

Long non-coding RNA OIP5-AS1 promotes the progression of esophageal cancer by regulating miR-30a/VOPPI expression

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Abstract. Long non-coding RNAs (lncRNAs) serve an important role in the development of esophageal cancer (EC), which is the eighth most common type of cancer worldwide. lncRNA opa-interacting protein 5 antisense transcript 1 (OIP5-AS1) is associated with human malignancy. However, the biological roles of OIP5-AS1 in the development of EC remain unclear. In the present study, transfection was conducted, and reverse transcription-quantitative PCR and western blot analysis were used for the detection of mRNA and protein expression, respectively. Furthermore, dual-luciferase reporter and RNA immunoprecipitation assays were used to study the interaction between miRNA and lncRNA or genes. The results revealed that OIP5-AS1 expression in EC tissues and cultured EC cells was upregulated, microRNA-30a (miR-30a) expression was downregulated. OIP5-AS1-knockdown suppressed the proliferation, migration and invasion of EC9706 and EC109 cells. miR-30a was confirmed to interact with OIP5-AS1, and miR-30a-mimics transfection ameliorated the effects of OIP5-AS1 in EC cells. Vesicular overexpressed in cancer prosurvival protein 1 (VOPPI) was verified as the direct target of miR-30a. VOPPI expression was positively correlated with OIP5-AS1 expression in EC cells. Overexpression of VOPPI ameliorated the negative effects of OIP5-AS1-knockdown on EC9706 and EC109 cells. In conclusion, OIP5-AS1 promoted the proliferation, migration and invasion of EC cells by increasing VOPPI expression by sponging miR-30a.

Introduction

Esophageal cancer (EC), accompanied by high morbidity and mortality, has become one of the most common malignancies in the world (1). Esophageal squamous cell carcinoma (ESCC) accounts for ~80% of EC cases (1,2). ESCC is highly aggressive and is characterized by a poor prognosis (1,2). There is a high incidence of EC in China due to numerous factors, including inappropriate diets (low fruit and vegetable intake and low fruit intake), drinking alcohol and tobacco smoking (3). The 5-year survival rate of patients with EC is 15-25% (4), and patients with advanced EC have a poor prognosis (4). Therefore, it is essential to identify new biomarkers for early diagnosis and to explore the possible molecular mechanisms for target-specific drug development.

Long non-coding RNAs (lncRNAs) are a group of RNA molecules >200 nucleotides in length (5). Dysregulation of lncRNAs is often observed in several types of cancer, such as EC (6). For example, lncRNA PVT1 promotes the proliferation, invasion, colony formation and tumor sphere formation of EC cells by regulating Yes-associated protein 1 signaling (7). lncRNA EIF3J-AS1 increases the invasion of EC cells by mediating AKT1 expression through interaction with microRNA (miRNA/miR)-373-3p (8). lncRNA opa-interacting protein 5 antisense transcript 1 (OIP5-AS1) is derived from the anti-sense of the OIP5 gene, and it promotes epithelial-mesenchymal transition (EMT), migration and invasion in cisplatin-resistant oral squamous cell carcinoma cells (9). OIP5-AS1 may effectively facilitate the progression of ovarian cancer by mediating CCNG1 expression through sponging of miR-128-3p (10). Recently, OIP5-AS1 has been reported to promote the development of EC (11). However, the underlying mechanism remains unclear.

The expression levels of vesicular overexpressed in cancer prosurvival protein 1 (VOPPI) are often increased in cancer (12). VOPPI enhances cell proliferation, migration and invasion in lung adenocarcinoma (13). Additionally, it has become a putative oncogene in gastric cancer (14). VOPPI also interacts with cytoplasmic NF- κ B protein (15). Recently, VOPPI has been identified as one of the key factors associated with EC development (1). However, the pathological roles of VOPPI in EC remain unknown. The present study aimed to investigate the biological activities of OIP5-AS1 and VOPPI in the progression of EC.

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Materials and methods

Tissue collection. A total of 32 pairs of human EC tissue samples and corresponding adjacent non-tumor tissues (5 cm away from the tumor tissue) were collected from newly diagnosed patients (mean age, 61.6±4.8 years; range, 54 to 75 years) at The First Affiliated Jiujiang Hospital of Nanchang University (Jiujiang, China) between March 2016 and July 2019. The patients had not received any chemotherapy. The clinicopathological data of patients with EC is shown in Table SI. The EC tissue samples were histologically confirmed as ESCC by two pathologists. The tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until further use. All patients signed the consent forms prior to the use of their tissues in the present study. The experimental protocols have been approved by the Ethics Committee of The First Affiliated Jiujiang Hospital of Nanchang University according to the Declaration of Helsinki Principles.

