Original Research

Tumor-Suppressor Gene Transmembrane Protein 98 Promotes Proliferation and Inhibits Apoptosis in Ovarian Cancer

San-Gang Wu[1],[†], Jing-Ying Xu[2],[†], Jian Lei[2],[†], Min Hu[2], Juan Zhou[2],*

[1]Department of Radiation Oncology, Xiamen Cancer Center, Xiamen Key Laboratory of Radiation Oncology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, 361003 Xiamen, Fujian, China
[2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, 361003 Xiamen, Fujian, China
*Correspondence: zhoujuan@xmu.edu.cn (Juan Zhou)
[†]These authors contributed equally.

Abstract

Background: Ovarian cancer (OC) is the most deadly tumor in gynecology and there is no effective biomarker for diagnosis and treatment. The role of Transmembrane Protein 98 (TMEM98) in ovarian cancer is still unclear. **Methods**: The expression and prognostic effect of TMEM98 in OC were analyzed using the public database. Cell Counting Kit-8 proliferation experiment, scratch experiment, Transwell invasion experiment, flow cytometry, TUNEL staining, and *in vivo and vitro experiment* were used. **Results**: TMEM98 was significantly downregulated in OC tissues and cell lines compared to the normal ovarian tissue and cells lines. In addition, patients with lower TMEM98 levels exhibited inferior survival. Low expression of the TMEM98 promoted proliferation, migration, invasion, vasculogenic mimicry, and inhibited apoptosis in OC cells. The expression of Caspase-3 was significantly downregulated and the expression of Bcl-2 was significantly increased in the silencing-TMEM98 group. Moreover, low expression of TMEM98 promotes OC development *in vivo*. Bioinformatics analysis showed that TMEM98 expression was negatively correlated with poly ADP-ribose polymerase expression. **Conclusions**: This study demonstrates that TMEM98 is low expressed in OC and impacts the prognosis of OC patients. TMEM98 inhibits proliferation and promotes apoptosis and finally exerts a certain tumor-suppressor effect on OC.

Keywords: TMEM98; ovarian cancer; tumor suppressor; prognosis

1. Introduction

Ovarian cancer (OC) is a gynecological tumor with the highest mortality rate among women in the world [1]. Early-stage OC has a favorable prognosis, while the 5-year survival rate of advanced-stage OC is only 20–30% [2]. Due to the insidious onset of OC, approximately 85% of the OC cases were diagnosed as advanced stage (stage III–IV disease) [3]. Therefore, OC has become a 'silent killer', threatening the life and health of women. Although effective treatment strategies have made progress in recent years, the survival rate has hardly improved [4]. In the current clinical practice, CA125 and HE4 are used to diagnose OC. However, the sensitivity of CA125 and HE in the diagnosis of early-stage OC is lower and the above markers can be also increased in some conditions [5]. Therefore, it is critical to investigate more specific biomarkers to improve the diagnostic accuracy and therapeutic efficacy for OC.

Human transmembrane proteins (TMEMs) are widely expressed in the membrane structure of human tissues and organs [6,7]. Moreover, TMEMs are involved in regulating several physiological processes, including apoptosis, autophagy, adhesion, ion transmembrane transport, mediating cell chemotaxis, and signal transduction [8–10]. However, few studies are reported regarding TMEM98, which has the highest RNA expression in the human normal ovary compared to other normal tissues or organs [11,12].

In the previous study, TMEM98 can promote endothelial cell adhesion by inducing the expression of ICAM-1/VCAM-1 and trigger vascular smooth muscle cell proliferation and migration through activation of ERK and AKT/GSK3β signaling pathways [13]. What's more, TMEM98 is able to reduce the protein level of FRAT2 and inhibit FRAT2-mediated induction of CTNNB1/TCF signaling, acting as a negative regulator of FRAT-mediated Wnt/β-catenin signaling [14]. It's also found that TMEM98 can promote the secretion of IFN-γ and inhibit inflammation [15].

In this study, we investigated the role of TMEM98 in regulating OC development and sought to identify its potential biological function.

2. Materials and Methods

2.1 Bioinformatics Analysis

The clinical sample data sets of OC were downloaded from The Cancer Genome Atlas (*TCGA*), GEO and GTEx database to analyze TMEM98 mRNA expression and RNA SEQ data. GEPIA website (http://gepia.cancer-pku.cn/) was used to analyze the difference of TMEM98 mRNA ex-

pression between OC and normal tissues in the TCGA and GTEx. TNMplot database (https://tnmplot.com/analysis/) was used to analyze the difference of TMEM98 mRNA expression between tumor and normal tissue in various tumors. TCGA database was used to analyze the expression of TMEM98 and clinical stage of OC patients. KM-plotter (http://kmplot.com/analysis/) was used to analyze the relationship between TMEM98 and progression-free survival (PFS). GSEA software was used for the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis to explore the biological function of TMEM98 in the progression of OC. The correlation between TMEM98 and other genes was analyzed online by the cbioportal website (https://www.cbioportal.org/).

2.2 Cell Culture

Two human cell lines, OC SKOV3 cells and ovarian epithelial IOSE80 cells were obtained from American Type Culture Collection (ATCC, USA). Cells were cultured in DMEM with 10% fetal bovine serum (FBS, Cellmax) at 37 °C in a humidified incubator filled with 5% CO_2.

2.3 Cell Transfection

To establish cell models with low-expressing/over-expressing TMEM98, the siRNA/Plasmid targeting TMEM98 with chemical modification and 10ul negative control products were transfected into 30%–50% confluence SKOV3 and IOSE80 cells, which were cultured in the 6-well plate by reagent system in the light of the product specifications. After incubated for 48 h, the expression of TMEM98 was detected by western blot (WB) and then the relative experiments were performed to check the influence of TMEM98 on biological behaviors.

2.4 WB Assay

Total proteins extracted from all cells were resolved on SDS-PAGE gels. And then, proteins on the gel were transferred to PVDF membranes (Millipore, Billerica, MA, USA) which were blocked with 5% BSA in TBST for 1 hour and incubated with TMEM98 antibody (1:1000, Proteintech) or β-actin antibody (1:1000, Cell Signaling Technology) overnight at 4 °C. The membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature. Finally, the bands were observed by Enhanced Chemiluminescence (ECL) Kit (Millipore, Billerica, MA, USA).

2.5 Cell Growth Assay

Cell growth was detected via Cell Counting Kit-8 (CCK-8, Dalian Meilun, Dalian, China) assay. Transfected cells were seeded into 96-well plates at a density of 3000 cells per well. CCK-8 experiment was performed according to the manufacturer's instructions. OD values were determined after 24, 48, 72, and 96 hours to evaluate cell growth. After adding 10 μL of CCK-8 to each well and incubating

for another 2 hours at 37 °C, absorbance at 490 nm was obtained by a microplate reader (BioTek Instruments, Inc). The cell viability rate was calculated as a percentage of control (the NC group).

Cell Cycle and Apoptosis Analysis Kit (Yeasen, Shanghai, China) was used for analyzing the cell cycle. Transfected cells were harvested and fixed with 70% ethanol overnight at 4 °C. Then the cells were treated with RNase A and stained with propidium iodide for 30 minutes at 37 °C. Flow cytometry was used to calculate the percentages of cells in the G0/G1, S, and G2/M phases to analyze the distribution of the cell cycle.

2.6 Cell Apoptosis Assay

Annexin-VFITC Apoptosis Detection Kit I (BD Biosciences) was used for analyzing apoptosis. Cells were resuspended by cold PBS at a density of 1×10^6 cells/mL, and incubated with PI and Annexin-VFITC staining, followed by incubation at room temperature for 15 minutes. Flow cytometry was used to analyze apoptosis.

2.7 Wound Healing Assay

Transfected cells were seeded into six-well plates with 10% FBS. When 80% of the well was covered with cells, a 100 μL pipette tip was used to scrape the cells. Defoliated cells were removed by washing three times with PBS and serum-free culture medium was added. As a result, wounds were generated. Then, cells were incubated at 37 °C. After 24 hours, the migration distance of cells was determined. The width ratio was calculated by the wound width/the distance measured at 0 hours.

2.8 Xenograft Mice Model

Female BALB/c nude mice aged 4 to 8 weeks old were used. Mice were divided into three groups and subcutaneously injected with silencing-TMEM98 (si-TMEM98) or overexpression-TMEM98 (oe-TMEM98) SKOV3 cells, respectively.

