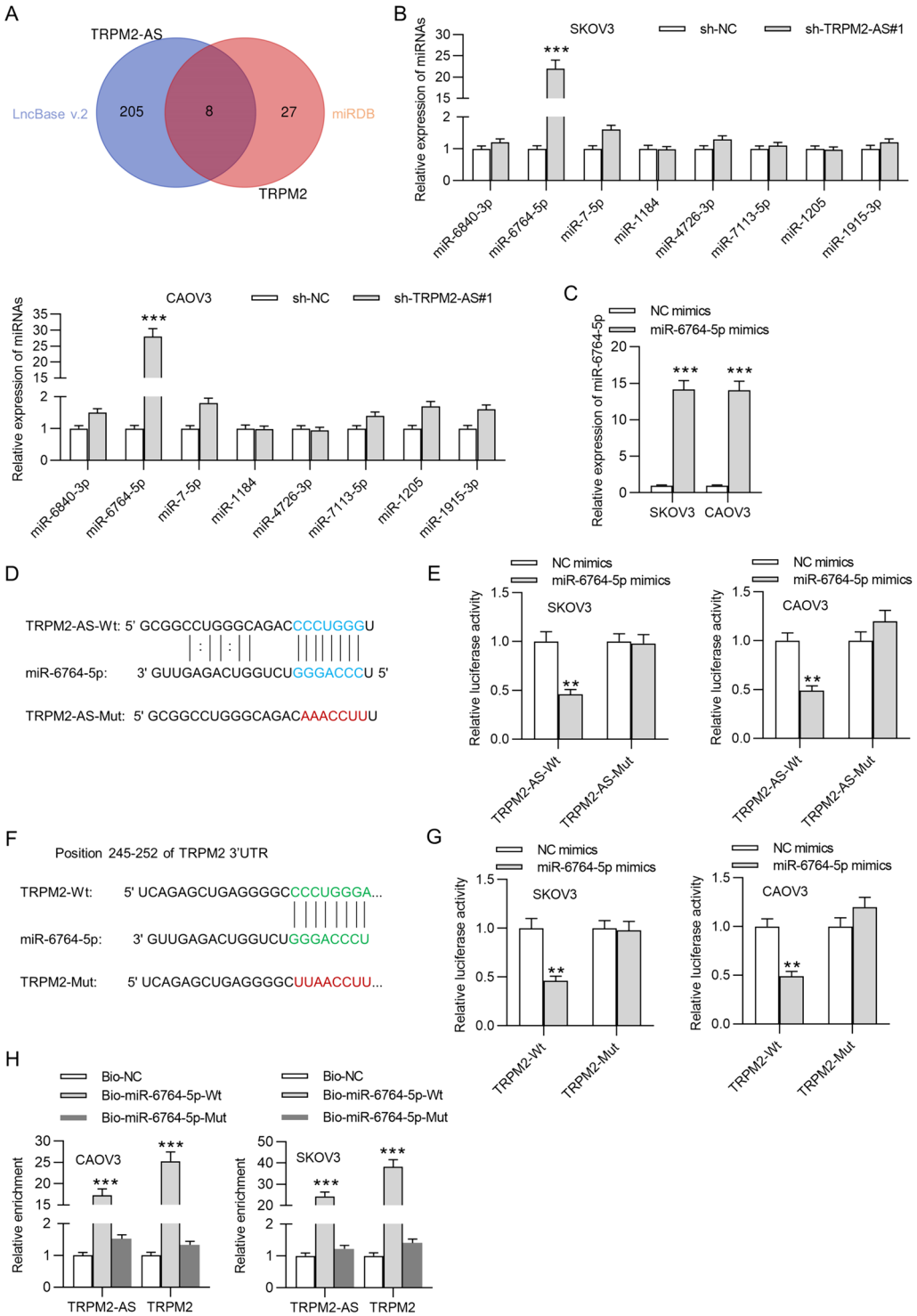

Fig. 4 TRPM2-AS binds to miR-6764-5p, and miR-6764-5p targets TRPM2. **A** The downstream miRNAs interacting with TRPM2-AS were predicted with the bioinformatics tool LncBase, and the upstream miRNAs targeting TRPM2 were predicted with another tool miRDB. Venn diagram is used to show the results of analysis. **B** Expression levels of eight overlapping miRNAs in OC cells with shRNA transfection were quantified using qPCR. **C** The efficiency of miR-6764-5p overexpression after miRNA mimics transfection was examined by qPCR. **D** The binding sequences of TRPM2-AS and miR-6764-5p was predicted using LncBase v2. **E** Luciferase reporter assays were conducted to evaluate the binding of TRPM2-AS and miR-6764-5p. **F** The binding area of miR-6764-5p and TRPM2 was predicted with Targetscan. **G** The binding of miR-6764-5p and TRPM2 was validated using luciferase reporter assays. **H** RNA pulldown assays were utilized for further verification of the relationship among the three molecules. $^{**}p < 0.01$, $^{***}p < 0.001$