Cell culture. Human esophageal carcinoma cells (EC109, EC9706 and TE-1) and human immortalized normal esophageal epithelial cells (Het-1A) were obtained from the American Type Culture Collection. RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.) and 100 U/ml penicillin and streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) was employed for cell culture. All cells were cultured in a humidified incubator containing 5% CO₂ at 37°C. For ensuring the phenotype of Het-1A, the first or second generation was used for investigation.

Cell transfection. Three short hairpin RNAs (shRNAs; sh-OIP5-AS1-#1, sh-OIP5-AS1-#2 and sh-OIP5-AS1-#3) and a scrambled shRNA negative control (sh-NC) were obtained from Shanghai GeneChem Co., Ltd. The shRNAs (100 nM) were inserted into pGPH1/Neo (40 nM; Shanghai GenePharma Co., Ltd.). Then, 75 pmol of constructed pGPH1/Neo were transfected into EC cells using Lipofectamine[®] 3000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Briefly, the plasmid pGPH1/Neo/shRNA and Lipofectamine[®] 3000 were diluted with Opti-MEM (serum-reduced medium) and incubated for 5 min at room temperature. The two transfection mixtures were mixed and incubated at room temperature for 20 min. The transfection mixture was then gently added to each well, and the cells were incubated at 37°C (5% CO₂). The cells were transfected with sh-OIP5-AS1 or sh-NC for 48 h prior to further experiments. Neomycin (cat. no. 1405-10-3; Sigma-Aldrich; Merck KGaA) at 400 µg/µl was used to select the stable transfected cells for 4 weeks.

miR-30a mimics (sense, 5'-UGUAAACAUCUCGACUG CAAG-3' and anti-sense, 5'-CUUCCAGUCGAGGAUCU UACA-3') and miR-NC (sense, 5'-UCACAACCUCCUAGA UAGAGUAGA-3' and anti-sense, 5'-UACUCUUUCUAGGAG GUAGUGAUU-3') were purchased from Guangzhou RiboBio Co., Ltd. EC cells at 60% confluence (1×10⁵ cells/well) were used for transfection using Lipofectamine[®] 3000 according to the manufacturer's instructions. The concentrations of miR-30a mimics and miR-NC in the final transfection system

were 50 nM. The transfected cells were incubated at 37°C (5% CO₂), and collected 48 h later for the following experiments.

Furthermore, 2000 ng pcDNA3.1-VOPPI vector (Guangzhou RiboBio Co., Ltd.) and the empty vector pcDNA3.1 (negative control) were prepared and then transfected into EC cells, respectively, using Lipofectamine 3000 according to the manufacturer's instructions. The transfected cells were incubated with 5% CO₂ at 37°C, and 48 h after transfection for the further experimentation.

MTT assays. Transfected EC cells (5×10³/well) in 96-well plates were cultured at 37°C for 48 h. The MTT assay was conducted according to the instructions of the MTT Cell Proliferation and Cytotoxicity Assay kit (cat. no. C0009S; Beyotime Institute of Biotechnology). Specifically, MTT (0.5 mg/ml) was added to each well and incubated at 37°C for 4 h. Then, the formazan crystals were dissolved in 150 µl DMSO in the dark. The wavelength of 490 nm was used for measurement using a microplate reader (Thermo Fisher Scientific, Inc.).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA from EC tissues and cultured cells was extracted using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Specifically, 2 µg RNA was reverse transcribed using random primers and M-MLV Reverse Transcriptase RNase H Minus (both Promega Corporation), according to the manufacturer's protocols. qPCR assays were conducted on Power SYBRs Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) to detect the mRNA expression levels of OIP5-AS1 and VOPPI. miR-30a expression was detected using the Taqman MicroRNA Reverse Transcription kit and Taqman Universal Master Mix II kit (both Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The procedure was carried out as follows: 95°C for 6 min, followed by 40 cycles at 95°C for 40 sec and 65°C for 30 sec, and finally 75°C for 8 min. Electrophoresis in 1.5% (wt/v) agarose gel was used to identify and confirm the PCR results. GAPDH and U6 were used as the endogenous reference genes for mRNA and miRNA, respectively. All the primers for the sense and anti-sense chains were obtained from Biomics. The primer sequences were as follows: OIP5-AS1 forward, 5'-TGCGAA GATGGCGCAGTAAG-3' and reverse, 5'-TAGTTCCTCTCG TCTGGCCG-3'; VOPPI forward, 5'-GATGAACCCTGTCCG GAAT-3' and reverse, 5'-GGCCTTCACTACCTGTTCGTA-3'; GAPDH forward, 5'-AGGTGAAGTCCGAGTCAACG-3' and reverse, 5'-AGGGTCAATTGATGGCAACA-3'; miR-30a forward, 5'-ACACTCCAGCTGGGTGTAACATCCTC GAC-3' and reverse, 5'-CAGTGCCTGTCTGGAGT-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTACGAATTTGCGT-3'. The gene expression of miRNA and mRNA was indicated as fold-changes using the 2^{-ΔΔC_q} method (16).