2.9 Immunohistochemistry

UltrasensitiveTM SP (Mouse/Rabbit) IHC Kit (Maixin Biotechnology Co., Fuzhou, China) was used to perform immunohistochemistry. The tumor tissue was made into 5 μm frozen sections. After adding peroxidase blocker and non-specific staining blocker sequentially, the sections were incubated with the primary antibodies (Caspase-3: 1:1000, Cell Signaling Technology; Bcl-2: 1:800, Cell Signaling Technology) at 4°C overnight and then incubated with biotin-labeled goat anti-mouse/rabbit IgG at room temperature for 10 minutes. Streptomyces anti-biotin protein-peroxidase was added into the sections and incubated for 10 minutes. The neutral resin was used to seal the sections after DAB and hematoxylin staining and the microscope was used to observe.
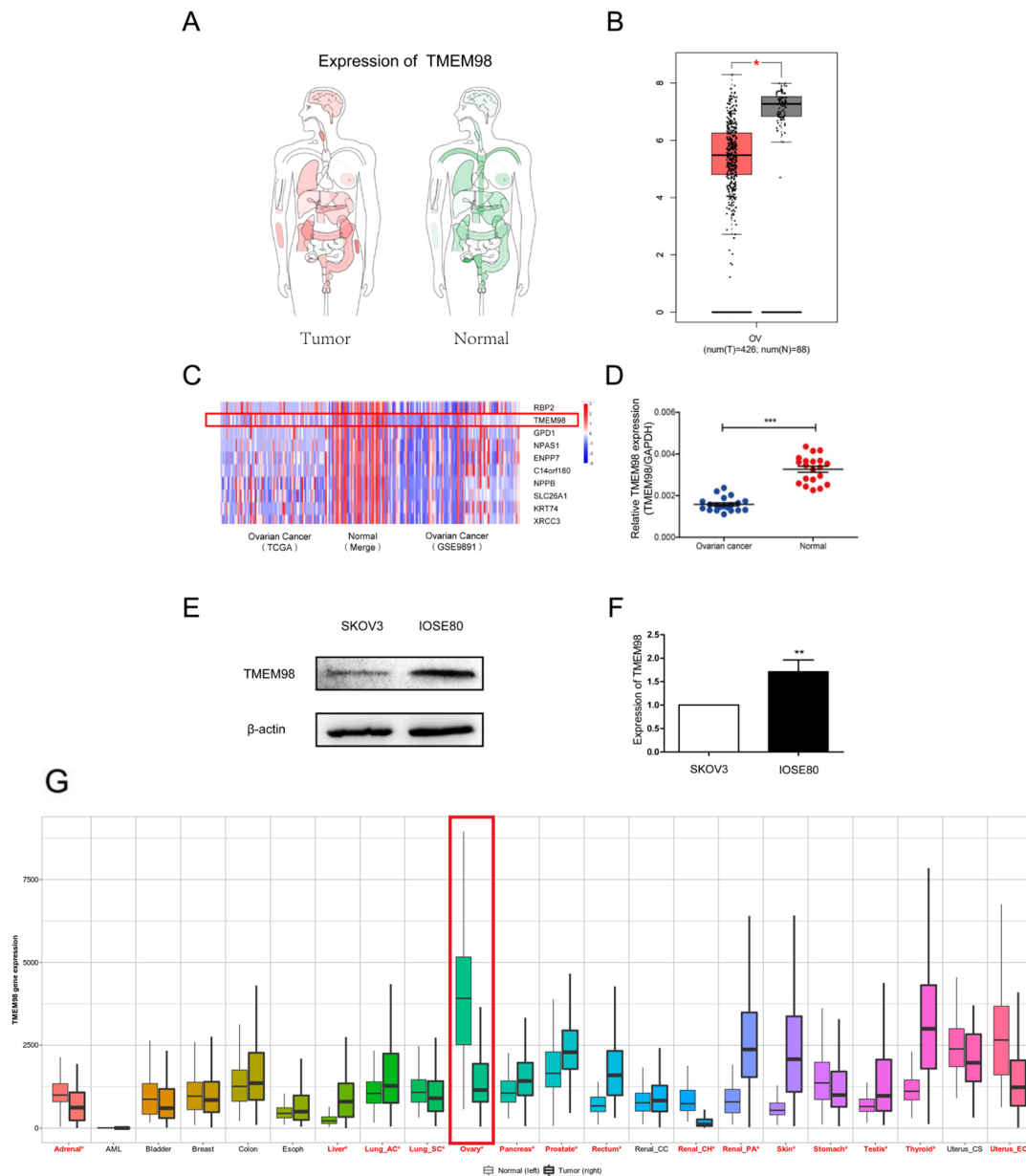
Fig. 1. The expression of TMEM98 in OC and normal ovary. (A,B) The expression of TMEM98 between OC tissue and normal ovarian tissue via the GEPIA online website (*$p < 0.05$). (C,D) Download the clinical sample data of high-grade serous OC from the TCGA and GSE9891 database and analysis via the GEPIA online website (***$p < 0.001$). (E,F) The expression of TMEM98 between SKOV3 and IOSE80 cells. (n = 3, **$p < 0.01$). (G) The expression of TMEM98 mRNA in various cancers and normal tissues.

2.10 Statistical Analysis

Data are presented as mean ± SEM. All experiments were performed at least three times. The chi-square test was used to analyze differences in categorical variables. One-way ANOVA, Students t-tests and Mann-Whitney-U tests were used to compare the different groups of data. Data were assessed using the software GraphPad Prism 5.0 (GraphPad, La Jolla, CA, USA) and SPSS statistical software, version 26.0 (IBM Corp, Armonk, NY, USA). $p < 0.05$ was regarded as a statistically significant difference.

3. Results

3.1 TMEM98 is Lowly Expressed in OC

By analyzing the data from TCGA, GSE9891, and GTEX databases, the TMEM98 gene has a higher expression in the normal ovary tissue compared to other human organs ($p < 0.05$) (Fig. 1A). In addition, the expression of TMEM98 was significantly higher in normal ovary tissue than in the OC tissues ($p < 0.001$) (Fig. 1B–D). These results showed that TMEM98 was a higher expression in normal ovarian tissue. Moreover, we also found that the
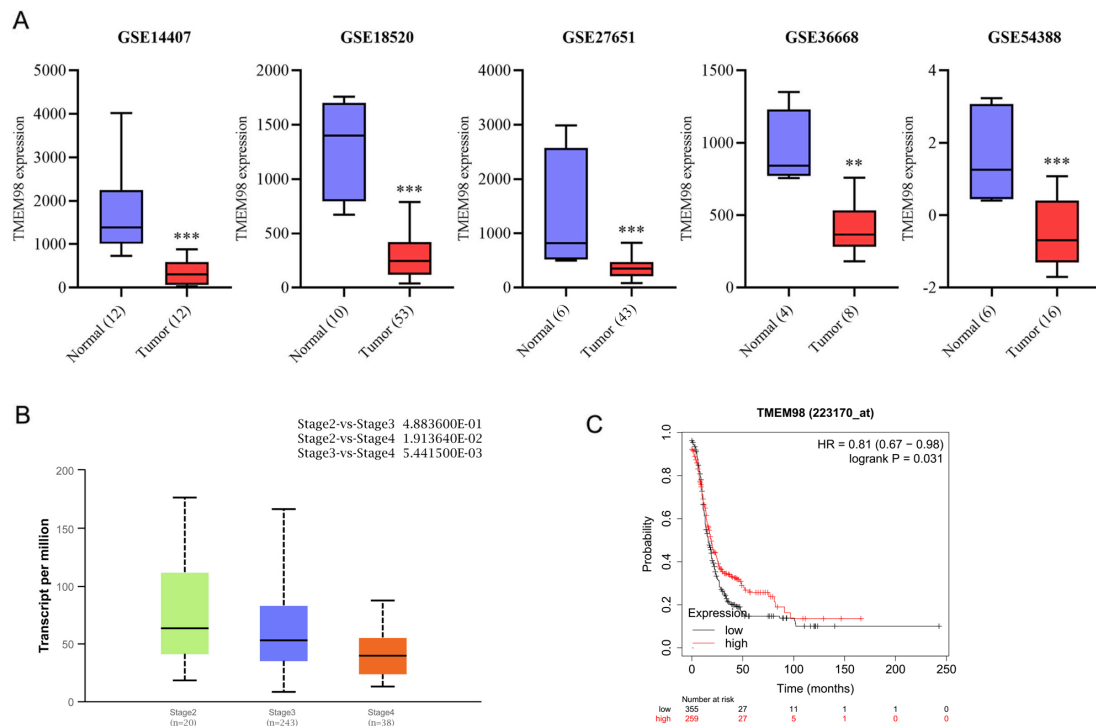
Fig. 2. Association of TMEM98 expression with clinicopathological features and survival. (A) Validation of TMEM98 expression levels in OC tissue compared with normal tissue from GEO datasets (**$p < 0.01$, ***$p < 0.001$). (B) TMEM98 was negatively correlated with the clinical stage of OC using the data from the TCGA database. (C) Kaplan-Meier plots of OC patients stratified by TMEM98 mRNA levels.

expression of TMEM98 in human normal ovarian epithelial cell lines (IOSE80) was significantly higher than that in human OC cells (SKOV3) (Fig. 1E,F) ($p < 0.01$). We found that the expression level of TMEM98 in normal ovarian tissue was the highest in 22 human normal tissues in the TNMplot database (Fig. 1G). And the data from the GEO database also showed that the expression level of TMEM98 in ovarian tissue was higher than in normal tissue (Fig. 2A), the details of the samples were shown in the **Supplementary Table 1**. It's indicated that TMEM98 may play a potential tumor-suppressing role in OC development. But the biological functions are required for further verification.

