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Upregulation of TRPS1 promotes proliferation, migration, and invasion in ovarian clear cell carcinoma and correlates with poor patient prognosis

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Abstract

Objective Tricho-rhino-phalangeal syndrome-1 (TRPS1), an atypical GATA transcription factor, plays a critical role in diverse physiological and pathological processes and holds potential as a biomarker for diseases and targeted tumor therapies. This study explores TRPS1 expression in ovarian clear cell carcinoma (OCCC), its correlation with patient prognosis, and its involvement in OCCC pathogenesis.

Research objectives and methods To investigate TRPS1 expression, we analyzed ovarian tissues from 50 OCCC patients and 25 normal tissues (from patients with uterine leiomyoma) via immunohistochemistry. Statistical methods, including Chi-square tests, Kaplan-Meier survival analysis, and Cox regression, were employed to evaluate the correlation between TRPS1 expression and clinicopathological parameters. In OCCC cell lines (TOV21G and ES-2), TRPS1 expression was quantified using qRT-PCR and Western blot. Functional studies were conducted by silencing TRPS1 in TOV21G cells with small interfering RNA and inducing overexpression in ES-2 cells using a plasmid. Cellular proliferation and migration were assessed through CCK-8, colony formation, and Transwell assays. Finally, Western blot analysis was performed to investigate the link between TRPS1 and EMT-related molecular pathways.

Results TRPS1 protein expression was significantly higher in OCCC tissues compared to normal tissues and was positively associated with lymph node metastasis and advanced clinical stage. High TRPS1 expression was linked to shorter overall and recurrence-free survival in OCCC patients. In vitro, TRPS1 knockdown suppressed cell proliferation, migration, and invasion, accompanied by reduced levels of invasion-promoting proteins (N-cadherin, MMP2, MMP9) and increased expression of the invasion-inhibiting protein E-cadherin. Conversely, TRPS1 overexpression promoted the expression of invasion-promoting proteins.

Conclusions TRPS1 is overexpressed in OCCC and is associated with poor prognosis, serving as an independent predictor of patient outcomes. Its elevated expression enhances OCCC cell proliferation, migration, and invasion by regulating proteins involved in the epithelial-to-mesenchymal transition (EMT) pathway. These findings highlight

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TRPS1 as a critical player in OCCC pathogenesis and a potential biomarker and therapeutic target for disease management.

Keywords Ovarian clear cell carcinoma, TRPS1, Clinical prognosis, Cell proliferation, Migration, Invasion

Introduction

Ovarian cancer (OC) remains one of the most lethal gynecologic malignancies worldwide. In the United States alone, an estimated 12,810 deaths from ovarian cancer were reported in 2022, accounting for approximately 4% of all cancer-related deaths among women [1]. Ovarian clear cell carcinoma (OCCC) is one of the most aggressive subtypes of ovarian cancer and is the only epithelial ovarian cancer (EOC) subtype with a rising incidence among Black, Hispanic, and Asian women in North America [2]. While most OCCC cases (57–81%) are diagnosed at an early stage (I/II) with better overall survival than stage-matched high-grade serous (HGS) ovarian cancer, advanced-stage OCCC responds poorly to standard platinum-based chemotherapy compared to advanced HGS ovarian cancer [3]. OCCC is characterized by unique molecular features, including frequent ARID1A mutations, as well as PI3K and PTEN mutations [4]. Notably, ARID1A deletion sensitizes cancer cells to PI3K/Akt pathway inhibition, offering potential for overcoming platinum resistance in OCCC [5]. However, no definitive therapeutic strategies have been established for advanced OCCC. These challenges underscore the urgent need to identify novel therapeutic targets and develop effective treatment strategies for this aggressive cancer subtype.

Tricho-rhino-phalangeal syndrome-1 (TRPS1) is an autosomal dominant disorder characterized by sparse scalp hair, slow growth, and craniofacial and skeletal abnormalities. These features result from mutations or deletions in the TRPS1 gene [6]. Located on human chromosome 8q23-24, the TRPS1 gene spans approximately 260.5 kb and encodes a protein with a GATA-type DNA-binding domain flanked by two nuclear localization signals (NLS) and two C-terminal zinc finger domains [7]. This structural composition classifies TRPS1 as a novel member of the GATA transcription factor family. Members of the GATA transcription factor family are crucial regulators of cell proliferation, differentiation, oncogenesis, and gene expression. GATA1/2/3 play roles in the differentiation of mesoderm- and ectoderm-derived tissues, including the hematopoietic system and central nervous system, while GATA4/5/6 are involved in the development and differentiation of endoderm- and mesoderm-derived tissues, such as embryonic stem cell differentiation, cardiovascular development, and adult epithelial cell differentiation [8]. Unlike other GATA family members (GATA1–6), which possess two C4-type GATA zinc finger domains, TRPS1 contains only one, indicating its unique transcriptional functions [9].

The functions of TRPS1 have been extensively studied in tissues such as bone, hair follicles, and the kidney. In bone, TRPS1 plays a critical role in chondrocyte proliferation and differentiation by repressing the expression of PTHrP [10] and osteocalcin [11] through direct promoter interactions and by physically interacting with Runx2 to block Runx2-mediated transcriptional activation [12]. Suemoto et al. demonstrated that TRPS1 regulates chondrocyte proliferation and apoptosis by directly inhibiting STAT3 signaling [13]. In the hair follicle, TRPS1 inhibits the follicular stem cell regulator Sox9, controlling the proliferation of hair follicle epithelial cells [14]. In the kidney, TRPS1 functions as a key target of BMP-7, promoting mesenchymal-to-epithelial transition (MET) during glomerular development via activation of p38MAPK signaling [15] and regulating ureteric budding by modulating TGFβ/SMAD3 signaling [16].Beyond its developmental roles, TRPS1 has been implicated in various cancers, including prostate cancer [17, 18], breast cancer [19–22], osteosarcoma [23], colon cancer [24], lung cancer [25], hepatocellular carcinoma [26]. TRPS1 has been associated with tumor cell proliferation, invasion, migration, metastasis, and multidrug resistance [21, 23, 27]. Despite its involvement in several cancers, the expression pattern of TRPS1 in ovarian clear cell carcinoma (OCCC) and its clinical significance remain unknown.