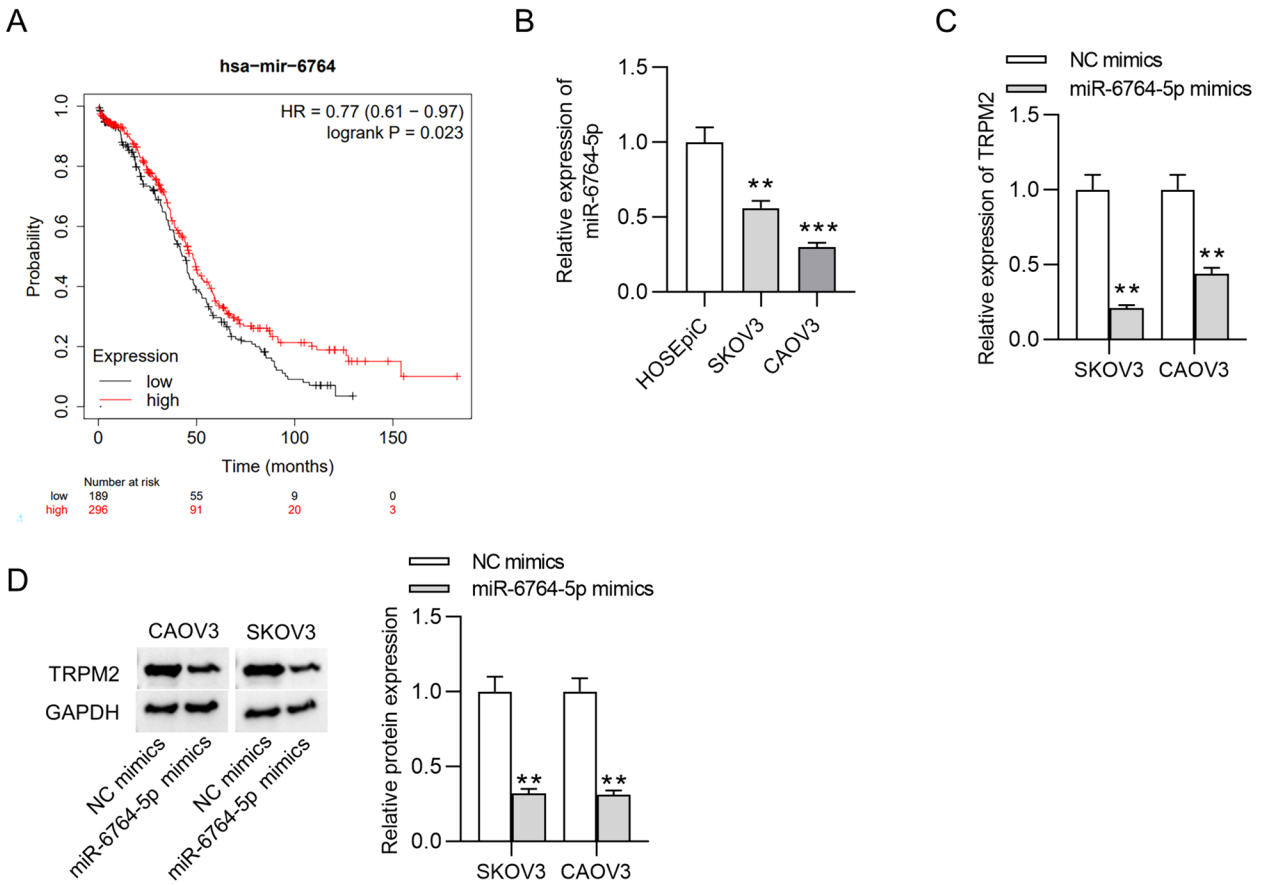

Fig. 5 miR-6764-5p inversely regulates TRPM2 expression. **A** The association between miR-6764 level and the survival outcome of OC patients was predicted with Kaplan–Meier Plotter. **B** MiR-6764-5p levels in OC cells and normal ovarian epithelial cells were detected by qPCR. **C**, **D** PCR and western blot were conducted to measure TRPM2 mRNA and protein expression in OC cells with miRNA mimics. $^{**}p < 0.01$, $^{***}p < 0.001$

Discussion

According to bioinformatics analysis, TRPM2-AS, the antisense RNA of TRPM2, is upregulated in OV tissues and higher TRPM2-AS expression is related to worse survival of patients with OC. Consistently, TRPM2-AS was found to show high expression in OC cells and was mainly localized in the cytoplasm. TRPM2-AS deficiency hampered the viability and proliferation of OC cells while augmenting cell apoptosis. Furthermore, TRPM2-AS expression was positively related to TRPM2 expression in OC cells. TRPM2-AS upregulated the nearby gene TRPM2 by interacting with miR-6764-5p.

TRPM2 is regarded as a cancer biomarker and is highly expressed in many types of cancer such as breast, pancreatic, prostate cancer, and melanoma [17]. TRPM2 deficiency induces lung cancer apoptosis and G2/M arrest by activating JNK pathway to elevate intracellular levels of reactive oxygen and nitrogen species [1]. TRPM2 promotes the development of pancreatic cancer by activating MAPK/ERK signaling [13]. Consistently, in this work, TRPM2 expression was increased in OC cells. TRPM2 overexpression rescued the decrease in cell proliferation and reversed the increase in cell apoptosis caused by TRPM2-AS deficiency. Furthermore, TRPM2 has recently been identified as a prognostic gene related to immune filtration in OC, and TRPM2 is positively related to immune checkpoints and pyroptosis-related genes [10, 44]. In this study, the downstream pathways mediated by TRPM2 in OC cells have not been explored, which could be a direction for future work.

MiRNAs are small noncoding RNAs closely related to cancer progression by post-transcriptionally regulate gene expression [35]. Recently, miR-651-3p has been reported to hamper OC cell growth, migration, epithelial-mesenchymal transition, and invasiveness by targeting ZNF703 and regulating the MEK/ERK pathway [38]. MiR-135b augments OC growth, metastasis, and invasiveness while hampering cisplatin sensitivity [36]. Downregulation of miR-6764-5p in OC cells was discovered in the current work. Previously, miR-6764-5p was reported to be dysregulated in plasma samples of patients with pituitary adenomas [19]. MiR-6764-5p is identified