3.2 TMEM98 is Negatively Correlated with the Clinical Stage and Grade of OC

In the TCGA database, 301 clinical samples of different stages of OC were downloaded, including 20, 243, and 38 patients who had stage II, III, and IV diseases, respectively. The expression of TMEM98 according to the clinical stages was analyzed online using the Ualcan website (http://ualcan.path.uab.edu/). The results showed that TMEM98 expression was negatively correlated with the clinical stage. The expression of TMEM98 decreased as the clinical stages progressed (Fig. 2B). The expression of TMEM98 was significantly lower in stage III ($p < 0.001$) and stage IV ($p < 0.001$) diseases compared to stage II disease (Fig. 2B). Meanwhile, the patient clinical charac-

teristic parameters have listed in Table 1. The levels of TMEM98 expression were significantly related to grade ($p = 0.035$). Patients with higher tumor grade had lower expression of TEME98 level. These results suggested that the TMEM98 gene might inhibit the development of OC.

Table 1. The correlation between clinical features and the expression of TMEM98 in ovarian cancer.

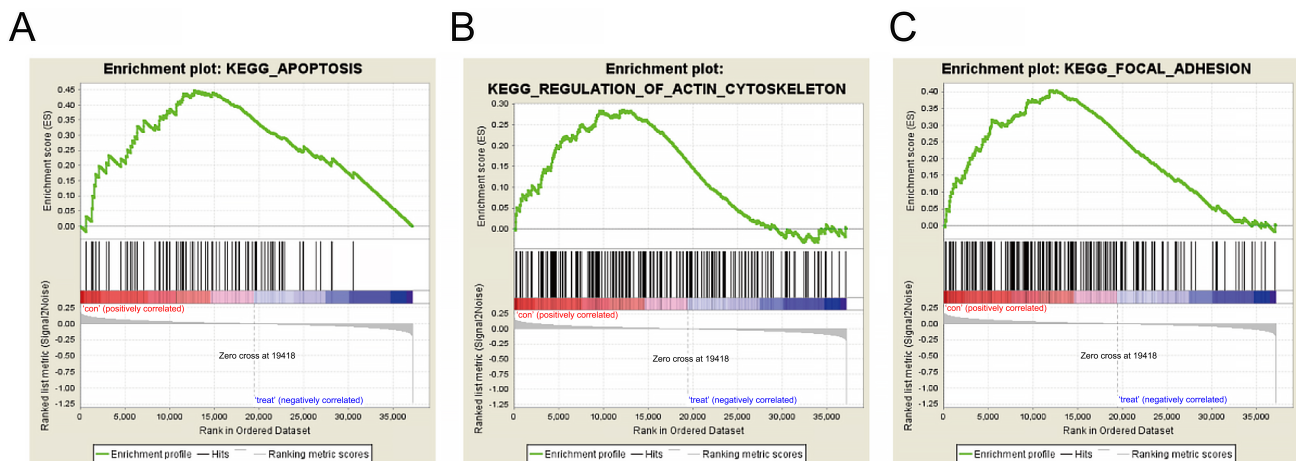| Variables	n	TEME98 expression		p
		Low (%)	High (%)	
Age (n = 376)				
<65 years	245	127 (67.6)	118 (62.8)	0.330
≥65 years	131	61 (32.4)	70 (37.2)	
Stage (n = 373)				
I–II	23	12 (6.4)	11 (5.9)	0.840
III–IV	350	175 (93.6)	175 (94.1)	
Grade (n = 366)				
1–2	43	15 (8.2)	28 (15.3)	0.035
3–4	323	168 (91.8)	155 (84.7)	
Lymphatic invasion (n = 148)				
No	48	23 (30.3)	25 (34.7)	0.562
Yes	100	53 (69.7)	47 (65.3)	
Vascular_invasion (n = 103)				
No	40	17 (34.7)	23 (42.6)	0.411
Yes	63	32 (65.3)	31 (57.4)	

Fig. 3. KEGG analysis of TMEM98 gene-related biological function. (A) Enrichment plots of gene expression signatures for apoptosis according to TMEM98 expression levels. (B) Enrichment plots of gene expression signatures for cytoskeleton according to TMEM98 expression levels. (C) Enrichment plots of gene expression signatures for cell adhesion according to TMEM98 expression levels.

3.3 An Increased Expression of TMEM98 Prolongs PFS

Since TMEM98 is correlated with the clinical stage of OC, the relationship between TMEM98 and PFS was further explored using the KM-plotter from the TCGA database. The results showed that PFS was significantly longer in patients with high mRNA levels of TMEM98 compared to those with low mRNA levels of TMEM98 ($p = 0.031$). The finding suggested that TMEM98 had a prognostic effect on OC (Fig. 2C).

3.4 Establish Transient OC Cell Models with Knockdown and Overexpression TMEM98

To further explore the effect of TMEM98 on the malignant behavior of OC, SKOV3 and IOSE80 cell lines were selected for comparison experiments. In this experiment, small interfering RNA (siRNA) was used to knock down the TMEM98 gene and TMEM98 expression level was detected using WB. The results showed that compared with the normal control (NC) group, after siRNA-TMEM98 knockdown in SKOV3 cells and IOSE80 cells, the TMEM98 protein expression in the si-TMEM98 group was significantly reduced in SKOV3 cells and IOSE80 cells (**Supplementary Fig. 1A**, **$**p < 0.01$; **B**, **$***p < 0.001$). We also used the plasmid to overexpress TMEM98 for subsequent experiments (**Supplementary Fig. 1C & D**, **$**p < 0.01$).

3.5 KEGG Analysis of TMEM98 Gene-Related Biological Function

We downloaded the RNA SEQ data of OC clinical samples from the TCGA database. According to the expression of TMEM98, we used GESA software for KEGG enrichment analysis. The results showed that TMEM98 was closely related to apoptosis, cytoskeleton, and cell adhesion (Fig. 3).

3.6 Low Expression of the TMEM98 Promotes Proliferation, Migration, and Invasion of OC Cells

SKOV3 cells and IOSE80 cells were treated with siRNA-TMEM98 and TMEM98 overexpression plasmid respectively. After the cell silencing and overexpression treatment, cell viability was detected. The cell viability of the si-TMEM98 group was compared with the NC group, and that of the oe-TMEM98 group was compared with the NC group. The results showed that compared with the NC group, the cell viability in the si-TMEM98 group was increased (Fig. 4A,B), while the cell viability was inhibited in the oe-TMEM98 group (Fig. 4C,D).

Moreover, cell healing was observed and the mobility of each group was calculated to investigate the ability of cell migration between si-TMEM98 and oe-TMEM98 groups. The results showed that the migration rate of SKOV3 cells in the si-TMEM98 group was significantly higher than that in the NC group and the ability of cell migration was significantly inhibited in the oe-TMEM98 group compared to the NC group (Fig. 5A,B,E,F). Interestingly, although cell migration ability is a malignant behavior of tumor cells, the cell migration ability of human normal ovarian cell IOSE80 was similar to those of OC cells SKOV3. Compared with the NC group, the cell migration rate of IOSE80 in the si-TMEM98 group was significantly higher and was significantly lower in the oe-TMEM98 group (Fig. 5C,D,G,H). These results indicated that TMEM98 knockdown could promote the development of OC and enhance the migration ability of OC. In addition, in normal ovarian cells, si-TMEM98 may promote the normal ovarian cell to have a trend of OC development, and after overexpression of the TMEM98 could further weaken this ability.