In this study, we investigated the molecular functions and roles of TRPS1 in OCCC, focusing on its impact on tumor cell proliferation and invasion. We also explored the relationship between TRPS1 expression and clinicopathological parameters in OCCC patients, providing new insights into its potential as a biomarker and therapeutic target.

Materials and methods

Human sample and tissue processing

This study was conducted with the informed written consent of all participants and adhered to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Human Ethics Committee of Zhejiang Cancer Hospital. Tissue samples were collected from 50 patients who underwent surgical resection of ovarian clear cell carcinoma (OCCC) between 2010 and 2021. Additionally, 25 normal ovarian tissues were obtained from patients who underwent total hysterectomy for uterine fibroids. None of the patients received local or systemic treatments prior to surgery. All tissue samples

were washed with sterile PBS, snap-frozen in liquid nitrogen, and stored at -80 °C for subsequent analysis. Pathological parameters were assessed by an experienced pathologist. Patient follow-up was conducted every three months during the first year post-surgery and every six months thereafter, concluding on September 1, 2022.

Immunohistochemical analysis

Immunohistochemistry was used to detect the subcellular localization and expression pattern of TRPS 1 on formalin-fixed, paraffin-embedded, 4 μ m thick tissue sections. The tissue slides were briefly proteolytically digested and peroxidase was blocked. The slides were then incubated with rabbit anti-TRPS1 primary antibody (1:500; Article No.: 21938-1-AP Proteintech) at 4 °C overnight. The proteins were visualized by staining with peroxidase-labeled polymers and substrate-chromogen after washing. Immunostaining was scored by two independent, experienced pathologists who were unaware of the clinicopathologic parameters and clinical outcome of the patients after hematoxylin restaining. Tumor specimens were semi-quantitatively scored, taking into account the homogeneity of target protein staining. The pathologists' scores were compared, and any inconsistencies were resolved by rechecking their staining to obtain a consistent score. Staining was ranked on a scale of 0 to 3, with 0 indicating no staining, 1 indicating light staining, 2 indicating moderate staining, and 3 indicating dark staining. The percentage of stained cells was also ranked on a scale of 0 to 3, with 0 indicating no stained cells, 1 indicating less than 25% stained cells, 2 indicating 25-50% stained cells, and 3 indicating greater than 50% stained cells. Five fields of view were randomly selected from each slide. TRPS1 expression was assessed by calculating the mean of the sum of the extent and proportion of stained cells. A score of less than 2 indicated low TRPS1 expression, while a score of 2 or greater indicated high TRPS1 expression. Progression-Free Survival (PFS) is defined as the time from surgery to the first recurrence of the disease or the last follow-up. Overall Survival (OS) is defined as the time interval from surgery to the patient's death.

Cell lines and culture conditions

The ES-2 cell line was obtained from the Zhejiang Cancer Hospital Research Institute and cultured in McCoy's 5 A complete medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The TOV21G cell line was purchased from Meisen CTCC and maintained in TOV21G complete medium, consisting of a 1:1 mixture of MCDB 105 medium and Medium 199 (85%) with 15% fetal bovine serum. All cell lines were cultured in a 37 °C incubator with 5% CO_2.

Transient transfection of plasmids and small interfering RNA

TRPS1 was cloned into the pcDNA3.1 expression vector (LABGIC) for overexpression. OCCC cells were transfected with TRPS1-targeting small interfering RNA (siRNA) using Lipofectamine 2000 (Invitrogen). OCCC cells were seeded into 6-well plates, and once cell density reached 50–60%, they were transfected with either the TRPS1 overexpression plasmid or an empty control plasmid. Plasmids and Lipofectamine 2000 were separately diluted in Opti-MEM medium and incubated at room temperature for 5 min, then mixed and incubated for an additional 10 min to form plasmid-lipid complexes. These complexes were added to the cells for transfection, which lasted 24–48 h before subsequent experiments.

The siRNAs targeting TRPS1 included:

- TRPS1 siRNA#1: GUCCCUUGAAUGUAGUAAATT.
- TRPS1 siRNA#2: GCACACAGCUGCUACAAAUTT.

RNA isolation, reverse transcription, and real-time quantitative PCR

Total RNA was extracted from the cell lines using the Vazyme Column RNA Extraction Kit (FastPure Cell/Tissue Total RNA Isolation Kit V2) according to the manufacturer's protocol. Reverse transcription (RT) and real-time quantitative PCR (qRT-PCR) were performed using Vazyme Taq Pro Universal SYBR qPCR Master Mix to assess the mRNA expression levels of the target genes. The primers used were as follows:

- GAPDH forward primer: 5'-GTCAGCCGCATCTTCTTT-3'.
- GAPDH reverse primer: 5'-CGCCCAATACGACCAAAT-3'.
- TRPS1 forward primer: 5'-CAAATCTCAGGCCTGAGTGA-3'.
- TRPS1 reverse primer: 5'-GTGAAGAGCTGATATCCTGCAG-3'.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference. Relative gene expression levels were calculated using the 2^-ΔΔCT method based on CT values.

Western blot analysis

Cells were collected and washed three times with cold PBS. Protein extraction reagents (RIPA with phosphatase inhibitor, protease inhibitor, and PMSF at a ratio of 1:100, Solarbio) were added, and protein concentration was determined using a bicinchoninic acid (BCA) protein quantification kit (Solarbio) according to the

manufacturer's instructions. The proteins were then mixed with 5× loading buffer and boiled at 100 °C for 10 min. Total protein samples (20 μg per well) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Merck Millipore). The membranes were blocked with 5% skimmed milk powder in Tris-buffered saline containing Tween-20 (TBST) for 2 h, followed by incubation with anti-TRPS1 primary antibody overnight at 4 °C. Afterward, the membranes were incubated with a secondary antibody for 30 min at 37 °C. Following three washes with TBST, the membranes were visualized using a chemiluminescence imager.

