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NAT10 promotes ovarian cancer cell migration, invasion, and stemness via N4-acetylcytidine modification of CAPRIN1

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Abstract

Ovarian cancer (OC) is the most lethal gynecological tumor. N4-acetylcytidine (ac4C) modification, catalyzed by the acetyltransferase NAT10, is involved in the occurrence and development of cancers. This study aimed to investigate the role of NAT10 in OC and the underlying molecular mechanisms. The expression of NAT10 and CAPRIN1 in OC cells lines were measured using quantitative real-time polymerase chain reaction and immunoblotting. Biological behaviors of OC cells were evaluated using EdU, Transwell, sphere formation, and immunoblotting assays. The molecular mechanism of NAT10 function was analyzed using bioinformatics, ac4C- RNA immunoprecipitation, and actinomycin D treatment assay. The effect of NAT10 on OC progression in vivo was evaluated using xenograft tumor model. The results indicated that NAT10 and CAPRIN1 were highly expressed in OC cells. NAT10 knockdown suppressed OC cell proliferation, migration, invasiveness, stemness, and epithelial-mesenchymal transition in vitro, and impeded tumor growth in vivo. Additionally, CAPRIN1 expression was found to be positively related to NAT10 expression in OC. Silencing of NAT10 inhibited ac4C levels of CAPRIN1 and reduced its RNA stability. Moreover, overexpression of CAPRIN1 reversed the suppression of migration, invasion, and stemness caused by NAT10 knockdown, while knockdown of CAPRIN1 alone inhibited these malignant behaviors of OC cells. In conclusion, NAT10 promotes OC progression by promoting cellular migration, invasion, and stemness via upregulating CAPRIN1 expression. Mechanistically, NAT10 stabilizes CAPRIN1 by promoting its ac4C modification. These findings suggest that NAT10 may be a promising therapy target for OC.

Keywords Ovarian cancer, N4-acetylcytidine modification, NAT10, Tumor stemness, CAPRIN1

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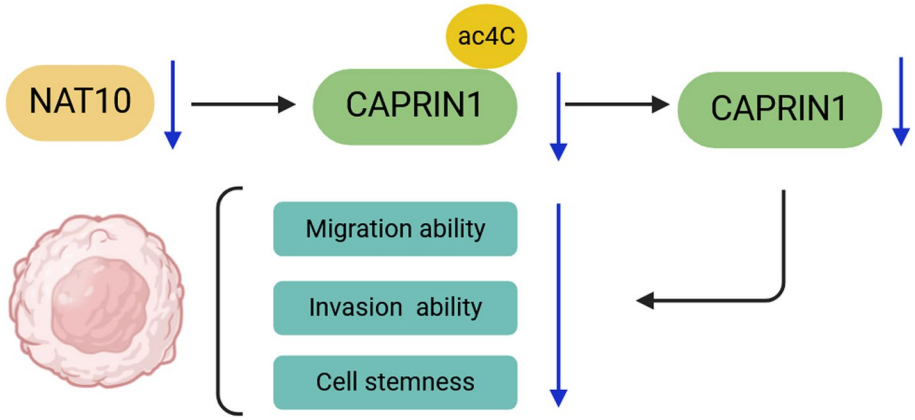
Graphical abstract



Introduction

Ovarian cancer (OC) is the deadliest of all gynecological malignancies. According to statistics, the incidence of OC exceeds 300,000, and the number of death cases exceeds 200,000 globally in 2020 [22]. High-grade serous OC is the main clinical subtype of OC that accounts for 70–80% of OC-related death [3]. Due to the lack of effective early screening strategies, more than two-thirds of patients are in the advanced stage [6, 8]. Therefore, traditional treatments, such as surgery and chemotherapy, are not effective for these patients. Immunotherapy and targeted therapy are emerging treatments for OC [25, 30]. However, the efficiency of these treatments has fallen short of expectations due to drug resistance and metastasis, which are attributed to cancer stem cells (CSCs) [19]. Hence, targeting CSCs to block tumor metastasis, may contribute to reducing OC mortality.

Epigenetic changes are reasons to drive OC progression, especially RNA epigenetic modifications linked to OC occurrence and development [31]. N4-acetylcytidine (ac4C) is a rare kind of RNA modification catalyzed by acetyltransferase Kre33 (in yeast) or NAT10 (in human) that regulates mRNA stability and translation [1, 10]. NAT10-catalyzed ac4C modification is associated with the initiation, progression, and prognosis of numerous human diseases, especially malignancies [11, 17]. NAT10 is a member belongs to the GNZT family that contains 1,025 amino acids. This protein consists of N-terminal acetylase structural domain, ATP/GTP binding motif, and ATPase structural domain [29]. Previous studies have revealed that NAT10 is implicated in several processes, such as tumor metastasis, chemoresistance, and immune response [5, 15, 28]. NAT10 is involved in microtubule-related processes and regulates cell growth in OC [23]. However, the effect of NAT10 on tumor metastasis and the underlying mechanism is still not well documented.

In this study, we analyzed the function of NAT10 in OC and the underlying molecular mechanism. We speculated that NAT10 regulated OC cell migration, invasion, and stemness by ac4C modification of CAPRIN1. This study may provide a novel insight into the pathogenesis of OC.