Furthermore, the Transwell chamber was used to determine the effect of TMEM98 on the invasion ability of SKOV3 cells. The results showed that compared with the NC group, the invasive ability of the si-TMEM98 group
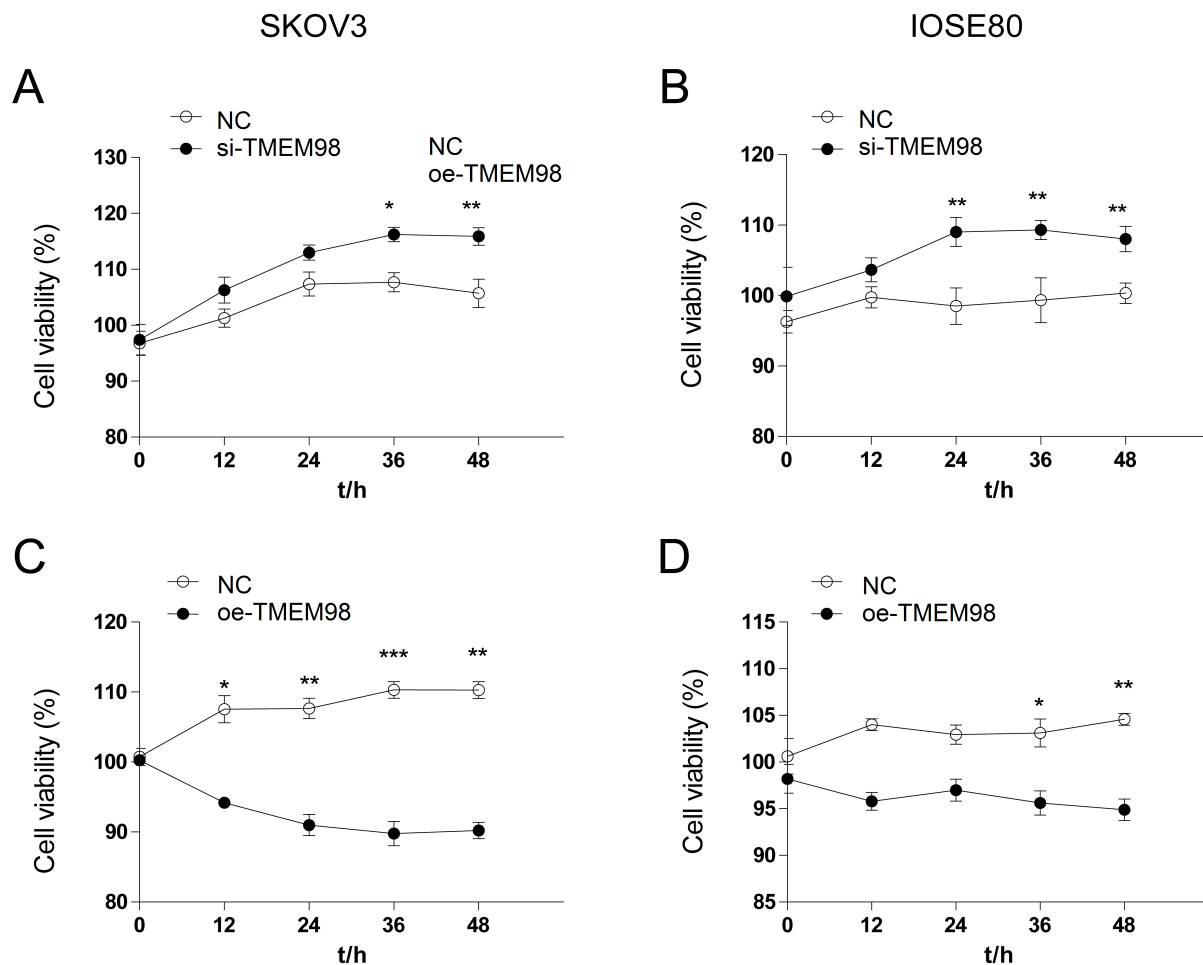
SKOV3   IOSE80

Fig. 4. Cell viability by down-regulation and up-regulation of TMEM98. (A) Compared with the NC group, the cell viability of SKOV3 cells in the si-TMEM98 group increased significantly. (B) Compared with the NC group, the cell viability of IOSE80 cells in the si-TMEM98 group increased significantly. (C) Compared with the NC group, the cell viability of SKOV3 cells in the oe-TMEM98 group decreased significantly. (D) Compared with the NC group, the cell viability of SKOV3 cells in the oe-TMEM98 group decreased significantly (n = 3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

was significantly increased, while the invasive ability of the oe-TMEM98 group was significantly inhibited (Fig. 6A,B). These results indicate that knockdown of TMEM98 promotes the invasion ability of SKOV3 cells.

3.7 Low Expression of the TMEM98 Promotes the Vasculogenic Mimicry in OC cells

In this study, the vascular mimicry model of OC was established by using a matrix glue simulation environment to investigate the effect of TMEM98 on the angiogenesis of OC cells. The results showed that compared to the NC group, the si-TMEM98 group had significantly increased vascular mimicry, and the cells were closely connected and had small microtubules, which could form a ring grid (Fig. 6C). Opposite results were found in the oe-TMEM98 group (Fig. 6D). The findings indicated that the low TMEM98 could significantly promote the angiogenesis of OC cells.

3.8 Low Expression of the TMEM98 Promotes Cell Cycle Progression

To further explore the effect of TMEM98 on the cell cycle. The effects of TMEM98 on the cell cycle of SKOV3 and IOSE80 were detected by flow cytometry. In the SKOV3 cells, the results showed that compared with the NC group, the proportion of G2 phase cells in the si-TMEM98 group was significantly increased (Fig. 7A), and the proportion of S phase and G2 phase cells in the oe-TMEM98 group were significantly decreased (Fig. 7C), which indicated that TMEM98 knockdown promoted the cycle progression. In the IOSE80 cells, compared with the NC group, the proportion of S phase and G2 phase cells in the si-TMEM98 group was significantly increased, which promoted the cycle progression (Fig. 7B). The proportion of S phase cells in the oe-TMEM98 group was significantly increased, and the proportion of G2 phase cells was significantly decreased (Fig. 7D), which indicated that over-
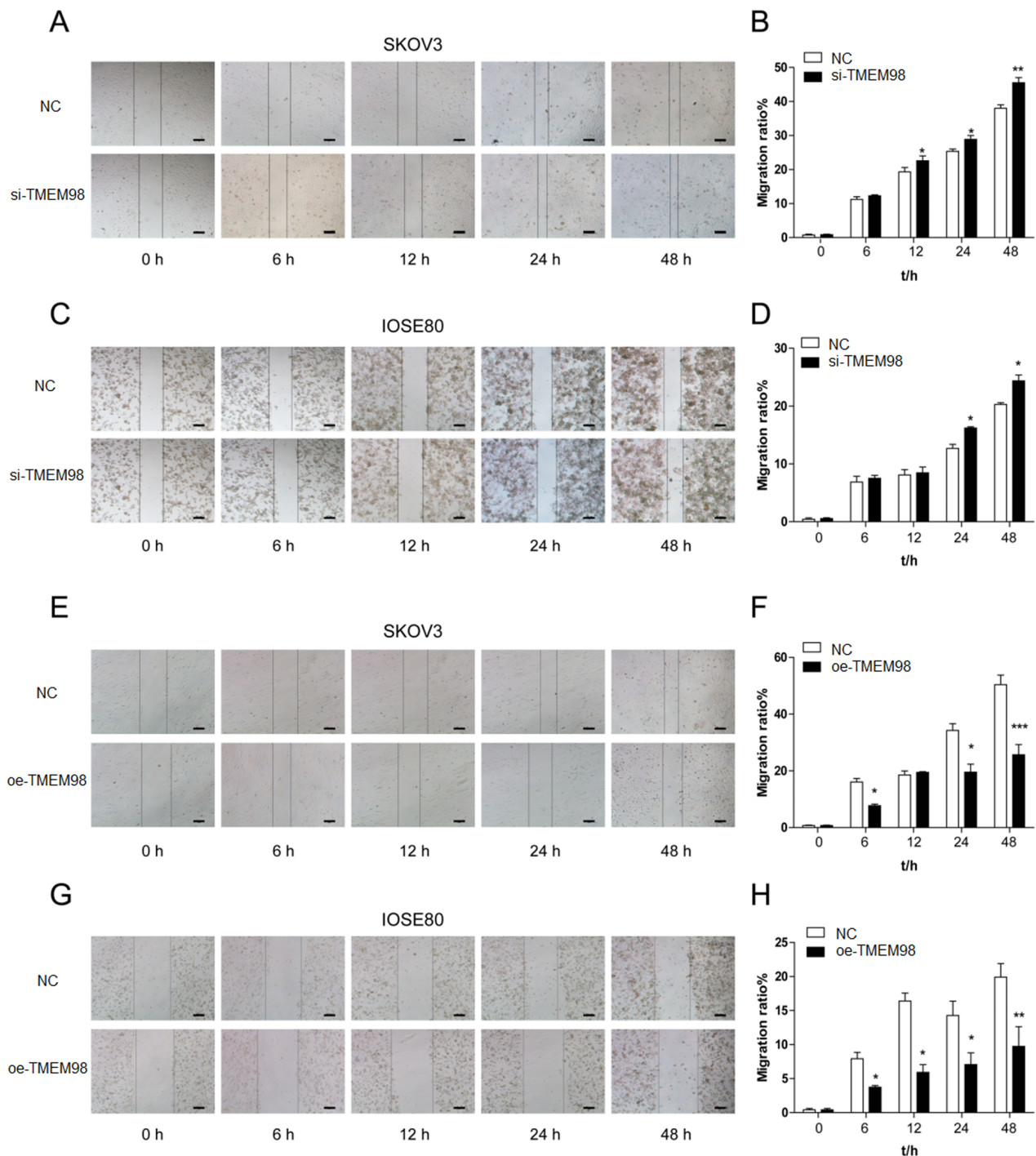
Fig. 5. Cell migration by down-regulation and up-regulation of TMEM98. (A,E) Test the migration ability of SKOV3 cells by scratch test. (B,F) Quantitative analysis of the migration rate of SKOV3 cells. (C,G) Test the migration ability of IOSE80 cells by scratch test. (D,H) Quantitative analysis of migration rate of IOSE80 cells (n = 3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