Cell proliferation assay

TOV21G and ES-2 cells (2×10^3 cells per well) were seeded 24 h prior to the assay and transfected with the targeted siRNA or plasmid. Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) kit (FDBio) following the manufacturer's instructions. All experiments were performed in triplicate, and proliferation curves were generated based on absorbance measurements at each time point.

The formula for calculating cell doubling time (DT) using the CCK-8 assay is as follows:

$$DT = \frac{T?\ln(2)}{\ln\left(\frac{OD_t}{OD_0}\right)}$$

DT: Cell doubling time; **T**: Time interval (in hours).
OD_0: OD value at the initial time point; **OD_t**: OD value at time T.

Ln(2) A constant, approximately 0.693.

Colony formation assay

Forty-eight hours after transfection, cells were digested with trypsin to obtain single-cell suspensions. For the colony formation assay, 1,000 cells were seeded into 6-well plates and incubated at 37 °C in medium supplemented with 10% FBS. After 10 days, the cells were fixed and stained with 0.1% crystal violet, and visible colonies were manually counted. Three replicate wells were analyzed for each treatment group.

Table 1 Expression of TRPS1 in ovarian clear cell carcinoma and normal ovarian tissue

Protein expression/*n*	OCCC	Normal ovaries.
(-)	23/27(85.2%)	21/25(84%)
(+)	4/27(14.8%)	4/25(16%)
(++)	10/23(43.5%)	
(+++)	13/23(56.5%)	

Transwell assay

Cell migration and invasion assays were performed using Transwell chambers (8.0 μm, Corning) in 24-well plates. For migration assays, the top chamber was seeded with 2×10^4 cells in 200 μL of serum-free medium, while the bottom chamber contained medium supplemented with 10% FBS. Cells were incubated at 37 °C for 48 h. After incubation, non-migrated cells on the upper surface were removed by scraping and washing, and cells on the lower surface were fixed with 4% methanol and stained with 0.1% crystal violet. Migrated cells were counted in five randomly selected fields under a microscope.

Invasion assays were conducted similarly, with the upper chamber coated with matrix gel (1:7, Corning). All experiments were performed independently in triplicate.

Statistical analysis

Statistical analyses were performed using SPSS 26.0 software (IBM Corp). Data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Differences in TRPS1 protein levels between ovarian clear cell carcinoma tissues and normal ovarian tissues were analyzed using the Student's t-test. Comparisons among multiple groups were conducted using one-way ANOVA followed by a post hoc least significant difference (LSD) test. GraphPad Prism 8.0 software was used for group comparisons and data visualization. The correlation between TRPS1 expression and clinicopathological parameters in OCCC patients was assessed using the chi-square test. Statistical significance was set at $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$).

Results

Association between TRPS1 expression and clinicopathological parameters in clear cell ovarian carcinoma

To explore the role of TRPS1 in OCCC, we analyzed its protein expression in OCCC tissues using immunohistochemistry. TRPS1 was detected with varying nuclear intensity in OCCC cells in 27 of 50 cases (54.0%). In contrast, as shown in Table 1, TRPS1 expression was minimal or low in normal ovarian tissues, observed in only 4 of 25 cases (16.0%). Notably, TRPS1 expression was significantly higher in cancer tissues compared to normal tissues ($p < 0.05$, Fig. 1).

To examine the correlation between TRPS1 expression and clinicopathological features in OCCC, we divided 50 cases into two groups based on immunohistochemical TRPS1 staining scores: the high-expression group (TRPS1 score ≥ 2) and the low-expression group (TRPS1 score < 2). As shown in Table 1, high TRPS1 expression was significantly associated with positive lymph node metastasis ($p = 0.027$). Furthermore, since OCCC patients with pathological stage II or above have poorer prognoses