Materials and methods

Cell culture

Human ovarian epithelial cell line IOSE-80 and OC cell line A2780 were obtained from Biovector NTCC (Beijing, China). OC cell lines (CaOV3 and SKOV3) were purchased from ATCC (Manassas, VA, USA). A2780, CaOV3, and SKOV3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO BRL) and 1% penicillin–streptomycin. IOSE-80 cells were maintained in Roswell Park Memorial Institute (RPMI)−1640 (GIBCO BRL) supplemented with 10% FBS and 1% penicillin–streptomycin. All cells were cultured in an incubator with 5% CO2 atmosphere at 37 °C.

Cell transfection

Short hairpin RNA targeting NAT10 (sh-NAT10), sh-CAPRIN1, the negative control (sh-NC), empty vector, and CAPRIN1 overexpressing plasmids were designed and synthesized by GenePharma (Shanghai, China). CaOV3 and SKOV3 cells were seeded into six-well plates one day before transfection. These cells were transfected with plasmids using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 48 h.

Transwell assay

Cell migration and invasion were analyzed using Transwell chambers (24-well, 8 μm pore size, Corning, Corning, NY, USA) without or with Matrigel, respectively. OC cell suspensions containing 10^5 cells were added in the upper Transwell chambers, and DMEM supplemented with 10%FBS was filled with the lower chambers. After 24 h of incubation at 37 °C, the cells through the pore were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The cells were visualized under an inverted microscope, and cell numbers were counted.

Sphere formation analysis

Stemness was analyzed using sphere formation analysis as previously described [24]. CaOV3 and SKOV3 cells were washed twice with PBS and seeded in six-well ultra-low attachment plates. The cells were maintained in serum-free DMEM/F-12 (GIBCO BRL) supplemented with 10 ng/mL β-FGF (Invitrogen), 20 ng/mL B27 (Invitrogen), 20 ng/mL EGF (Invitrogen) and 20 ng/ml IGF-1 (Cell Signaling) for 2 weeks. The sphere formation was viewed using an inverted microscope. The percentage of tumourspheres (diameter 50–150 μm) was quantified.

EdU assay

Cell proliferation was evaluated using the BeyoClick™ EdU-594 cell proliferation assay kit (Byotime, Shanghai, China). CaOV3 and SKOV3 cells were seeded in six-well plates and labeled with 10 μM EdU working solution for 2 h. Subsequently, the cells were fixed with immunol staining fix solution (Beyotime) and permeated using the enhanced immunostaining permeabilization buffer (Beyotime). For EdU detection, the cells were incubated with Click reaction solution for 30 min in the dark. After staining with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO, USA), the cells were observed under a fluorescence microscope.

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted from cells using the MolPure cell RNA kit (Yeason, Shanghai, China). RNA concentration and purity were measured using the NanoDrop spectrophotometry. Next, RNA (1 μg) was used to synthesize cDNA first chain using the Hifair AdvanceFast One-step RT-gDNA digestion superMix for qPCR (Yeason). qPCR was carried out using the Hieff qPCR SYBR Green master mix (Yeason) on the CFX96 real-time PCR system (BioRad, Hercules, CA, USA). RNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method as normalizing GAPDH.

Immunoblotting

Total proteins were extracted from cells using radioimmunoprecipitation assay lysis buffer (Beyotime), and their concentrations were measured using a BCA kit (Yeasen). The same amount of protein (30 μg each lane) was loaded on 10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes. Then, 5% fat-free milk was used to block the membranes for 1 h. Next, the membranes were incubated with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) against NAT10 (#66548, 1:1000), GAPDH (#2118, 1:1000), Nanog (#3580, 1:1000), OCT4 (#2750, 1:1000), SOX2 (#2748, 1:1000), CAPRIN1 (#18553, 1:1000), E-cadherin (#3195, 1:1000), N-cadherin (#4061, 1:1000), and Vimentin (#3932, 1:1000) at 4 °C overnight. On the second day, the membranes were incubated with anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technology) at room temperature for 2 h. The super ECL detection reagent (Yeasen) was used to develop protein bands.

Bioinformatics

mRNAs positively or negatively related to NAT10 were predicted using the LinkedOmics database (https://www.linkedomics.org/login.php). The correlation of NAT10 and TRIM44, CAPRIN1, or QSER1 was acquired from the GEPIA database (http://gepia.cancer-pku.cn/).

RNA binding protein immunoprecipitation (RIP)

The Imprint RIP kit (Sigma-Aldrich) was used to evaluate the relation between NAT10 and CAPRIN1. Briefly, cells were collected (2×10^6 cells) were lysed using 200 μL lysis buffer for 15 min. The supernatant was collected at 16,000 ×g for 10 min. The supernatant (10%) was stored as the Input. Protein A/G magnetic beads were precoated with antibodies (anti-NAT10 and anti-IgG). The supernatant was incubated with antibody prebound magnetic beads with rotation for 3 h at 4 °C. The mixture was washed with RIP wash buffer and RNA was purified. CAPRIN1 expression was measured using qPCR.

Ac4C-RIP

The ac4C levels of CAPRIN1 were measured using the GenSeq ac4C RIP kit (CloudSeq, Shanghai, China). Total RNA was fragmented by incubating with fragmentation buffer at 70 °C for 6 min. Three microgramme fragmented RNA was the Input. PGM beads were incubated with anti-ac4C and anti-IgG with rotation for 1 h. Fragmented RNA was incubated with nuclease-free water, IP buffer, and beads at 4 °C for 1 h. The beads were washed

with IP buffer. RNA was purified and CAPRIN1 expression was measured using qPCR.

Actinomycin D treatment assay

To measure RNA stability, the cells were treated with 5 μg/mL actinomycin D (Sigma-Aldrich) for 1, 4, 8, and 12 h. QPCR was used to measure CAPRIN1 expression at the specified point-in-time.