expression of TMEM98 blocked the progress of IOSE80 from the S phase to the G2 phase. The proliferation index was calculated by using the formula proliferation index and combined with the results of the cell proliferation test, the results showed that TMEM98 knockdown improved the proliferation activity of IOSE80 cells and promoted the cycle progression. Moreover, overexpression of

TMEM98 decreased the proliferative activity of SKOV3 cells and blocked the cycle progression (Fig. 7E).

3.9 Low Expression of TMEM98 Inhibits Apoptosis

The above results showed that TMEM98 could inhibit malignant proliferation and cell cycle progression, and en-
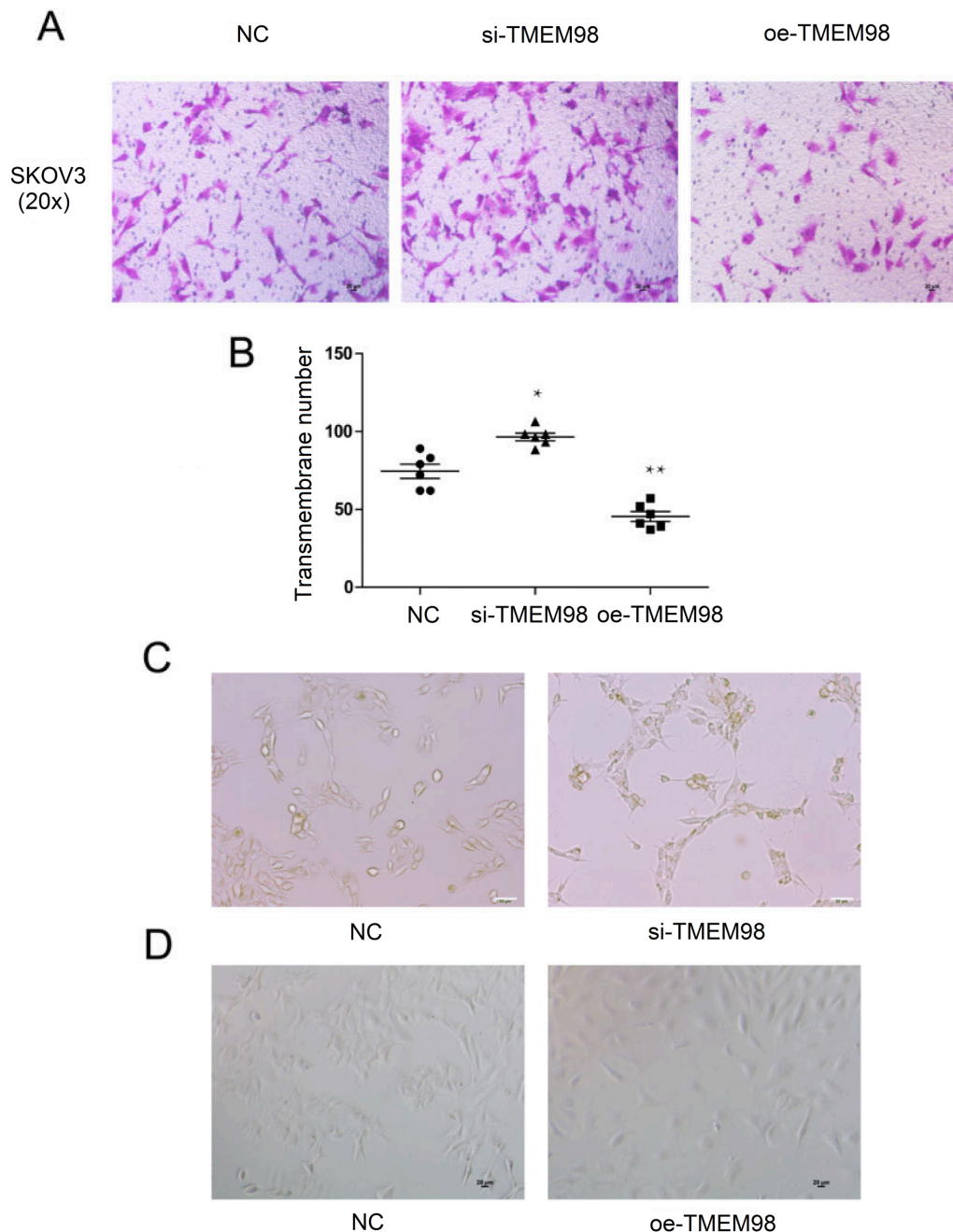
Fig. 6. Invasion ability and vascular mimicry by down-regulation and up-regulation of TMEM98. (A) The invasion ability of SKOV3 cells was detected by the Transwell chamber. (B) Quantitative analysis of SKOV3 cell invasion ability (n = 3, *$p < 0.05$, **$p < 0.01$). (C,D) vascular mimicry of SKOV3 cells.

richment analysis showed that TMEM98 was closely related to apoptosis. To explore whether TMEM98 can inhibit the proliferation of OC cells, we further explored the effect of TMEM98 on apoptosis. In the SKOV3 cells, the results showed that compared with the NC group, the number of early apoptotic cells in the si-TMEM98 group decreased significantly, the number of living cells in the oe-TMEM98 group decreased significantly and the number of early apoptotic and late apoptotic cells increased significantly in the oe-TMEM98 group. The results of IOSE80 cells showed