Western blotting. The total proteins were extracted from tissues and cultured cells in ice-cold RIPA lysis buffer (Beyotime Institute of Biotechnology), and the protein concentrations were determined using a BCA protein assay kit (Beyotime Institute of Biotechnology). Total proteins (30 µg/lane) of each experimental group were subjected to 10% SDS-PAGE

and then transferred onto polyvinylidene fluoride membranes (EMD Millipore). After blocking in TBS containing 5% skimmed milk for 1 h at room temperature, the membranes were incubated at 4°C overnight with primary antibodies against VOPPI (1:1,000; Sigma-Aldrich; Merck KGaA; cat. no. HPA038371) and GAPDH (1:1,000; Sigma-Aldrich; Merck KGaA; cat. no. SAB1410512). Subsequently, the membranes were incubated with the secondary antibody conjugated with peroxidase (1:2,000; Sigma-Aldrich; Merck KGaA; cat. no. AP510) for 1 h at 37°C. Protein bands were detected using the enhanced chemiluminescence detection system (Bio-Rad Laboratories, Inc.) and Quantity One software v4.6.2 (Bio-Rad Laboratories, Inc.).

Transwell migration and invasion assays. EC cells were prepared for cell suspensions using serum-free medium to a density of 3×10^4 cells/ml. The cell suspensions were added into the upper chambers of Transwell plates at room temperature, while RPMI-1640 medium supplemented with 20% FBS was added to the lower chambers. For the invasion assay, Transwell membranes were pre-coated with Matrigel (EMD Millipore) overnight at room temperature. Following incubation for 24 h at 37°C, the migratory and invasive cells were collected, washed and stained with 0.5% crystal violet (Sigma-Aldrich; Merck KGaA) for 15 min at room temperature. Finally, the cells were counted under an inverted light microscope (Olympus CK-40; Olympus Corporation) at a magnification of x100.

Dual-luciferase reporter assays. The online predicted system StarBase v2.0 (<http://starbase.sysu.edu.cn>) and TargetScan7.2 (<http://www.targetscan.org>) were employed to seek the targets of OIP5-AS1 and miR-30a, respectively. The recombinant luciferase plasmids were constructed by cloning the sequences of wild-type (WT) OIP5-AS1 and 3'-UTR of VOPPI, respectively, into the pGL-3 luciferase basic vector (Promega Corporation). In addition, their mutant-types (MUT) were also constructed as MUT-OIP5-AS1 and MUT-VOPPI, respectively. Each constructed plasmid was transfected into EC cells with miR-30a mimics or miR-NC using Lipofectamine 3000, as aforementioned. Following incubation for 48 h at 37°C, firefly and *Renilla* luciferase activities were detected using the Glomax 96 luminometer (Promega Corporation) according to the manufacturer's instructions. Firefly luciferase activity was normalized to *Renilla* luciferase activity.