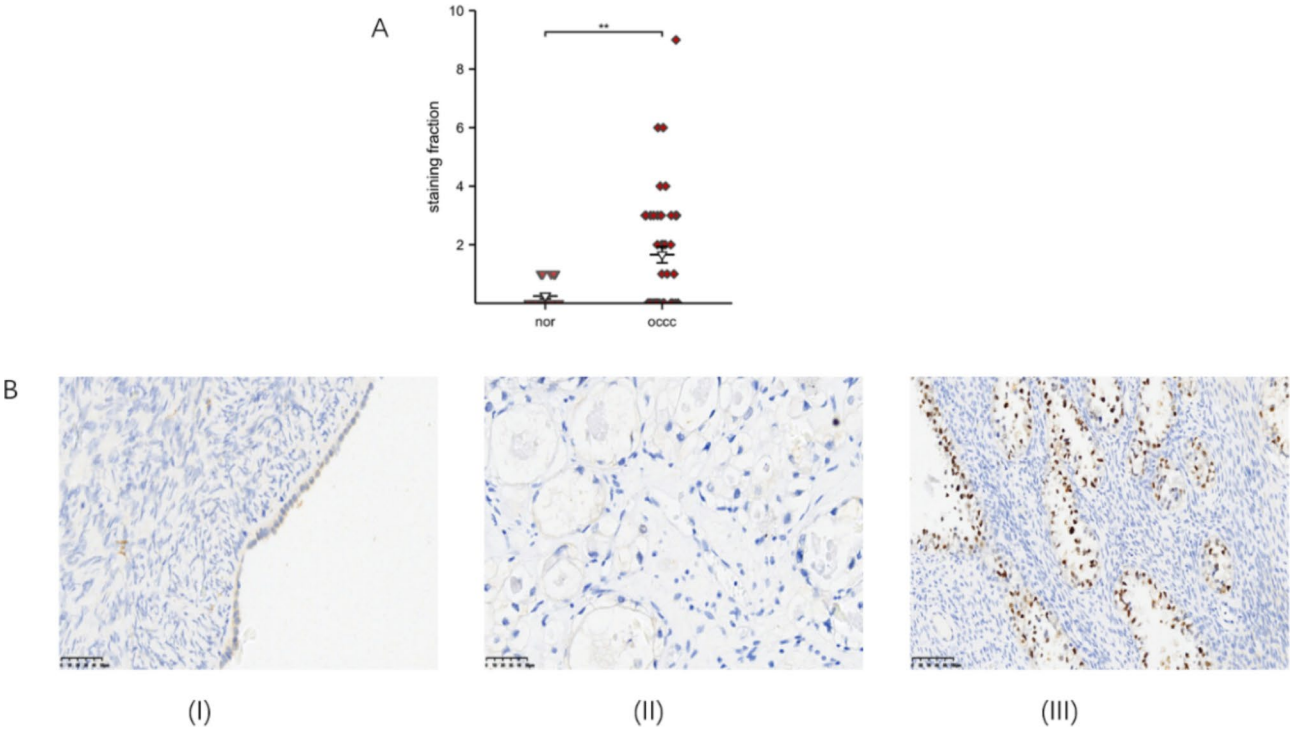

Fig. 1 TRPS1 was highly expressed in ovarian clear cell carcinoma tissues. **A**: TRPS1 expression was significantly higher in ovarian clear cell carcinoma tissues compared to normal ovarian tissues (1.66 ± 1.96 vs. 0.25 ± 0.44, $p = 0.0018$). **B**: Immunohistochemical analysis of TRPS1 expression in normal ovarian tissue and ovarian clear cell carcinoma (×400). (I) TRPS1 is minimally expressed in normal ovarian tissue; (II) TRPS1 shows a negative expression profile in OCCC; (III) TRPS1 exhibits a positive expression profile in OCCC

compared to those with stage I, we categorized patients into early-stage (stage I) and advanced-stage (stage II and above) groups. High TRPS1 expression was significantly correlated with advanced clinical stage ($p = 0.047$). However, no significant correlations were observed between TRPS1 expression and other clinicopathological features, including age, BMI, pathological grade, history of endometriosis, fertility, or family history ($p > 0.05$ for all), as shown in Table 2.

To evaluate the impact of TRPS1 expression on the prognosis of OCCC patients, we analyzed its correlation with overall survival (OS) and progression-free survival (PFS) using Kaplan-Meier survival curves. The analysis revealed that patients with high TRPS1 expression had significantly shorter overall survival compared to those with low TRPS1 expression ($P = 0.007$, Fig. 2A). Similarly, progression-free survival was also significantly shorter in patients with high TRPS1 expression ($P = 0.006$, Fig. 2B).

In the Overall Survival analysis of OCCC patients, TRPS1 was identified as a critical independent prognostic factor (Table 3). Univariate analysis revealed a significant association between TRPS1 expression and survival, with a hazard ratio (HR) of 4.234 (95% CI: 1.475–12.158, $p = 0.007$). Multivariate analysis further emphasized its importance, with an HR of 7.701 (95% CI: 1.686–35.185, $p = 0.008$). TRPS1 retained statistical significance even after adjusting for variables such as pathological stage and residual tumor, underscoring its central role in patient outcomes. Pathological stage and residual tumor also emerged as strong independent predictors, with advanced stages (III and IV) significantly associated with worse survival. Conversely, factors such as lymph node metastasis and family history did not reach statistical significance in multivariate analysis. Overall, high TRPS1 expression significantly increases mortality risk, positioning it as a potential molecular marker for predicting OCCC prognosis and informing clinical decisions.

Subsequently, Progression-Free Survival (PFS) analysis in ovarian clear cell carcinoma (OCCC) patients identified TRPS1 as a critical independent prognostic factor (Table 4). Univariate analysis revealed that high TRPS1 expression was significantly associated with reduced PFS (HR = 3.913, 95% CI: 1.490–10.274, $p = 0.006$). This association persisted in multivariate analysis, where TRPS1 remained a statistically significant predictor of poor PFS (HR = 3.815, 95% CI: 1.187–12.259, $p = 0.025$). Pathological stage also demonstrated a strong correlation with PFS, with stage IV patients having a markedly elevated risk (HR = 17.363, 95% CI: 1.955–154.202, $p = 0.010$) in multivariate analysis. While lymph node metastasis was significant in univariate analysis (HR = 3.216, 95% CI: 1.347–7.678, $p = 0.008$), it did not retain significance

Table 2 Correlation between TRPS1 expression and clinicopathologic features of patients with ovarian clear cell carcinoma

Characteristics	TRPS1-low	TRPS1-high	*P* value
n	27	23	
Age, mean ± sd	51.852 ± 10.365	51.043 ± 9.4363	0.776
BMI, median (IQR)	23.634 (22.049, 25.304)	21.514 (19.9, 25.381)	0.119
Pathologic.stage, n (%)			0.143
IV	2 (4%)	4 (8%)	
III	7 (14%)	7 (14%)	
II	1 (2%)	4 (8%)	
I	17 (34%)	8 (16%)	
Clinical stage, n (%)			0.047
I	17 (34%)	8 (16%)	
II-IV	10 (20%)	15 (30%)	
Residual tumor, n (%)			0.518
0	25 (50%)	19 (38%)	
1	2 (4%)	4 (8%)	
Lymph node metastasis, n (%)			0.027
0	22 (44%)	12 (24%)	
1	5 (10%)	11 (22%)	
GP, n (%)			1.000
0	3 (6%)	2 (4%)	
1	24 (48%)	21 (42%)	
Endometriosis, n (%)			1.000
0	23 (46%)	20 (40%)	
1	4 (8%)	3 (6%)	
Family history, n (%)			0.460
0	27 (54%)	22 (44%)	
1	0 (0%)	1 (2%)	

Notes: GP: Childbirth history. 1: at least 1 birth, 0: no births

Table 3 Univariate and multifactorial overall survival analysis for OCCC patients