that compared with the NC group, the number of late apoptotic cells in the si-TMEM98 group was significantly decreased, the number of living cells in the oe-TMEM98 group was significantly decreased, and the number of late apoptotic cells was significantly increased (Fig. 8A–H). The results showed that the knockdown of TMEM98 could inhibit the apoptosis of SKOV3 and IOSE80 cells. Similar results were found using the TUNEL staining in the SKOV3 cells (Fig. 8I,J).
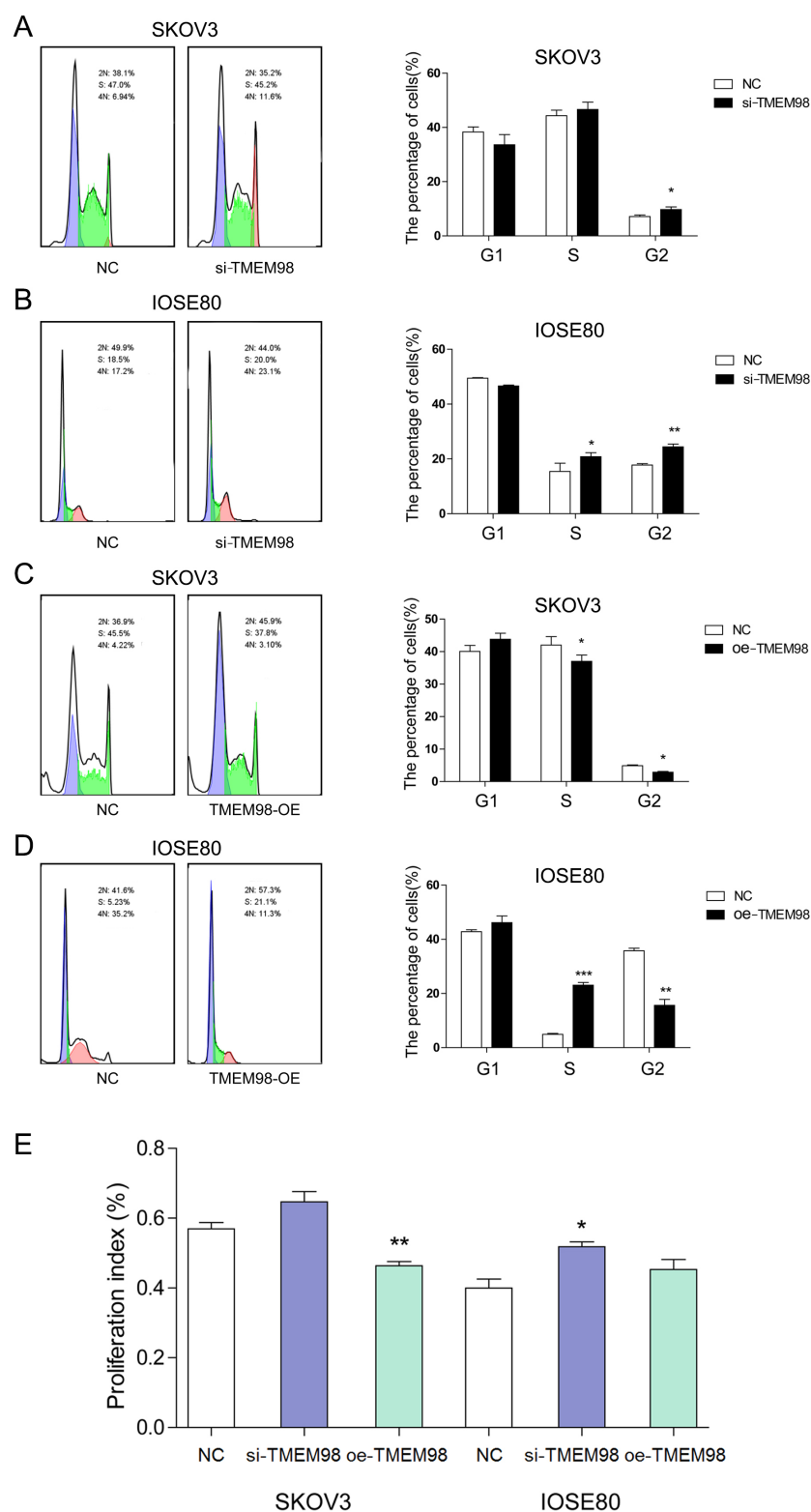
Fig. 7. Cell cycle progression by down-regulation and up-regulation of TMEM98. (A) The cell cycle of SKOV3 cells in the NC group and si-TMEM98 group. (B) The cell cycle of IOSE80 cells in the NC group and si-TMEM98 group. (C) The cell cycle of SKOV3 cells in the NC group and oe-TMEM98 group. (D) The cell cycle of IOSE80 cells in the NC group and oe-TMEM98 group. (E) Cell proliferation index of NC group, si-TMEM98 group, and oe-TMEM98 group of SKOV3 cells and IOSE80 cells (n = 3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
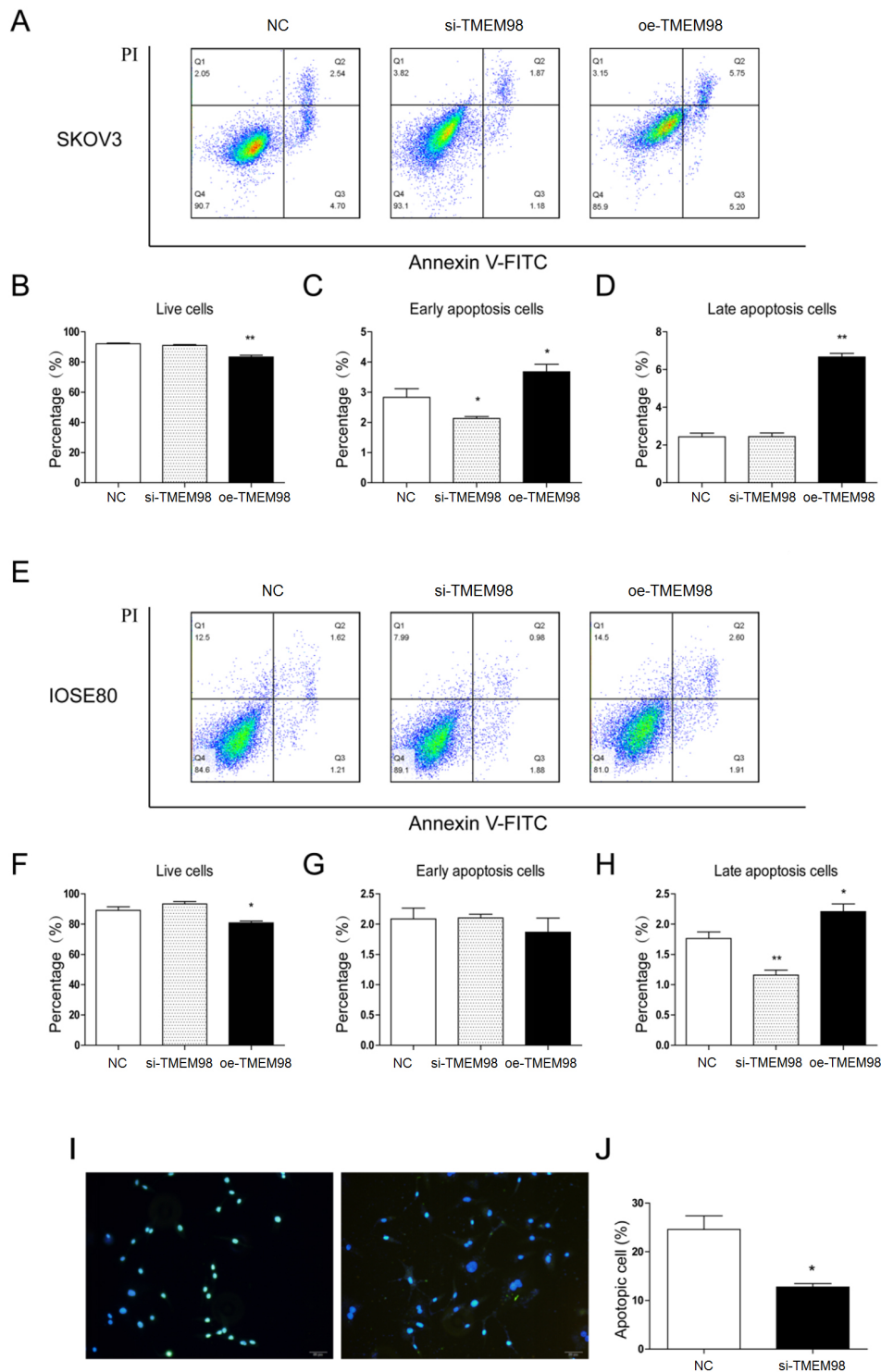
Fig. 8. Apoptosis status by down-regulation and up-regulation of TMEM98. (A) Flow cytometry for apoptosis of SKOV3 cells. (B) The percentage of viable SKOV3 cells in the NC group, si-TMEM98 group, and oe-TMEM98 group. (C) The percentage of early apoptotic SKOV3 cells in the NC group, si -MEM98 group, and oe-TMEM98. (D) The percentage of late apoptotic SKOV3 cells in the NC group, si-TMEM98 group, and oe-TMEM98 group. (E) Flow cytometry for apoptosis of IOSE80 cells. (F) The percentage of viable IOSE80 cells in the NC group, si-TMEM98 group, and oe-TMEM98 group. (G) The percentage of early apoptotic IOSE80 cells in the NC group, si-TMEM98 group, and oe-TMEM98 group. (H) The percentage of late apoptotic IOSE80 cells in the NC group, si-TMEM98 group, and oe-TMEM98 group. (I,J) TUNEL staining for apoptotic cells in the NC group and si-TMEM98 group (n = 3, *$p < 0.05$, **$p < 0.01$).
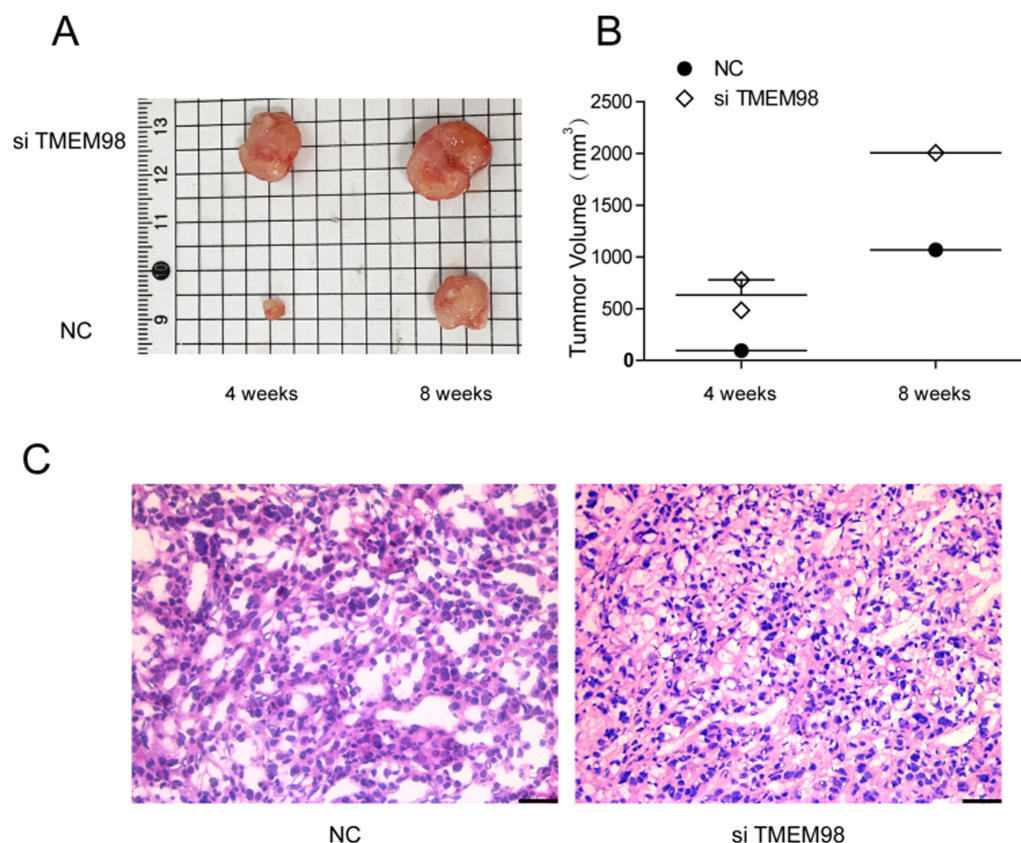
Fig. 9. Tumor growth of TMEM98 in OC. (A) The images of tumor injection with si-TMEM98 SKOV3 cells. (B) Knockdown of TMEM98 increased tumor volume. (C) Hematoxylin-eosin staining of tumor sections in NC group and si-TMEM98 group.