Animal experiments

Female BALB/c nude mice (body weight: about 20 g, six weeks old) were purchased from Charles River (Beijing, China). SKOV3 cells were stably transfected with sh-NC and sh-NAT10. The cells (5×10^6) were subcutaneously injected into the flank of mice. One week later, tumors were observed (week 0). Then, tumor length and width were measured every week until week 4, and volume was calculated using the $0.52 \times \text{length} \times \text{width}^2$ formula. After the last test, all mice were sacrificed. The tumors were collected, photographed, and weighed.

Statistical analysis

The data were analyzed using GraphPad Prism software (version 8.0) and the results were expressed as mean ± standard deviation. The student's t-test was applied to compare the differences between two groups, and one-way ANOVA followed by Tukey's post hoc test was used for the comparisons among multiple groups. P value less than 0.05 was statistically significant.

Results

NAT10 is highly expressed in OC cells

NAT10 has been reported to be essential for epithelial OC cell growth [23]. However, its effect on OC progression has not been fully elucidated. Therefore, we first measured NAT10 expression in OC cells. The results of qPCR and immunoblotting showed that NAT10 expression was elevated in OC cell lines, including CaOV3, A2780, and SKOV3 cells, compared with ovarian epithelial cell line IOSE-80 (Fig. 1A and B). Moreover, the expression of NAT10 was highest in the CaOV3 cell line and second in the SKOV3 cell line. Thus, the two cell lines were chosen for cell phenotype analysis. The results demonstrate that NAT10 is highly expressed in OC cells, suggesting it is involved in OC development.

NAT10 knockdown inhibits OC cell migration, invasion, and stemness

To investigate the function of NAT10, we knocked down its expression. As compared with sh-NC, sh-NAT10 transfection decreased NAT10 expression in CaOV3 and SKOV3 cells (Fig. 2A). Subsequently, Transwell assay was performed to determine cell migration and invasion. The results indicated that knockdown of NAT10 impeded CaOV3 and SKOV3 cell migration and invasion (Fig. 2B-E). Besides, sphere formation assay and immunoblotting were conducted to evaluate cancer stemness. As shown in Fig. 2F-H, silencing of NAT10 suppressed sphere formation capability and downregulated the protein levels of stemness-related markers, including Nanog, OCT4, and SOX2. In addition, the effect of NAT10 on cell proliferation was assessed by

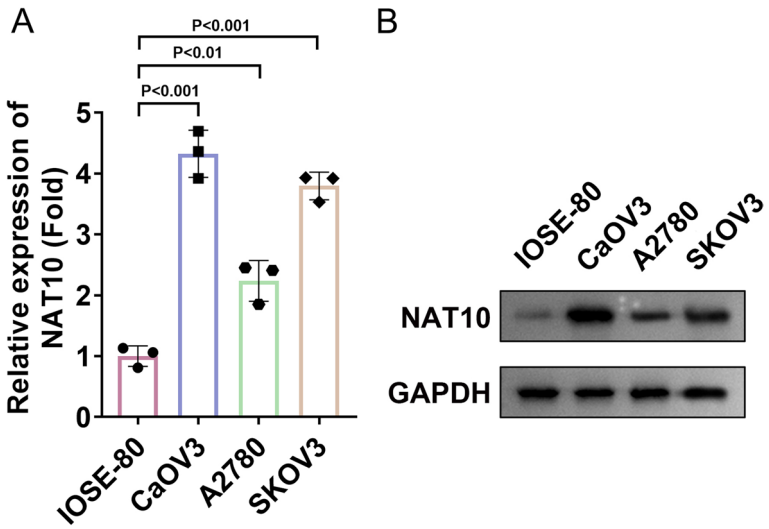


Fig. 1 NAT10 is highly expressed in OC cells. **A** NAT10 mRNA expression and (**B**) protein levels in OC cells (CaOV3, A2780, and SKOV3) and normal ovarian epithelial cell line IOSE-80 were examined using qPCR and immunoblotting, respectively