RNA immunoprecipitation (RIP) assays. RIP assays were conducted to further investigate the direct interaction between OIP5-AS1 and miR-30a using the Magna RNA immunoprecipitation kit (EMD Millipore), according to the manufacturer's instructions. Specifically, EC cells (2×10^7 cells) were harvested using trypsin and lysed in RIP cell lysis buffer (MilliporeSigma). The obtained supernatant was used for the RIP assays using the EZ-Magna RIPTM RNA-Binding Protein Immunoprecipitation Kit (MilliporeSigma); 10% cell extract was used as the input, and 100 μ l cell extract was incubated at 4°C with 50 μ l protein A/G-conjugated magnetic beads with antibodies against Argonaute2 (Ago2; Sigma-Aldrich; Merck KGaA; cat. no. MABE56) and anti-IgG (Sigma-Aldrich; Merck KGaA; cat. no. I5131) (the negative control) overnight.

The magnetic beads were harvested by centrifugation at 1,000 x g at room temperature for 2 min, and then rinsed with 1 ml RIP buffer. After detachment with proteinase K at 55°C for 30 min, the immunoprecipitated RNA was extracted and analyzed by RT-qPCR, as aforementioned. Finally, the expression levels of OIP5-AS1 and miR-30a in anti-IgG and anti-Ago2 groups were compared.

Statistical analysis. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. SPSS 20.0 software (IBM Corp.) was used for statistical analysis. Pearson's χ^2 test or Fisher's exact test were used to analyze the clinicopathological data of patients. Differences between EC and normal tissues were analyzed by paired Student's t-test. One-way ANOVA and Tukey's post-hoc test were used to compare differences among multiple groups. An unpaired Student's t-test was used to make statistical comparisons between two groups. Pearson's correlation coefficient was used to calculate the correlation. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

OIP5-AS1 expression is increased in EC tissues and cultured cells. To investigate the roles of OIP5-AS1 in EC, the expression levels of OIP5-AS1, VOPPI and miR-30a in EC tissues and cultured cells were detected by RT-qPCR. The clinicopathological data of patients with EC showed that OIP5-AS1 expression was significantly associated with differentiation, tumor invasion depth and histological grade (Table SI). In addition, OIP5-AS1 (Fig. 1A) and VOPPI (Fig. 1B) expression was significantly increased, while miR-30a (Fig. 1C) expression was significantly decreased in EC tissues compared with in adjacent normal tissues. Pearson's correlation analysis indicated that there was a positive correlation between OIP5-AS1 and VOPPI expression (Fig. 1D) and a negative correlation between OIP5-AS1 and miR-30a expression (Fig. 1E) in EC tissues (n=32). In the three cultured EC cells (EC109, EC9706 and TE-1) and normal Het-1A cell, OIP5-AS1 expression was also significantly upregulated in EC cultured cells than that in Het-1A cells (Fig. 1F).

Knockdown of OIP5-AS1 suppresses cell proliferation, migration and invasion in EC cells. To investigate the biological functions of OIP5-AS1 in EC cells, three shRNAs (sh-OIP5-AS1-#1, sh-OIP5-AS1-#2 and sh-OIP5-AS1-#3) against OIP5-AS1 and sh-NC were constructed and transfected into EC cells. RT-qPCR analysis was performed to confirm the transfection efficiencies (Fig. 2A). All shRNAs exhibited inhibitory effects on OIP5-AS1 expression in EC9706 and EC109 cells, and since sh-OIP5-AS1-#1 exhibited the most effective activity, it was selected for subsequent experiments. MTT assays indicated that OIP5-AS1-knockdown by sh-OIP5-AS1 transfection resulted in suppression of EC9706 (Fig. 2B) and EC109 (Fig. 2C) cell proliferation at 48-96 h. The effects of OIP5-AS1 on the migration and invasion of EC9706 and EC109 cells were investigated. The results indicated that knockdown of OIP5-AS1 expression significantly decreased the capacities of migration (Fig. 2D and E) and invasion (Fig. 2F and G) in EC9706 and EC109 cells.