3.10 Low Expression of TMEM98 Promotes OC Development in vivo

To further explore the effect of TMEM98 on the malignant behavior of OC *in vivo*, 20 female nude mice were used in this experiment. SKOV3 cells were transfected and treated separately. The cells were collected and inoculated under the skin of nude mice to establish the model of OC heterotopic transplantation. The results showed that there was no tumor in the oe-TMEM98 group. Then the subcutaneous ovarian tumors of the NC group and si-TMEM98 group were taken out and compared with the volume, and then embedded sections were stained with it. The results showed that the volume of subcutaneous tumors in the si-TMEM98 group was significantly larger than that of the NC group at 4 and 8 weeks after inoculation. HE staining showed that there are many vacuoles and no obvious necrotic lesions in the tumor tissue (Fig. 9).

3.11 TMEM98 Expression is Negatively Correlated with poly ADP-ribose polymerase (PARP) Expression

According to the above results and enrichment analysis, TMEM98 may inhibit the development of OC by regulating apoptosis. It has been confirmed that PARP activity is necessary for repairing single-strand DNA breakage by base excision and repair. Therefore, when PARP function is inhibited, cell death increases. PARP inhibitor can inhibit the repair of DNA damage, and then promote the apoptosis of tumor cells. In addition, PARP is the substrate of most members of the caspase family, and the shear fracture is considered as the symbol of apoptosis and is also generally regarded as the indicator of Caspase-3 activation. To further explore the relationship between TMEM98 and apoptosis and the preliminary study of its pathway, the correlation between the TMEM98 gene and PARP family was analyzed online by the cbiportal website (Fig. 10). The results showed that TMEM98 was significantly negatively correlated with PARP family members, and the most significant correlation was PARP4, PARP9, PARP10, PARP12, PARP14, and PARP15 (all $p < 0.001$).

3.12 Low Expression of TMEM98 Inhibits OC Apoptosis in vivo

Finally, we investigated the association between the expression level of TMEM98 and OC apoptosis *in vivo*. The expression of Caspase-3 and Bcl-2 in OC was detected by immunohistochemistry to assess the apoptosis status. The results showed that compared with the NC group, the expression of Caspase-3 was significantly downregulated and the expression of Bcl-2 was significantly increased in the si-TMEM98 group (Fig. 11). These findings suggested
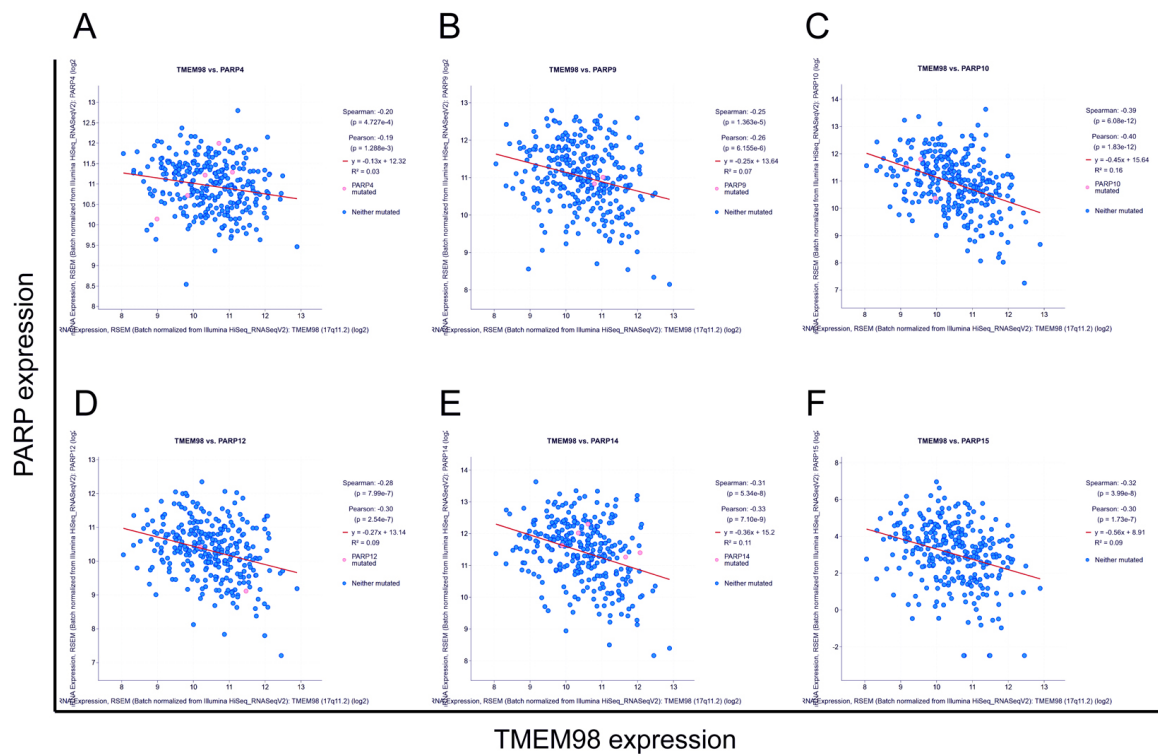
Fig. 10. TMEM98 expression is negatively correlated with PARP expression. (A) TMEM98 expression was significantly negatively correlated with PARP4 expression. (B) TMEM98 expression was significantly negatively correlated with PARP9 expression. (C) TMEM98 expression was significantly negatively correlated with PARP10 expression. (D) TMEM98 expression was significantly negatively correlated with PARP12 expression. (E) TMEM98 expression was significantly negatively correlated with PARP14 expression. (F) TMEM98 expression was significantly negatively correlated with PARP15 expression (***$p < 0.001$).
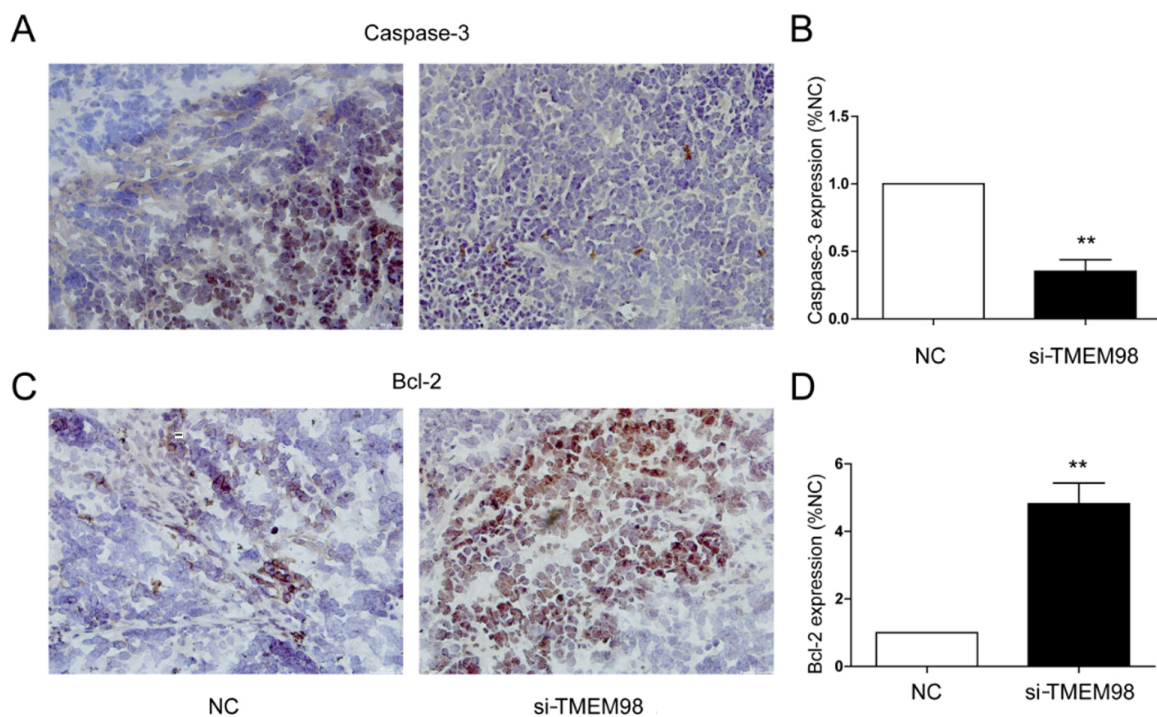