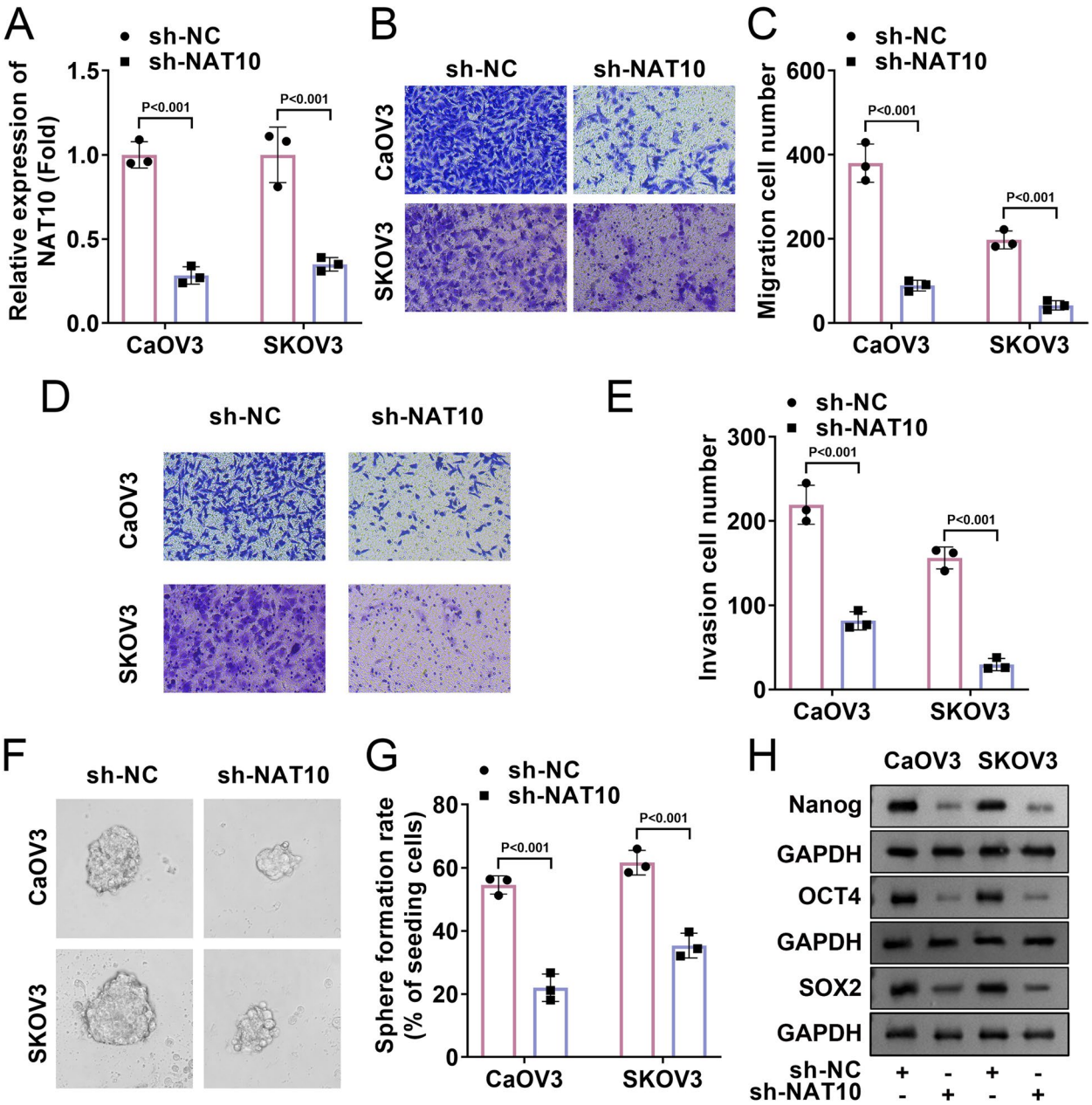


Fig. 2 NAT10 knockdown inhibits OC cell migration, invasion, and stemness. **A** NAT10 expression in CaOV3 and SKOV3 cells following sh-NC and sh-NAT10 transfection. **B** Effect of NAT10 on OC cell migration was assessed using Transwell assay, and (**C**) the number of migrated cells was counted. **D** Effect of NAT10 on OC cell invasion was evaluated using Transwell assay, and (**E**) invasive cell number was counted. **F**, **G** Cancer stemness was assessed using sphere formation assay. **H** The levels of stemness-related proteins (Nanog, OCT4, and SOX2) were measured using immunoblotting

EdU assay. The results showed that NAT10 knockdown reduced EdU positive cells, suggesting that OC cell proliferation was suppressed by NAT10 silence (supplementary Fig. 1). Epithelial–mesenchymal transition (EMT) plays a pivotal role in tumor invasion, metastasis, and stemness [14]. Therefore, we analyzed the effect of NAT10 on EMT of CaOV3 and SKOV3 cells. The results of immunoblotting showed that silencing of NAT10 upregulated E-cadherin expression, as well as decreased N-cadherin and Vimentin levels (supplementary Fig. 2). Together, NAT10 acts as an oncogene in OC to facilitate cell proliferation, migration, invasion, stemness, and EMT process.

NAT10 facilitates ac4C modification of CAPRIN1

NAT10 is a well-known ac4C writer during RNA ac4C modification [29]. Hence, to investigate the underlying mechanism of NAT10, we predicted which RNA can be modified by it. We used the LinkedOmics database to obtain mRNAs associated with NAT10. Multiple mRNAs associated with NAT10 were predicted to have positive or negative correlations, among which TRIM44, CAPRIN1, and QSER1 exhibited the highest positive correlation with NAT10 (Fig. 3A). Next, the correlation of NAT10 and TRIM44, CAPRIN1, or QSER1 was evaluated using the GEPIA database. TRIM44 had no correlation with NAT10 ($R=0.081$, $P=0.45$; Fig. 3B), whereas CAPRIN1 ($R=0.28$, $P=0.0.0087$; Fig. 3C) or QSER1 ($R=0.23$, $P=0.03$; Fig. 3D) was weakly correlated with NAT10. Moreover, we evaluated the effect of NAT10 on the expression of TRIM44, CAPRIN1, and QSER1. The results showed that NAT10 knockdown markedly decreased CAPRIN1 expression, but had no significant effect on TRIM44 and QSER1 expression (Fig. 3E-G). Thus, we speculated that NAT10 downregulated CAPRIN1 expression in an ac4C-dependent manner. To verify the speculation, we performed ac4C-RIP assay. As illustrated in Fig. 3H, interfering with NAT10 reduced the ac4C enrichment in CAPRIN1. Moreover. NAT10 interacted with CAPRIN1 (Fig. 3I and J). The mRNA stability of CAPRIN1 was reduced after NAT10 knockdown (Fig. 3K). In summary, NAT10 promotes CAPRIN1 ac4C modification and stabilizes CAPRIN1.