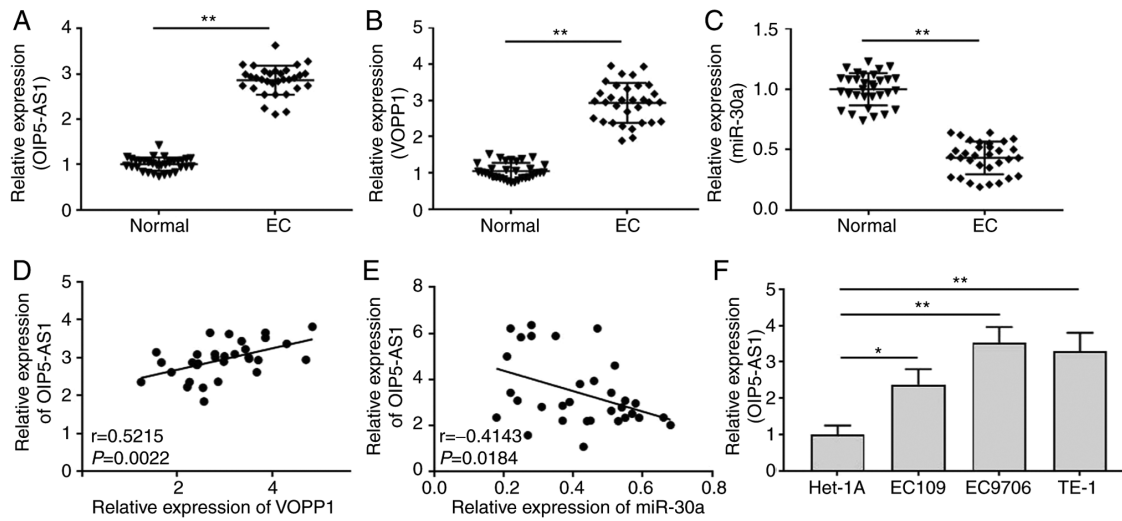


Figure 1. OIP5-AS1/miR-30a/VOPP1 expression in EC tissues and cells. Relative expression levels of (A) OIP5-AS1, (B) VOPP1 and (C) miR-30a in EC tissues (n=32) and adjacent normal tissues (n=32) were detected by RT-qPCR. Pearson's correlation analysis between OIP5-AS1 and (D) VOPP1 or (E) miR-30a expression in EC tissues was conducted. (F) OIP5-AS1 expression in human EC cell lines (EC109, EC9706 and TE-1) and human immortalized normal esophageal epithelial cells (Het-1A) cells was determined by RT-qPCR. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. * $P<0.05$ and ** $P<0.01$. EC, esophageal cancer; RT-qPCR, reverse transcription-quantitative PCR; OIP5-AS1, opa-interacting protein 5 antisense transcript 1; miR-30a, microRNA-30a; VOPP1, vesicular overexpressed in cancer pro-survival protein 1.