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
TRPS1	4.234 (1.475–12.158)	**0.007**	7.701 (1.686–35.185)	**0.008**
Pathologic.stage				
I				
II	11.528 (2.123–62.595)	**0.005**	15.291 (1.933–120.958)	**0.010**
III	10.121 (2.540–40.336)	**0.001**	38.072 (2.535–571.893)	**0.008**
IV	7.803 (1.506–40.432)	**0.014**	25.331 (1.276–502.843)	**0.034**
Residual tumor	5.820 (1.787–18.958)	**0.003**	4.857 (1.127–20.933)	**0.034**
Lymph node metastasis	3.825 (1.451–10.085)	**0.007**	0.346 (0.045–2.690)	0.311
GP	0.826 (0.188–3.620)	0.800		
Endometriosis	0.826 (0.188–3.620)	0.800		
Family history	3.862 (0.498–29.936)	0.196		

in multivariate analysis ($p = 0.228$). These findings underscore that high TRPS1 expression and advanced pathological stage are the most significant factors affecting PFS in OCCC patients, suggesting TRPS1 as a valuable biomarker for prognosis and a potential target for therapeutic intervention.

Inhibition of TRPS1 hinders the proliferative capacity of OCCC cells

To determine the function of TRPS1, we analyzed its expression in OCCC cell lines (Fig. 3A-B). TOV21G cells displayed high levels of TRPS1 expression, whereas ES-2 cells expressed relatively lower levels. To manipulate TRPS1 expression, we designed two small interfering

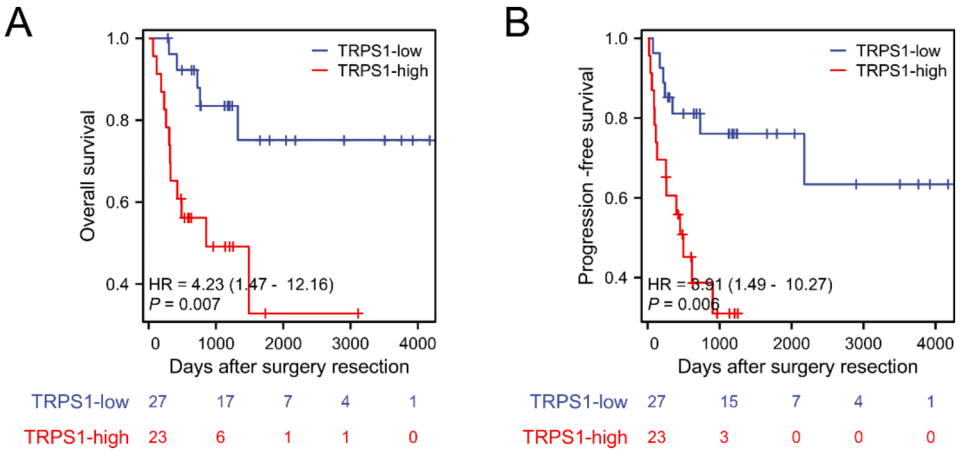

Fig. 2 Kaplan-Meier survival curves illustrating the impact of TRPS1 expression on the prognosis of OCCC patients. **A**: High TRPS1 expression is associated with shorter overall survival and worse prognosis. **B**: High TRPS1 expression is associated with shorter progression-free survival and worse prognosis

Table 4 Unifactorial and multifactorial analysis of progression-free survival of OCCC patients

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
TRPS1	3.913 (1.490–10.274)	**0.006**	3.815 (1.187–12.259)	**0.025**
Pathologic stage				
I				
II	4.862 (1.184–19.968)	**0.028**	2.784 (0.647–11.991)	0.169
III	4.236 (1.383–12.981)	**0.012**	9.568 (1.570–58.313)	**0.014**
IV	7.495 (2.235–25.137)	**0.001**	17.363 (1.955–154.202)	**0.010**
Residual tumor	2.635 (0.751–9.246)	0.130		
Lymph node metastasis	3.216 (1.347–7.678)	**0.008**	0.309 (0.046–2.087)	0.228
GP	0.794 (0.183–3.435)	0.758		
Endometriosis	0.554 (0.129–2.392)	0.429		
Family history	2.923 (0.384–22.253)	0.300		

RNAs (siRNAs) targeting the TRPS1 mRNA sequence, along with a control RNA. Additionally, a TRPS1 overexpression plasmid was constructed and transfected into ES-2 cells using Lipofectamine 2000. Western blot and RT-qPCR were used to evaluate the knockdown efficiency of siRNAs and the effect of overexpression. The results demonstrated that the TRPS1 overexpression plasmid significantly upregulated TRPS1 levels in ES-2 cells compared to the empty plasmid control (Fig. 3C-D). In contrast, siRNA-2 effectively reduced TRPS1 expression in TOV21G cells compared to siRNA-NC (Fig. 3E-F).

Immunohistochemical analysis indicates that TRPS1 is highly expressed in OCCC; however, its functional role in OCCC remains poorly understood. To explore the effects of dysregulated TRPS1 expression on cell proliferation, we employed the CCK-8 assay and plate colony formation assay. The doubling time of TOV21G cells, as determined using the CCK-8 assay, is 19 h, whereas that of ES-2 cells is 32 h. TRPS1 expression was modulated using transiently transfected plasmids and small interfering RNAs