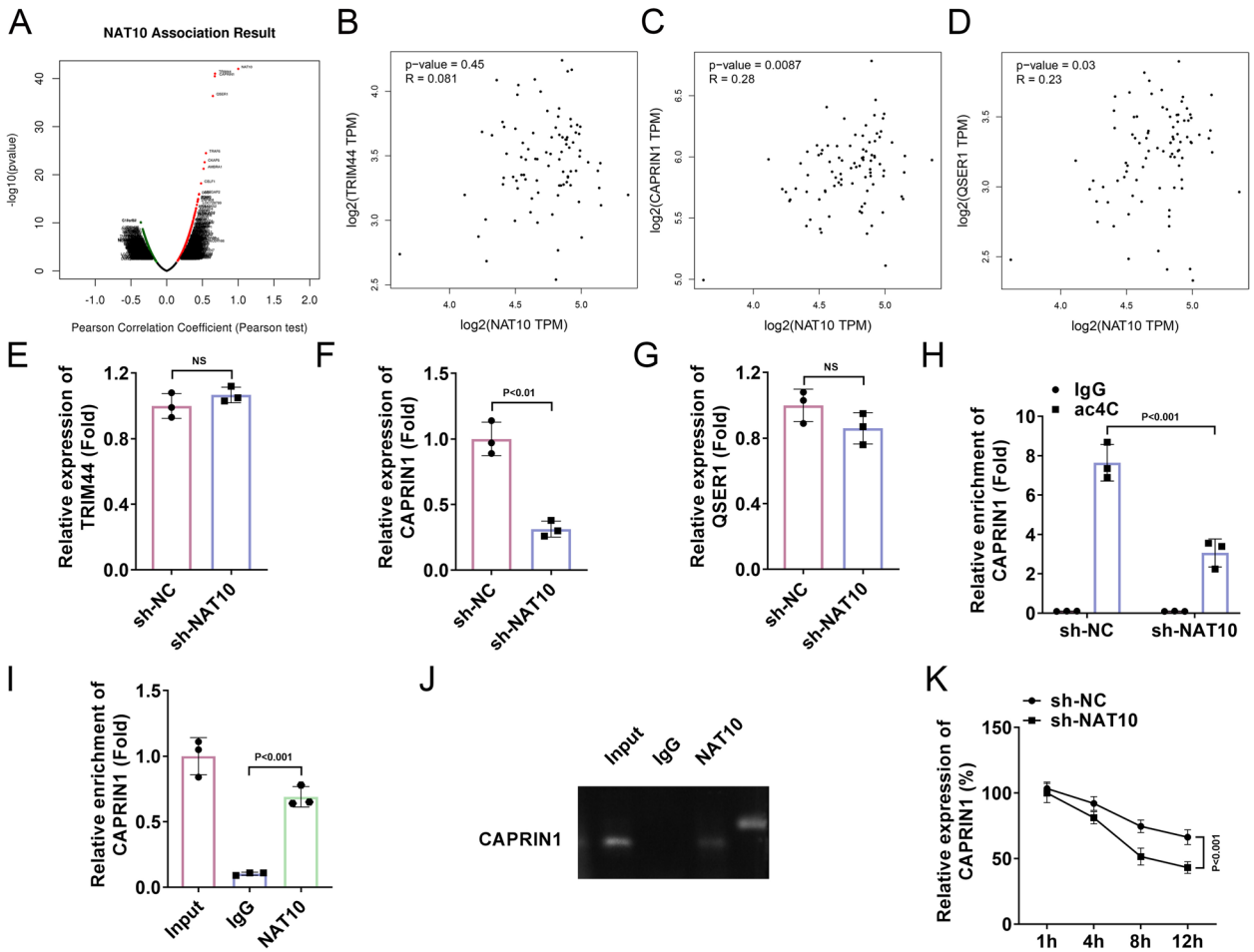


Fig. 3 NAT10 facilitates ac4C modification of CAPRIN1. **A** NAT10-associated genes were predicted using the LinkedOmics database through Pearson correlation coefficient. Correlation between NAT10 and (**B**) TRIM44, (**C**) CAPRIN1, and (**D**) QSER1 was predicted using the GEPIA database. The expression of (**E**) TRIM44, (**F**) CAPRIN1, and (**G**) QSER1 was measured using qPCR after NAT10 knockdown. **H** The regulation of NAT10 knockdown on ac4C levels of CAPRIN1 was measured using ac4C-RIP assay. **I** The relation of NAT10 and CAPRIN1 was evaluated using RIP. **J** A representative photograph of electrophoresis results in RIP. **K** Effect of NAT10 on CAPRIN1 mRNA stability was measured using actinomycin D treatment assay. NS: no significance

Overexpression of CAPRIN1 counteracts the inhibition of OC cellular behaviors caused by NAT10 knockdown

The expression of CAPRIN1 in OC cells was detected using qPCR and immunoblotting. The results showed that CAPRIN1 mRNA and protein levels were higher in CaOV3, A2780, and SKOV3 cells than that in ovarian epithelial cells IOSE-80 (Fig. 4A and B). Next, the effects of CAPRIN1 on NAT10-mediated cellular behaviors were investigated. To overexpress CAPRIN1, CAPRIN1 overexpressing plasmids were transfected, and its expression was increased (Fig. 4C). The results of Transwell assay showed that NAT10 knockdown suppressed cell migration and invasion, whereas CAPRIN1 overexpression counteracted this suppression (Fig. 4D-G). Additionally, the results of sphere formation assay and immunoblotting indicated that overexpression of CAPRIN1 reversed the decrease of cancer stemness induced by NAT10 knockdown (Fig. 4H-J). To sum up, NAT10 knockdown inhibits OC cell migration, invasion, and stemness by decreasing CAPRIN1 expression.