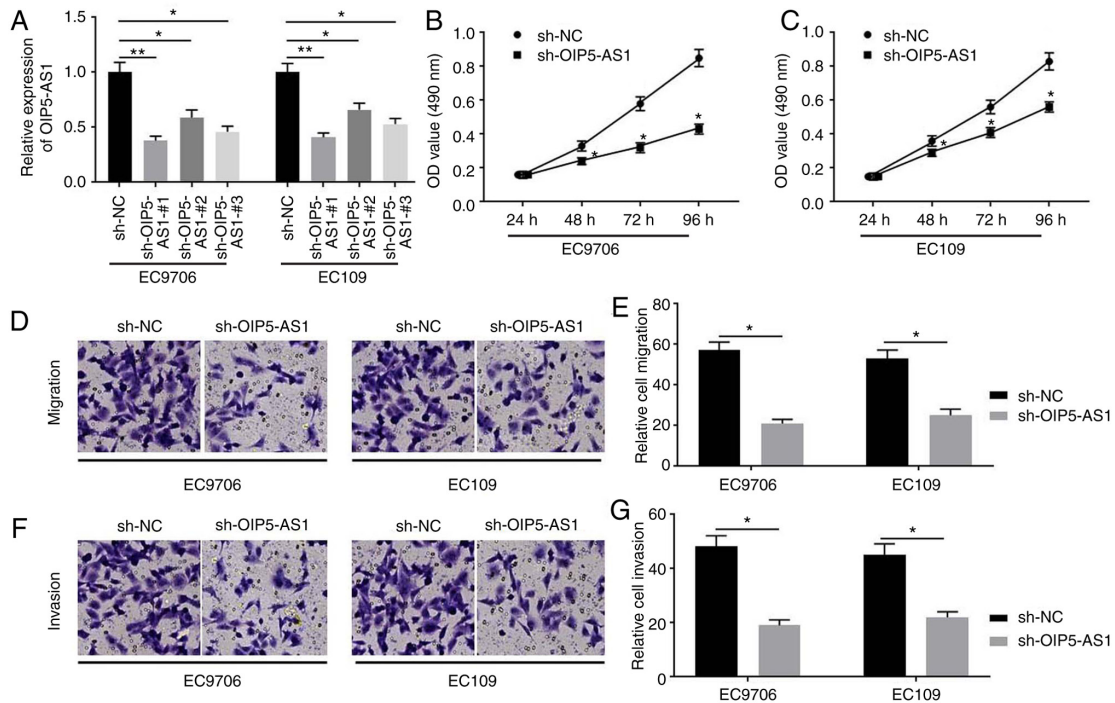


Figure 2. Knockdown of OIP5-AS1 expression decreases the proliferation, migration and invasion of EC9706 and EC109 cells. (A) Efficiency of sh-OIP5-AS1-knockdown was determined by reverse transcription-quantitative PCR. MTT assays were employed to detect cell viability in (B) EC9706 and (C) EC109 cells. Transwell assays were used to determine the (D and E) migration and (F and G) invasion in sh-OIP5-AS1-transfected EC9706 and EC109 cells; magnification, x400. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. * $P<0.05$ and ** $P<0.01$. OD, optical density; OIP5-AS1, opa-interacting protein 5 antisense transcript 1; sh, short hairpin RNA; NC, negative control.

OIP5-AS1 interacts with miR-30a. To further investigate the possible underlying mechanism of OIP5-AS1 in EC cells, the potential miRNAs that bind to OIP5-AS1 were investigated, since lncRNAs exhibit regulatory activity by sponging miRNAs (17). Starbase2.0 software was employed to predict the target miRNAs that interacted with OIP5-AS1. As a result, miR-30a was identified as a possible target of

OIP5-AS1 (Fig. 3A). The dual-luciferase reporter assays revealed that the luciferase activity in the reporter containing the WT-OIP5-AS1 was significantly using miR-30a mimics; by contrast, no significant differences were observed in the relative luciferase activities with the reporter containing the MUT-OIP5-AS1 (Fig. 3B and C). In addition, RIP assays indicated that OIP5-AS1 could interact with miR-30a