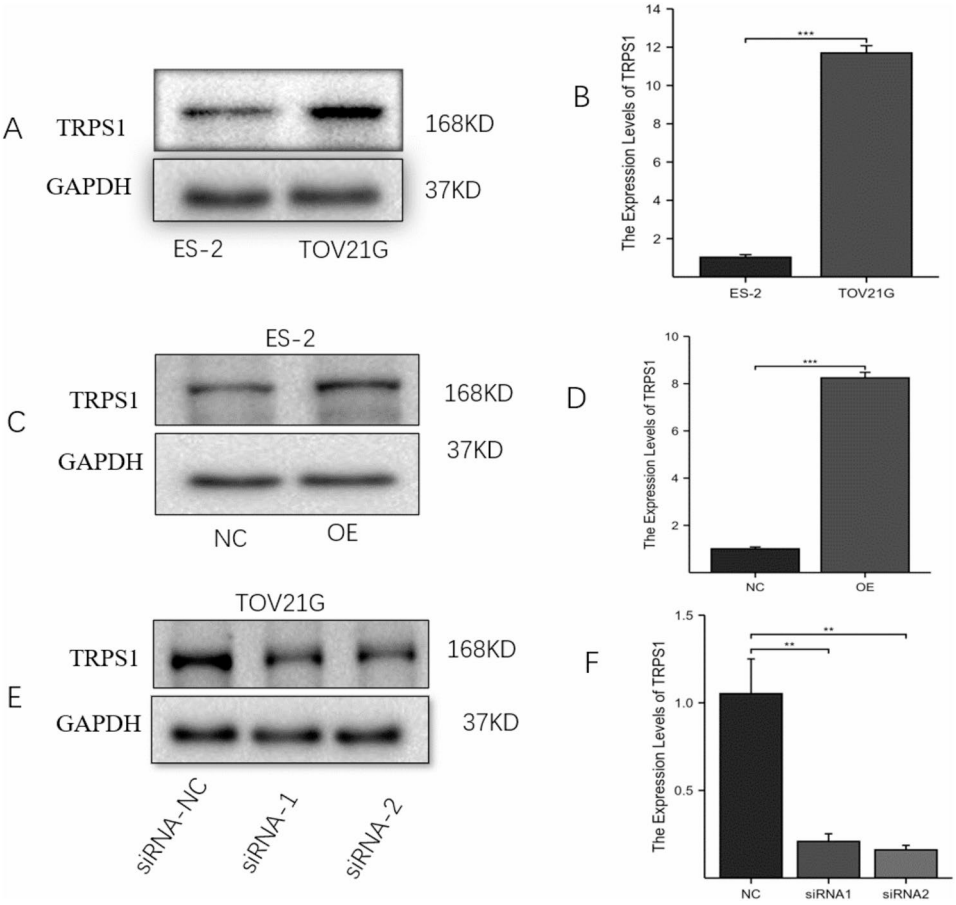

Fig. 3 TRPS1 expression in ovarian clear cell carcinoma cells. **A-B**: Western blot (WB) and real-time quantitative PCR (RT-qPCR) were used to measure TRPS1 expression in TOV21G and ES-2 cells. **C-D**: TRPS1 overexpression efficiency in ES-2 cells was confirmed using RT-qPCR and Western blot after transfection with the TRPS1 overexpression plasmid. **E-F**: Knockdown efficiency in TOV21G cells transfected with TRPS1-siRNA1 or TRPS1-siRNA2 was validated by RT-qPCR and Western blot. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

(siRNAs). The CCK-8 assay revealed that TRPS1 overexpression significantly enhanced the proliferation of ES-2 cells (Fig. 4A), while TRPS1 knockdown via two distinct siRNAs significantly suppressed TOV21G cell growth (Fig. 4C). Consistently, TRPS1 overexpression promoted colony formation in OCCC cells (Fig. 4B), whereas TRPS1 silencing substantially reduced their colony-forming ability (Fig. 4D).

TRPS1 facilitates the invasion and migration of OCCC cells

In our previous analysis of TRPS1's impact on prognosis, we found that high TRPS1 expression was associated with a higher likelihood of lymph node metastasis. To investigate whether TRPS1 also promotes invasion and migration in OCCC cells, we conducted transwell assays. The results showed that TRPS1 knockdown in TOV21G cells significantly reduced the number of invading cells compared to the control group (Fig. 5A), whereas TRPS1 upregulation in ES-2 cells enhanced their invasive ability (Fig. 5B). Similarly, TRPS1 downregulation decreased the migration ability of TOV21G cells (Fig. 5C), while TRPS1 overexpression increased the migration capacity of ES-2 cells (Fig. 5D).

Studies have demonstrated that the EMT-related pathway plays a critical role in tumor metastasis [28]. To determine whether TRPS1 promotes tumor cell metastasis, we analyzed its association with EMT-related molecules using western blot. The results showed that TRPS1 knockdown reduced the expression of invasion-promoting proteins, including N-cadherin, MMP2, and MMP9, while increasing the expression of the invasion-inhibiting protein E-cadherin. Conversely, TRPS1 overexpression increased the expression of invasion-promoting proteins (N-cadherin, MMP2, MMP9) and decreased E-cadherin expression (Fig. 6A-B). These findings suggest that TRPS1 may facilitate OCCC cell migration and invasion through the EMT pathway. Collectively, the in vitro and molecular results suggest that TRPS1 may promote OCCC cell migration and invasion.

Discussion

OCCC is a highly malignant subtype of epithelial ovarian cancer with a poor prognosis. Unlike HGSC, the most common epithelial ovarian cancer, OCCC is largely resistant to conventional platinum-based chemotherapy, with an effective response rate of only 11–56% compared to approximately 80% for HGSC [29]. Consequently, OCCC progresses rapidly after diagnosis and is characterized by a high propensity for infiltration, metastasis, and recurrence. Therefore, identifying biomarkers for early diagnosis, therapeutic intervention, and prognosis, as well as unraveling the molecular mechanisms underlying OCCC development and metastasis, is crucial.

TRPS1, a non-specific transcriptional repressor of the GATA family, plays a critical role in tumorigenesis and progression across various cancers. Radvanyi et al. reported TRPS1 expression in over 90% of early and advanced breast cancers, as determined by immunohistochemical analysis [19]. Chen et al. further demonstrated significant correlations between TRPS1 and ER/PR/