Interfering with CAPRIN1 impedes OC cell malignant functions

The role of CAPRIN1 alone was also explored. After sh-CAPRIN1 transfection, its mRNA and protein levels were decreased (Fig. 5A and B). Subsequently, we found that knockdown of CAPRIN1 did not change the levels of NAT10 (Fig. 5B). Moreover, CAPRIN1 knockdown inhibited the migration (Fig. 5C and D), invasion (Fig. 5E and F), and stemness (Fig. 5G-I) of CaOV3 and SKOV3 cells, suggesting that CAPRIN1 functions as an oncogene in OC.

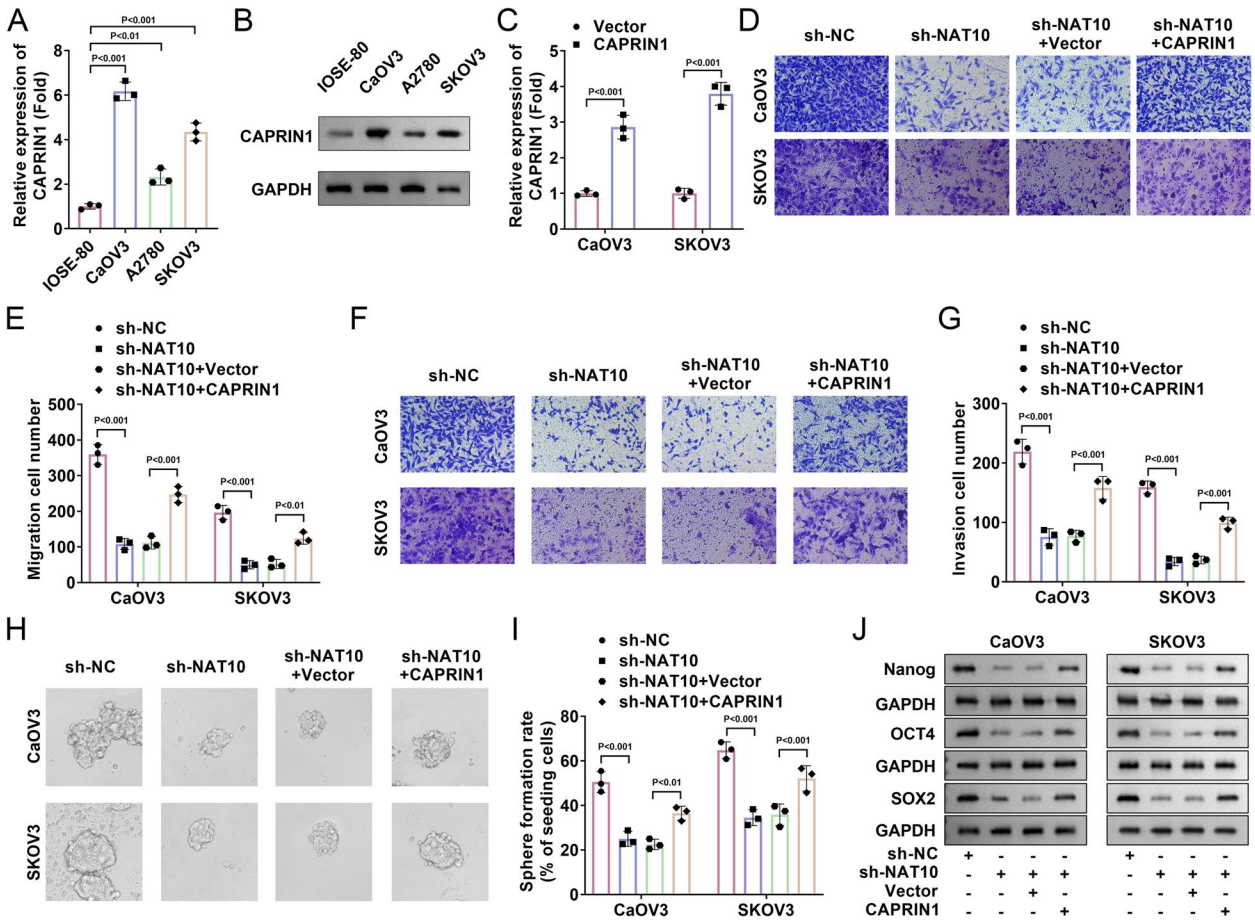


Fig. 4 Overexpression of CAPRIN1 counteracts the inhibition of OC cellular behaviors caused by NAT10 knockdown. **A** CAPRIN1 mRNA expression and (**B**) protein levels in OC cells (CaOV3, A2780, and SKOV3) and normal IOSE-80 cells were examined using qPCR and immunoblotting, respectively. **C** CAPRIN1 expression in CaOV3 and SKOV3 cells following empty vector and CAPRIN1 overexpressing plasmids transfection. After NAT10 knockdown and CAPRIN1 overexpression, (**D**) cell migration was evaluated using Transwell assay, and (**E**) migrated cells were counted; (**F**) invasion was evaluated using Transwell assay, and (**G**) invaded cells were counter; (H, I) sphere formation analysis was used to assess stemness; and (J) immunoblotting was conducted to examine the levels of stemness-related factors, including Nanog, OCT4, and SOX2