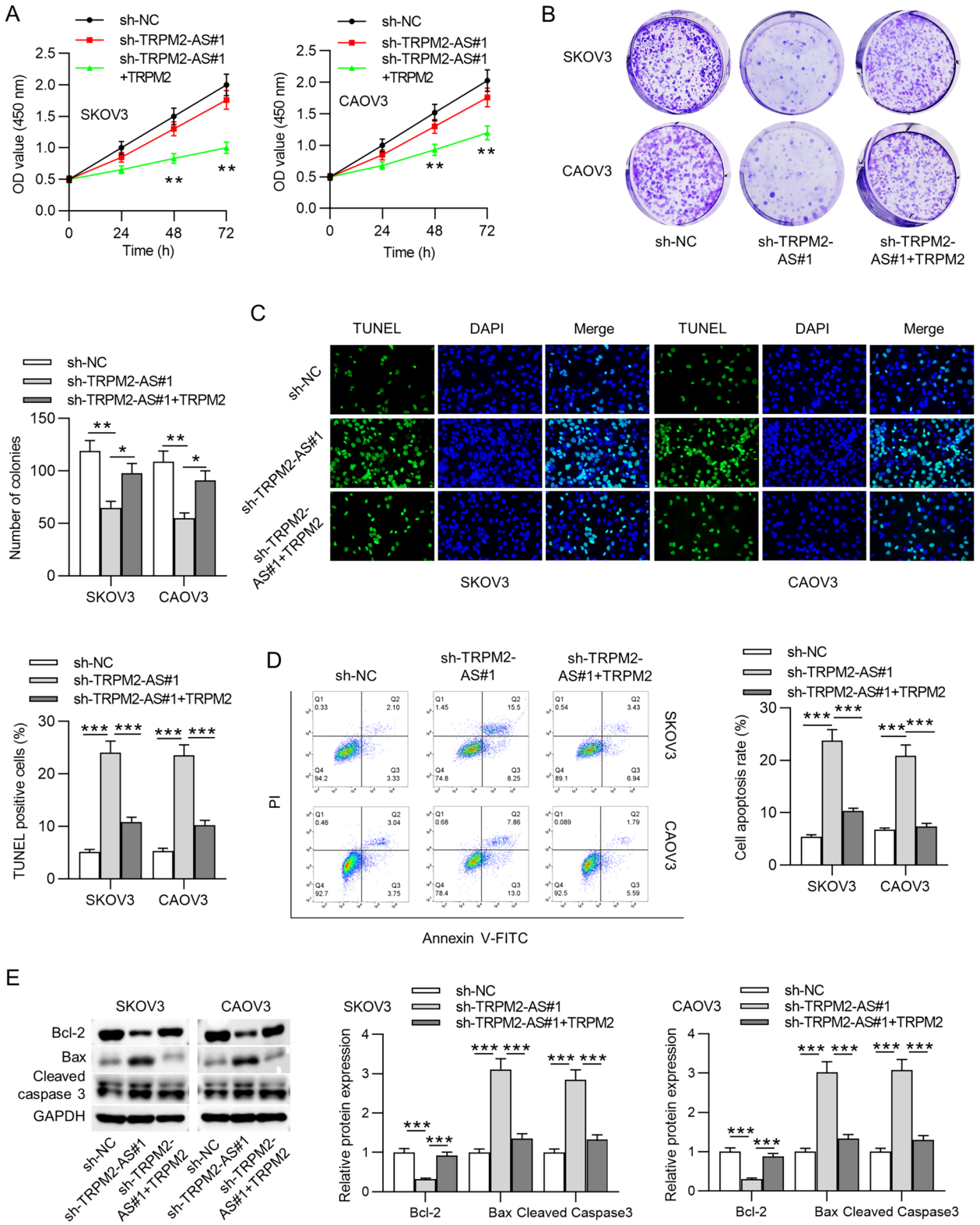

Fig. 6 Overexpressed TRPM2 rescues the repressive effect of TRPM2-AS depletion on OC cell growth. **A**–**D** OC cell viability (**A**), proliferation (**B**), and apoptosis (**C, D**) were measured in sh-TRPM2-AS1 group, sh-TRPM2-AS1 +TRPM2 group, and sh-NC group using CCK-8 assays (**A**), colony forming assays (**B**), as well as TUNEL assays (**C**) and flow cytometry analyses (**D**). **E** Western blotting was conducted to examine the protein expression of apoptotic markers in OC cells of sh-TRPM2-AS1 group, sh-TRPM2-AS1 +TRPM2 group, and sh-NC group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

as an important interacting miRNA in different hypoxic cancer cell lines and is dysregulated under hypoxia [27]. In our study, TRPM2-AS knockdown increased miR-6764-5p level in OC cells, indicating their negative expression correlation. Overexpressed miR-6764-5p downregulated TRPM2 mRNA and protein levels in OC cells.