Fig. 11. Apoptosis status by down-regulation of TMEM98 *in vivo*. (A,B) Immunohistochemistry and quantitative analysis of Caspase-3 protein expression. (C,D) Immunohistochemistry and quantitative analysis of Bcl-2 expression (n = 3, **$p < 0.01$).

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that down-regulation of TMEM98 could inhibit the apoptosis of OC cells *in vivo* and *in vitro*. Fig. 12 is the summary of this experiment.
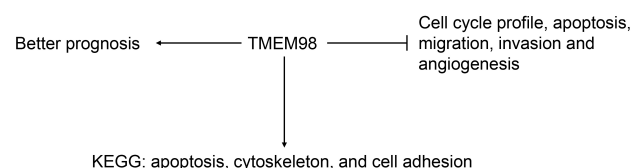

Fig. 12. Summary of this study.

4. Discussion

In this study, we found that TMEM98 was a low expression in OC, and a low level of TMEM98 was associated with lower survival outcomes compared to those with a high level of TMEM98. The biological function study showed that low expression of the TMEM98 could promote proliferation, migration, invasion, vasculogenic mimicry, and cell cycle progression in OC cells. In addition, knocking down TMEM98 could inhibit apoptosis. Our study suggests that TMEM98 plays an important role in the development of OC.

In the TNMplot database [11] and the Human Protein Atlas database [12], the gene expression of TMEM98 in normal ovarian tissue is significantly higher than that in various normal human tissues, suggesting that TMEM98 has higher specificity in the human normal ovary. In our study, we found that TMEM98 expression was significantly lower in primary OC than in the normal ovarian tissue. The prognosis in patients with low TMEM98 expression was inferior to those with high TMEM98 expression, which showed that TMEM98 was involved in the development and metastasis of OC. However, TMEM98 was overexpressed in hepatocellular carcinoma and was significantly associated with advanced stage and poor prognosis [16]. In those with cervical adenocarcinoma [17], lung cancer [18], gastric cancer [19], and head and neck carcinoma [20], TMEM98 was also overexpressed in tumors compared to the normal tissues. In the TNMplot database, there were also significant differences in the expression of TMEM98 in different tumors. The above studies suggest that TMEM98 has high heterogeneity in various tumors.

Several studies have confirmed that TMEM98 was related to the differentiation of T helper 1 cells [15], normal eye development [21], and atherosclerosis [22]. In previous studies, TMEM98 was associated with the ERK, AKT/GSK3β and Wnt/β-catenin signaling pathway [13, 14]. However, the function of TMEM98 in OC remains unclear. In lung cancer, Mao *et al*. [18] found that si-TMEM98 effectively suppressed the proliferation, invasion, and migration of lung cancer cells. Li *et al*. [20] also found that down-regulation of TMEM98 represses prolif-

eration ability and migration ability of head neck cancer cells. In gastric cancer, Ao *et al*. [19] showed that cell proliferation and invasion were promoted by upregulation of TMEM98 mRNA. In our study, we found the exact opposite function by si-TMEM98 in OC. Our results showed that si-TMEM98 promoted proliferation, migration, invasion, and cycle progression of OC cells. Interestingly, although migration ability is a biological characteristic of tumor cells, we found that the migration ability of IOSE80 cells and SKOV3 cells were enhanced after knockdown of TMEM98, which indicated that knockdown of TMEM98 would increase the migration ability of normal ovarian epithelial cells IOSE80, and may have the tendency to gradually transform into cancer cells. We found that knockdown of TMEM98 would promote cell cycle progression and inhibit apoptosis, and cell proliferation and death also have an effect on the width of the wound in wound healing assay. Although we used serum-free medium to reduce this effect, it is undeniable that this effect still exists. Our study suggests that TMEM98 may be a potential prognostic biomarker and therapeutic target for OC patients. The diagnostic value of TMEM98 in OC is also valuable for further characterization.

In this study, we found that low expression of the TMEM98 promotes vasculogenic mimicry in OC cells. Although antiangiogenic therapy can inhibit tumor growth by inhibiting tumor angiogenesis. However, the antiangiogenic drug bevacizumab did not prolong overall survival in patients with OC as a first-line setting [23]. One theory is that antiangiogenic drugs are ineffective because tumors may have other blood supply patterns, known as "vasculogenic mimicry". Antiangiogenic drug with bevacizumab promotes a hypoxic response and vasculogenic mimicry [24], which may be related to limited efficacy and poor response to antiangiogenic therapy in OC. Angiogenesis mimicry has recently been demonstrated in a variety of human malignancies such as hepatocellular carcinoma [25], gastric cancer [26], glioblastoma [27], breast cancer [28], and head and neck cancers [29]. Vasculogenic mimicry also exists in OC, which is closely related to tumor progression and poor survival outcomes [30,31]. Therefore, targeting TMEM98 to inhibit vasculogenic mimicry may overcome the limited efficacy of antiangiogenic therapy for OC patients.

Using the KEGG function enrichment analysis, we found that TMEM98 had a closer relationship with apoptosis. Our study also showed that low expression of TMEM98 could inhibit apoptosis of OC *in vivo* and *in vitro*. The previous study has shown that PARP activity is necessary for repairing single-strand DNA breakage [32]. Therefore, inhibition of PARP function can lead to apoptosis of homologous recombinant tumor cells [33]. PARP inhibitors are the biggest advance in the treatment of OC in recent years [34]. In addition, PARP overexpression was associated with poor survival outcomes in patients with OC [35]. In our

study, we found that TMEM98 was negatively related to the PARP family. PARP is a substrate of caspase, and PARP is the marker of apoptosis initiation by caspase [36]. Therefore, TMEM98 is closely related to the caspase apoptosis pathway. Our *in vivo* study showed that the expression of Caspase-3 was significantly downregulated and the expression of Bcl-2 was significantly increased in the si-TMEM98 group.

Our study has some limitations. First, we didn't use the TMEM98 knockout cell line, which is more efficient and persuasive in exploring the role of TMEM98 in OC cell proliferation and apoptosis. Second, although we have proved that TMEM98 is related to the occurrence and development of ovarian cancer and has a tumor suppressor effect on ovarian cancer, the exact pathway is still unclear and requires further validation. Third, we did not conduct further studies on PARP and the role of PARP in TMEM98-dependent regulation of apoptosis is still not clear.

5. Conclusions

In conclusion, our study demonstrates for the first time that TMEM98 is downregulated in OC, and patients with lower TMEM98 mRNA levels show inferior survival. In addition, our study suggests that tumor-suppressor gene TMEM98 promotes proliferation and inhibits apoptosis of OC.

Author Contributions

SGW, JYX, JL, and MH are lead authors who participated in manuscript drafting, tables/figures creation, and manuscript revision. SGW aided in data collection. JZ is the corresponding author who initially developed the concept and drafted and revised the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2707210.

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