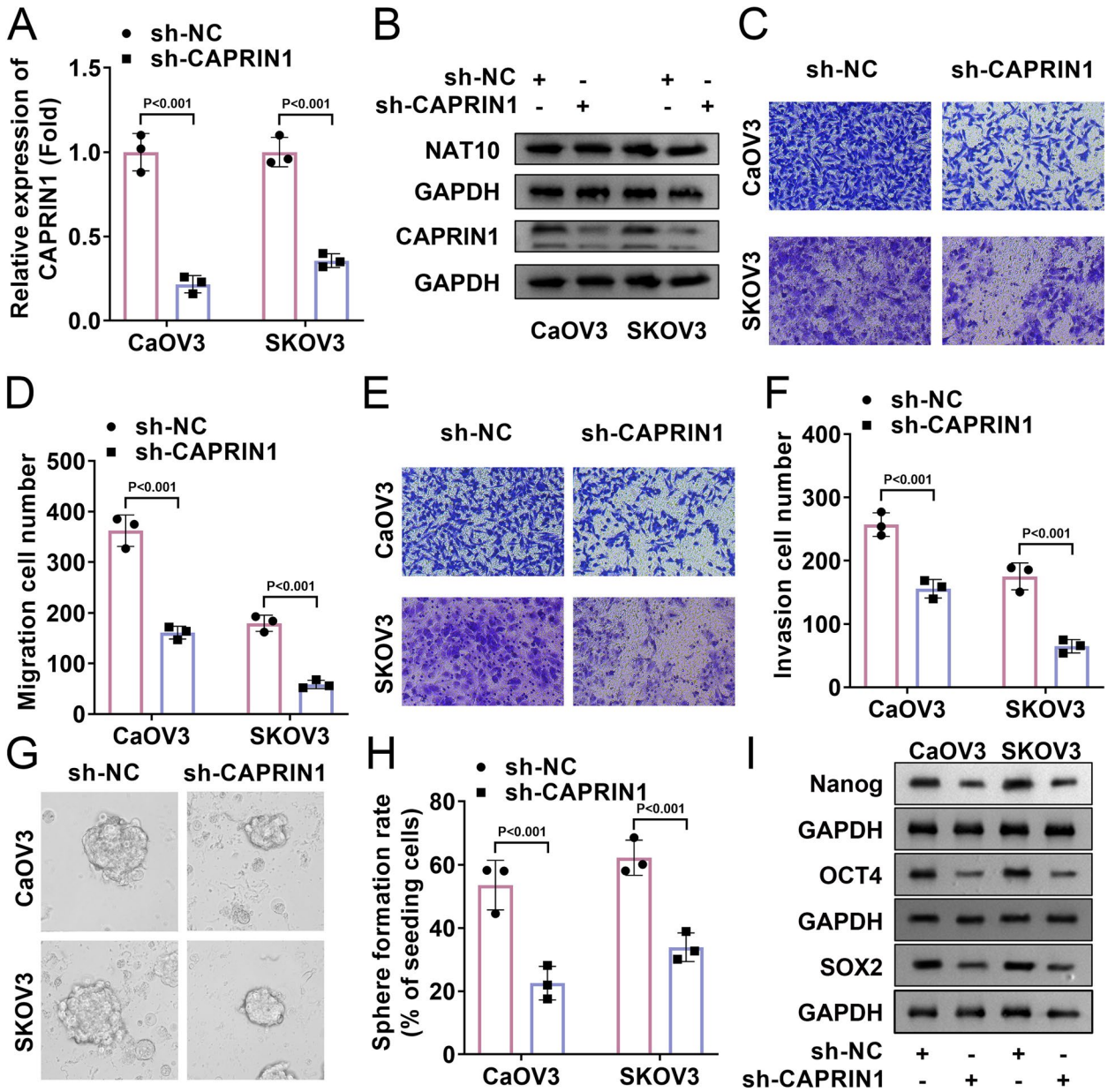


Fig. 5 Interfering with CAPRIN1 impedes OC cell malignant functions. **A** After sh-NC and sh-CAPRIN1 transfection, qPCR was performed to measure CAPRIN1 expression in CaOV3 and SKOV3 cells. **B** After CAPRIN1 knockdown, the protein levels of NAT10 and CAPRIN1 were measured using immunoblotting. **C** Effect of CAPRIN1 on OC cell migration was assessed using Transwell assay, and (**D**) the number of migrated cells were counted. **E** Effect of CAPRIN1 on OC cell invasion was evaluated using Transwell assay, and (**F**) invasive cell number was counted. **G**, **H** Cancer stemness was assessed using sphere formation assay. **I** The levels of stemness-related proteins (Nanog, OCT4, and SOX2) were measured using immunoblotting

Knockdown of NAT10 retards tumor growth in vivo

Finally, we explored the role of NAT10 in vivo. Nude mice were injected with sh-NC or sh-NAT10 transfected cells to generate xenograft tumor model. The results showed that silencing of NAT10 reduced tumor size, weight, and volume, compared with the sh-NC group (Fig. 6A-C). The results demonstrate that NAT10 promote the progression of OC.