Conclusion

In conclusion, TRPM2-AS promotes OC cell growth while inhibiting OC cell apoptosis by acting as a ceRNA for miR-6764-5p to regulate TRPM2-AS expression. The findings may provide new insight into functions and mechanism of TRPM2-AS in OC progression. Animal experiments were not conducted in this work, and the ceRNA network mediated by TRPM2-AS should be verified in vivo for future work.

Methods and related materials

Cell lines

Human OC cell lines (SKOV3 and CAOV3) were obtained from the American Type Culture Collection (Manassas, USA), and human ovarian surface epithelial cell line (HOSEpiC) was obtained from TongPai Biotechnology Cooperation (Shanghai, China). These cells were incubated in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Thermo Fisher, Rochester, USA) in a humidified atmosphere (5% CO_2 at 37℃).

Cell transfection

Short hairpin RNA targeting TRPM2-AS (sh-TRPM2-AS#1/2), miR-6764-5p mimics, and pcDNA3.1 vectors inserted with full TRPM2 sequence were adopted to knockdown TRPM2-AS expression, overexpress miR-6764-5p, and amplify TRPM2 expression, respectively. The negative controls (NCs) for the above plasmids are sh-NC, NC mimics and empty pcDNA vectors, respectively. All these plasmids were provided by GenePharma (Shanghai, China). OC cells were transfected with shRNA, miRNA mimics or pcDNA vectors utilizing Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) for 2 days as instructed. The transfection efficiency was evaluated by quantifying RNA expression using reverse transcription qPCR before and after plasmid transfection.

RT-qPCR

RNA extraction in OC cells was achieved utilizing TRIzol reagent (Invitrogen). Next, a Reverse Transcription Kit (Roche, Basel, Switzerland) was utilized to reverse transcribe the extracted RNA into cDNA. PCR was conducted on ABI biosystem (Applied Biosystems, USA) utilizing the SYBR Premix Ex Taq (Takara, Japan). Relative RNA level was calculated using the $2^{-\Delta\Delta Ct}$ method. GAPDH is the internal reference for TRPM2-AS and genes, while U6 snRNA act as the normalization for miRNAs. Primer sequences were presented in Table 1.

Subcellular fractionation assay

To isolate RNA in cytoplasmic part and nuclear part of SKOV3 and CAOV3 cells, a PARIS™ kit (Invitrogen) was utilized according to the instructions. TRPM2-AS expression in cytoplasmic and nuclear fractions were measured by qPCR analysis.

Measurement of cell viability

OC cells were plated in culture plates (2×10^4 cells/well, 96-well). At 0, 24, 48 and 72 h of cell culture, each well was added with ten microliters of the Cell Counting Kit-8 solution (Dojindo, Japan) for 2 h of incubation at 37 °C, and the absorbencies were measured at 450 nm using a microplate reader.

Colony forming assay

OC cell culture was performed on 6-well plates (1×10^3 cells/well) for 12 days. Culture medium was replaced every three days. Afterwards, formed colonies were fixed by methanol and stained using crystal violet solution (Sigma-Aldrich, USA). The number of colonies was manually counted.

TUNEL assay

OC cells with indicated plasmids were rinsed with phosphate buffered saline and fixed with 4% paraformaldehyde. For the TUNEL assay, the cells were incubated with TdT reaction cocktail followed by treatment of Click-iT reaction cocktail as instructed (Roche, Switzerland). Cell nuclei were stained with DAPI.