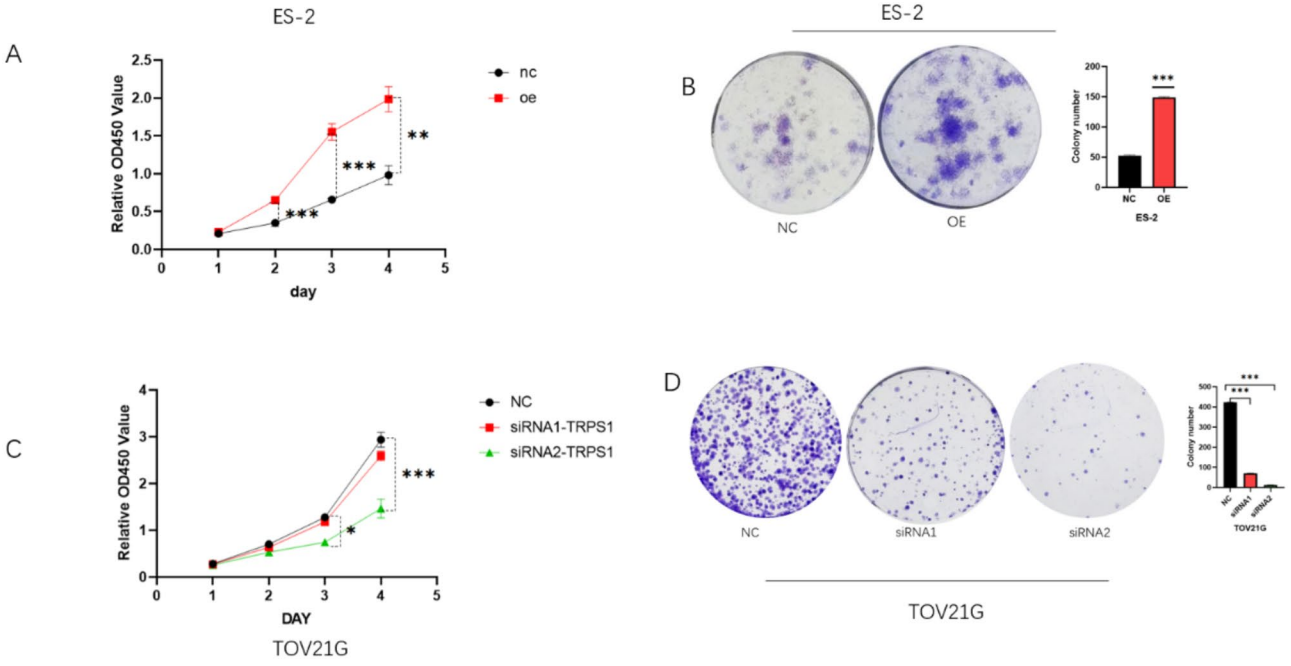

Fig. 4 TRPS1 promotes cell proliferation. **A-C**: CCK-8 assay was used to assess the effect of TRPS1 on cell proliferation. **B-D**: Plate colony formation assay was conducted to evaluate the impact of TRPS1 on the colony-forming ability of OCCC cells

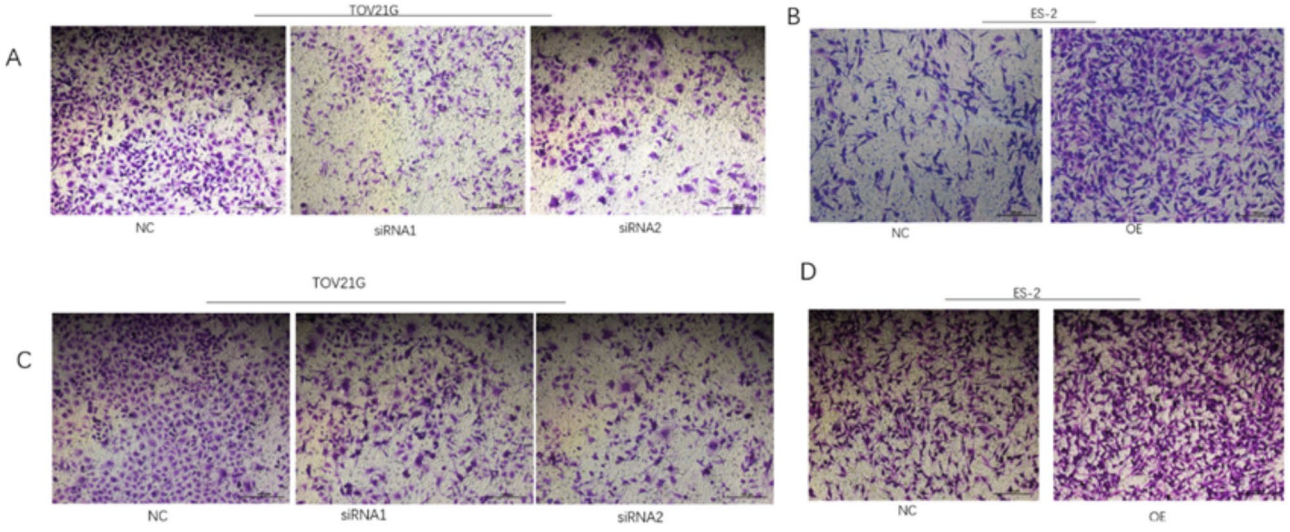

Fig. 5 TRPS1 enhances cell invasion and migration in vitro. **A**: Transwell assay confirmed the reduced invasive ability of TOV21G cells following TRPS1 knockdown. **B**: Transwell assay confirmed the increased invasive ability of ES-2 cells after TRPS1 overexpression. **C**: Knockdown of TRPS1 impaired the migratory ability of TOV21G cells. **D**: Overexpression of TRPS1 enhanced the migratory ability of ES-2 cells