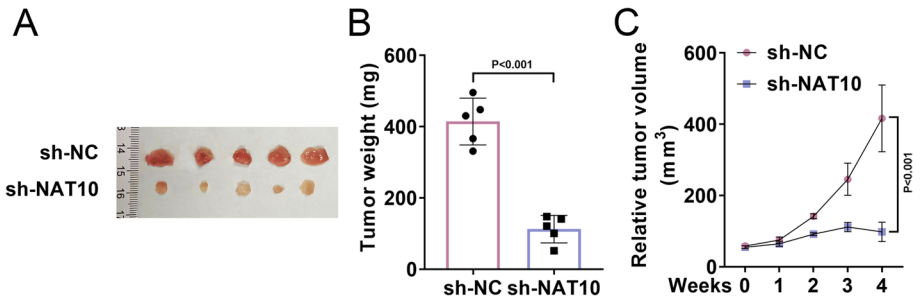


Fig. 6 Knockdown of NAT10 retards tumor growth in vivo. **A** Photograph of all xenograft tumors in each group of mice. **B** Tumors were weighed after mice were sacrificed. **C** Tumor volume was detected every week

Discussion

In this study, we identified the role of NAT10 in OC. We found that NAT10 functioned as an oncogene in OC to promote cell invasiveness, migration, and stemness by ac4C modification of CAPRIN1.

NAT10 is the only known ac4C writer to catalyze ac4C acetylation. In malignancies, NAT10 regulates malignant cellular processes. For instance, NAT10 expression is increased in patients with bladder cancer and is essential for the proliferation, migration, and invasion of bladder cancer cells [26]. Besides, NAT10 accelerates colorectal cancer progression by inhibiting cell apoptosis and promoting invasion and migration [12]. Additionally, knockdown of NAT10 inhibits the growth and invasion of cervical cancer cells [16]. However, the functions of NAT10 in OC is not understood. OC-induced mortality is partly due to tumor metastasis, which involves cell migration and invasion [4]. Moreover, CSCs are one of the major factors to promote tumor metastasis and are also related to tumor recurrence [2]. EMT has been demonstrated to be associated with OC tumor metastasis and chemoresistance [7]. Therefore, we measured the effects of NAT10 on OC cell migration, invasion, stemness, and EMT. We found that NAT10 expression was increased in OC cells, and knockdown of NAT10 suppressed the proliferation, migration, invasiveness, EMT, and stemness of OC cells. Moreover, our animal experiments showed that NAT10 knockdown inhibits tumor growth in vivo. The findings suggest that NAT10 is a tumor promoter to aggravate OC.

Based on the ac4C modification catalytic activity of NAT10, we identified which mRNA ac4C acetylation could be mediated by NAT10. We found that silencing of NAT10 inhibited ac4C levels of CAPRIN1 to reduce its mRNA stability, thereby downregulating CAPRIN1 expression. CAPRIN1 is a member of the cytoplasmic activation/proliferation-associated protein family. It has similar homologous regions with CAPRIN2. Accumulating evidence has revealed that CAPRIN1 regulates cellular proliferation, cell cycle, and synaptic plasticity [27]. CAPRIN1 is associated with numerous diseases, such as neurodevelopmental disorders, hearing loss, and inflammation [13, 20, 21]. Importantly, CAPRIN1 has been reported to be involved in the progression of several cancers as an oncogene. For example, reduced CAPRIN1 expression inhibits the proliferation and glycolysis of esophageal carcinoma cells, and impedes tumor growth [9]. Besides, miR-621 inhibits liver cancer cell proliferation and arrests G0/G1 cell cycle by targeting CAPRIN1 [32]. In OC, it has been reported that lncRNA SNHG8 facilitates cell growth, metastasis, and stemness by positively regulating CAPRIN1 [18]. Herein, knockdown of CAPRIN1 suppressed OC cell migration, invasion, and stemness, confirming that CAPRIN1 is an oncogene in OC. Moreover, overexpression of CAPRIN1 counteracted the inhibition of cellular malignant behaviors caused by NAT10 silencing. The findings suggest that NAT10 promotes the progression of OC by increasing CAPRIN1 expression.

However, there are still several limitations in this study. We have not investigated the ac4C acetylation sites in CAPRIN1. Additionally, whether NAT10 affects tumor metastasis in vivo by regulating CAPRIN1 remains unknown. We will further study the functions of NAT10 and CAPRIN1 in OC in our future work.

In conclusion, NAT10 is highly expressed in OC, and knockdown of NAT10 suppresses the migration, invasion, and stemness of OC cells, as well as inhibits tumor growth in vivo. Mechanistically, NAT10 promotes ac4C modification and stabilizes CAPRIN1, thereby positively regulating CAPRIN1 expression. The findings suggest that targeting ac4C acetylation may be an effective strategy, and NAT10 may be a therapeutic target for OC.

Abbreviations

ac4C	N4-acetylcytidine
ANOVA	analysis of variance
ATCC	American Type Culture Collection
BCA	bicinchoninic acid
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's modified Eagle's medium
ECL	enhanced chemiluminescence

EdU	5-ethynyl-2′-deoxyuridine
FBS	fetal bovine serum
NC	negative control
OC	ovarian cancer
PVDF	polyvinylidene fluoride
qPCR	quantitative real-time polymerase chain reaction
RIP	RNA binding protein immunoprecipitation
RPMI	Roswell Park Memorial Institute
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Sh	Short hairpin RNA

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12905-025-03567-9.

Additional file 1. Supplementary Figure 1. NAT10 knockdown inhibits OC cell proliferation. After sh-NC and sh-NAT10 transfection, the proliferation capability was evaluated using EdU assay.

Additional file 2. Supplementary Figure 2. NAT10 knockdown inhibits EMT process of OC cells. After sh-NC and sh-NAT10 transfection, the levels of EMT markers including E-cadherin, N-cadherin, and Vimentin were measured by immunoblotting.

Additional file 3.

Acknowledgements
Not applicable.

Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by YS and MC. The first draft of the manuscript was written by YS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Cancer Hospital, Chinese Academy of Medical Sciences. All animal experiments should comply with the ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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