Table 1 Sequences of primers used for reverse transcription-quantitative PCR

Gene		Sequence (5′→3′)
TRPM2-AS	F	CAGATGGATCGGAGCTCTG
	R	CATTTGGGCGGAGTTACTG
TRPM2	F	ATGACAAGCAAGAAAGCCT
	R	GACAGTTTGGAACTTTCCAC
miR-6764-5p	F	TCCCAGGGTCTGGTCAGA
	R	CTCTACAGCTATATTGCCAGCCA
miR-7-5p	F	TGGAAGACTAGTGATTTTGTTGTT
	R	CTCTACAGCTATATTGCCAGCC
GAPDH	F	CATTTCCTGGTATGACAACGA
	R	GGGTCTTACTCCTTGGAGG
U6	F	GGATCAATACAGAGCAGATAAGC
	R	CTTTCTGAATTTGCGTGCC

F forward, *R* reverse

Flow cytometry analysis

After collected and washed with cold phosphate buffered saline, OC cells with indicated plasmids were stained with binding buffer containing propidine iodide and Annexin V-FITC (Beyotime, Shanghai, China) for 15 min at 4 ℃ without light exposure. A flow cytometer (BD Biosciences, USA) was adopted to measure cell apoptotic rate.

Western blotting

To extract protein samples, SKOV3 and CAOV3 cells were lysed using radio immunoprecipitation lysis buffer (Beyotime, Shanghai, China) containing phenylmethylsulfonyl fluoride. The proteins were separated by electrophoresis and then were loaded to PVDF membrane (Millipore, Billerica, USA). After blockage in 5% skim milk for 1 h, the membrane was incubated with primary antibodies at 4 °C overnight (Abcam, USA). The name and dilution of primary antibodies were listed in Table 2. After TBST-20 washing, secondary antibodies (Abcam) were supplemented for incubation with the membrane for 1 h. Chemiluminescent detection system (Roche, Switzerland) was used to visualize the blots on the membrane. The signal intensity of blots was quantified using ImageJ software with normalization to GAPDH. The uncropped blots are provided as supplemental figures.

Luciferase reporter assay

The wild type of TRPM2-AS sequence (TRPM2-AS-Wt) with binding site to miR-6764-5p and mutant TRPM2-AS sequence (TRPM2-AS-Mut) were cloned to pmirGLO vectors (Promega, USA). Similarly, TRPM2 3'UTR Wt sequence with binding area to miR-6764-5p or its Mut sequence was cloned to pmirGLO vectors. After the establishment of the luciferase reporters, miR-6764-5p mimics/control mimics were transfected into OC cells together with these reporters for 2 days. A dual-luciferase reporter assay system (Promega) was adopted for assessment of the relative activity (firefly/Renilla).

RNA pulldown assay

First, OC cells were transfected with biotinylated miR-6764-5p-Wt/Mut or the biotinylated control (Bio-NC) that obtained from Sangon (Shanghai, China). Next, the lysis buffer (Sigma-Aldrich, USA) was used for cell lysis, and the lysates were incubated with magnetic beads (Beyotime, China) for 15 min. RNA on the beads was measured by PCR after RNA extraction utilizing TRIzol.

Table 2 Information of primary antibodies

Name of antibody	Catalog number	Dilution
anti-Bcl-2	ab32124	1:1000
anti-Bax	ab182733	1:2000
anti-cleaved caspase 3	ab32042	1:2000
anti-GAPDH	ab9485	1:2500

Statistical analysis

Data analysis was performed using GraphPad 8.0.2 and SPSS software. Data from triplicate experiments are presented as the mean ± standard deviation. Significance comparison between two groups is analyzed by student's *t* test, and significance evaluation among three groups is achieved using one-way analysis of variance followed by Bonferroni post hoc test. The value of $p < 0.05$ was defined as threshold for statistical significance.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13008-024-00130-0.

Additional file 1.

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Author contributions

WZ conceived and was the main designer of this study, WZ, SQM and JJ conducted the experiments and analyzed the data. WZ, SQM and JJ wrote the manuscript. All authors read and approved the final version.

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None.

Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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