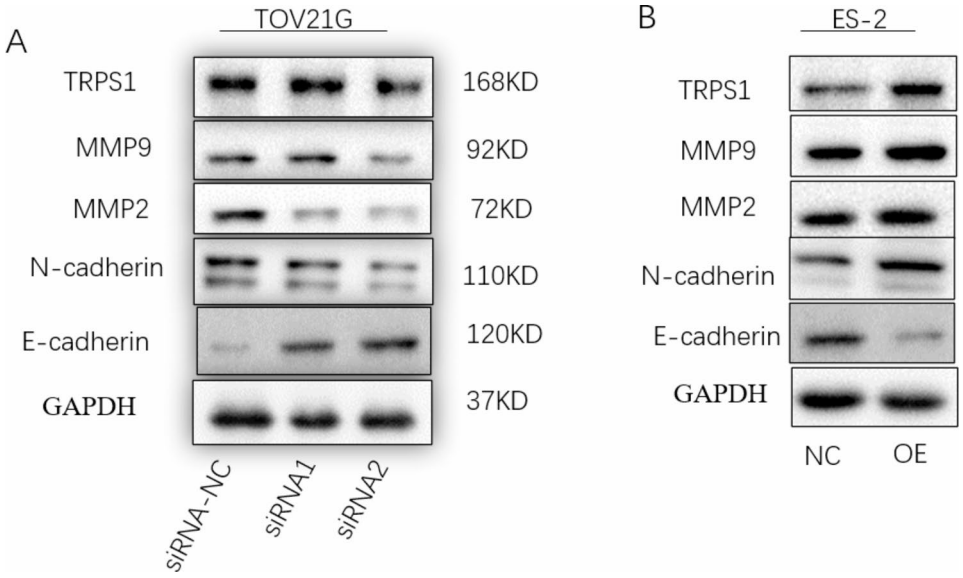

Fig. 6 TRPS1 promotes cell invasion and migration. **A-B**: Western blot analysis was performed to assess changes in the expression levels of N-cadherin, E-cadherin, MMP2, and MMP9 after modulation of TRPS1 expression, with GAPDH used as an internal control

GATA-3/HER2 expression, as well as clinical and pathological characteristics. Notably, high TRPS1 expression was associated with improved OS and disease-free survival (DFS) in stage I/II ER-positive breast cancer patients receiving endocrine therapy, establishing TRPS1 as an independent prognostic marker [30]. The data suggest that TRPS-1 serves as an independent prognostic marker for early breast cancer. In addition, it can differentiate patients with ER + breast cancer who have a prolonged response to adjuvant endocrine therapy [31]. In colorectal cancer, TRPS1 expression is significantly higher in tumor tissues compared to normal tissues ($P < 0.001$) and correlates with positive lymph node status and advanced pathological stage, suggesting its involvement in disease progression and its potential as a prognostic indicator [24]. Similarly, TRPS1 contributes to tumor angiogenesis and metastasis in osteosarcoma, making it a promising target for anti-angiogenic therapy [32]. Moreover, in hepatocellular carcinoma (HCC), TRPS1 overexpression is associated with vascular invasion and TNM staging, and its inhibition reduces cell invasion and proliferation, highlighting its therapeutic potential [26]. In conclusion, TRPS1 functions differently in different cancers and is strongly associated with prognosis. However, the

role of TRPS1 in the development of OCCC is not well understood.

Our findings suggest that TRPS1 indirectly influences the EMT pathway by modulating the expression of proteins associated with cell invasion and adhesion. Specifically, TRPS1 knockdown leads to a decrease in invasion-promoting proteins, such as N-cadherin, MMP2, and MMP9, alongside an increase in the invasion-inhibitory protein E-cadherin. In breast cancer, TRPS1 is positively correlated with the epithelial marker E-cadherin, stabilizing the epithelial phenotype [31]. Mechanistically, TRPS1 directly inhibits SUZ12 transcription, thereby maintaining stable E-cadherin expression and impeding EMT progression [33]. Additionally, TRPS1 suppresses EMT by binding to the ZEB2 promoter and repressing its expression, further reinforcing its role as an EMT inhibitor [20]. Research by Rangel et al. identified TRPS1 using the Sleeping Beauty transposon mutagenesis assay, demonstrating that TRPS1 inhibits lung metastasis by reducing the expression of EMT pathway genes and several serine protease inhibitors, including SERPINE1 and SERPINB2 [22]. However, TRPS1 exhibits a dual role in EMT regulation under specific contexts. For instance, Cornelissen et al. found that TRPS1 knockdown activates interferon signaling pathways, increasing STAT1 and STAT2 expression. This activation impedes mammary epithelial differentiation and promotes tumor formation induced by E-cadherin loss, suggesting a complex and context-dependent regulatory mechanism [34]. Furthermore, FOXA1, a negative regulator of EMT, is downregulated by TRPS1 knockdown. TRPS1 functions as a transcriptional activator by binding to the FOXA1 promoter and inducing its expression, thereby inhibiting tumor metastasis and stabilizing the epithelial phenotype [21]. In physiological contexts, Gai et al. found that TRPS1 deletion promotes TGF-β1-induced EMT and enhances renal fibrosis by downregulating Smad7 expression through Arkadia-mediated ubiquitination. These findings highlight TRPS1's multifaceted role in regulating EMT and its dual functionality in different cellular and pathological environments [35]. Consequently, TRPS1 emerges as a critical and nuanced player in EMT regulation, with its actions deeply intertwined with tumor progression, metastasis, and fibrosis. Its complex dual roles underscore the need for continued investigation to harness its therapeutic potential effectively.

Conclusions

In conclusion, our findings indicate that the upregulation of TRPS1 may contribute to the pathogenesis and progression of OCCC and holds potential as a prognostic biomarker for this malignancy. These results not only deepen our understanding of the molecular mechanisms underlying TRPS1's role in OCCC but also identify TRPS1 as a promising therapeutic target for the development of novel treatment strategies.

Supplementary Information

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Supplementary Material 1

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Author contributions

Conceptualization, Aijun Yu; Data organization, Jingfang Liu, Beier Wu, Shihan Wan; Formal analysis, Li Yang; Funding acquisition, Aijun Yu; Methodology, Yanlu Jin，Li Yang; Project management, Aijun Yu; Resources, Meijuan Wu and Aijun Yu; Software, Yanlu Jin; Supervision, Aijun Yu; Validation, Jie Xing and Jiejie Zhang; Visualization, Xin Chen; Writing the original version, Jingfang Liu.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

The study was approved by the Institutional Review Board of Zhejiang cancer hospital. Each patient signed a written informed consent in the study before surgery (IRB-2021-350). The study was performed in accordance with the Declaration of Helsinki. This study is not a clinical trial. Clinical trial numbers do not apply.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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