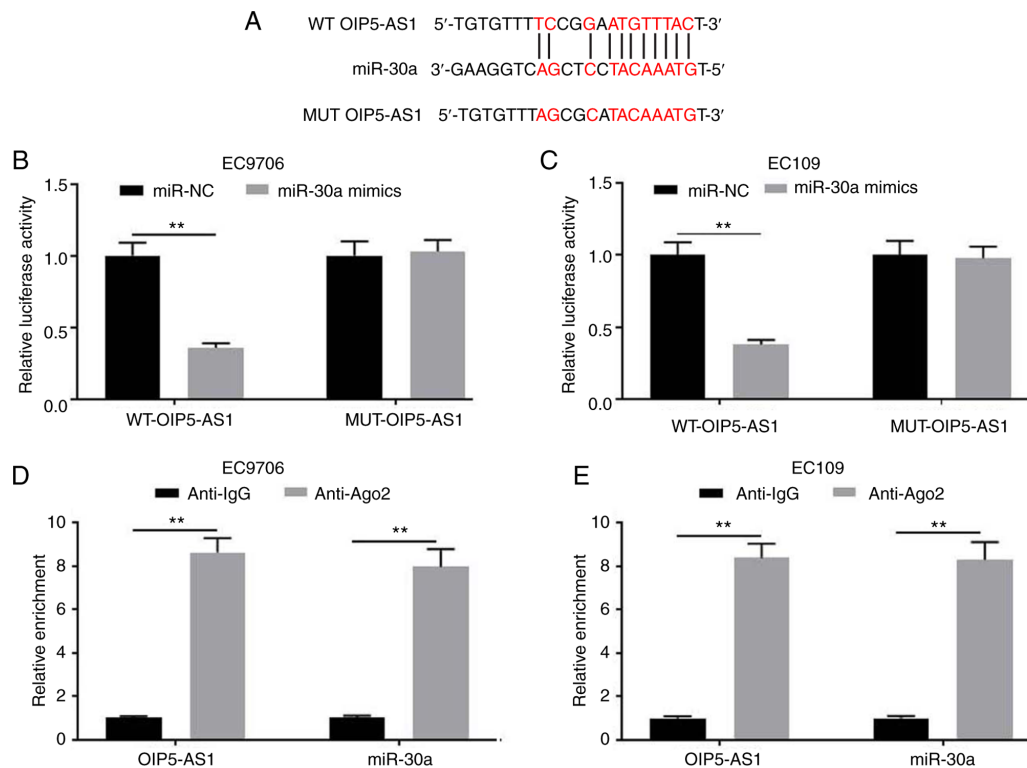


Figure 3. miR-30a is a target of OIP5-AS1. (A) Potential interaction between OIP5-AS1 and miR-30a was predicted by Starbase2.0 software. Relative luciferase activity was detected in (B) EC9706 and (C) EC109 cells co-transfected with both WT/MUT-OIP5-AS1 and miR-30a mimics/miR-NC. Firefly luciferase reporter activity was normalized to *Renilla* luciferase activity. Interaction between OIP5-AS1 and miR-30a was confirmed by RNA immunoprecipitation assays in (D) EC9706 and (E) EC109 cells. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. ** $P < 0.01$. OIP5-AS1, opa-interacting protein 5 antisense transcript 1; miR-30a, microRNA-30a; NC, negative control; WT, wild-type; MUT, mutant; Ago2, argonaute 2.

(Fig. 3D and E). Overall, miR-30a may be a potential target of OIP5-AS1.

Overexpression of miR-30a ameliorated the migration and invasion of EC cells. To investigate the roles of miR-30a in EC cells, RT-qPCR assays were used to detect miR-30a expression. Suppression of OIP5-AS1 significantly increased miR-30a expression (Fig. 4A). In turn, miR-30a mimics significantly decreased OIP5-AS1 expression (Fig. 4B). miR-30a expression detected by RT-qPCR suggested successful transfection (Fig. 4C). Furthermore, miR-30a overexpression suppressed the migration (Fig. 4D and E) and invasion (Fig. 4F and G) of EC9706 and EC109 cells.

VOPPI is a target of miR-30a. To further explore how miR-30a affected the biological functions in EC cells, the target genes of miR-30a were predicted by TargetScan7.2. As a result, VOPPI was identified as a potential target of miR-30a (Fig. 5A), which was verified using the dual-luciferase reporter assay (Fig. 5B and C). The relative luciferase activities did not show statistical difference with the reporter containing the mutant site of VOPPI; by contrast, the relative luciferase activities in the reporter containing the WT binding site of VOPPI were significantly decreased (Fig. 5B and C). The mRNA and protein expression levels of VOPPI were determined. It was revealed that miR-30a mimics significantly downregulated VOPPI mRNA (Fig. 5D) and protein (Fig. 5E and F) expression. Collectively, miR-30a may specifically target VOPPI to degrade it by binding to its 3'-UTR.

Overexpression of VOPPI rescues the biological functions induced by OIP5-AS1-knockdown in EC cells. Transfection of pcDNA3.1 and pcDNA3.1-VOPPI into EC cells was conducted. RT-qPCR (Fig. 6A) and western blot (Fig. 6B and C) assays confirmed the successful transfection. To further investigate the roles of the downstream factor VOPPI in sh-OIP5-AS1-transfected EC cells, pcDNA3.1-VOPPI was prepared for co-transfection. RT-qPCR (Fig. 6D) and western blot (Fig. 6E and F) assays were used to verify the successful co-transfection of sh-OIP5-AS1 and pcDNA3.1-VOPPI, as showed by up regulation of VOPPI expression at the mRNA and protein levels. VOPPI overexpression showed similar effects as OIP5-AS1 in EC9706 and EC109 cells. Specifically, overexpression of VOPPI ameliorated the decreased proliferative activity of EC9706 (Fig. 7A) and EC109 (Fig. 7B) cells induced by OIP5-AS1-knockdown. Similarly, VOPPI overexpression also improved cell migration (Fig. 7C and D) and invasion (Fig. 7E and F), which were attenuated by sh-OIP5-AS1 transfection in EC9706 and EC109 cells. Collectively, overexpression of VOPPI improved the negative effects of OIP5-AS1-knockdown in EC cells.

Discussion

EC is the 8th most common type of cancer (18) and has complex pathological mechanisms, which remain incompletely understood. The potential post-transcriptional interactions between lncRNAs and mRNAs generate an integrated lncRNA-mRNA network, orchestrating the oncogenic/oncosuppressive

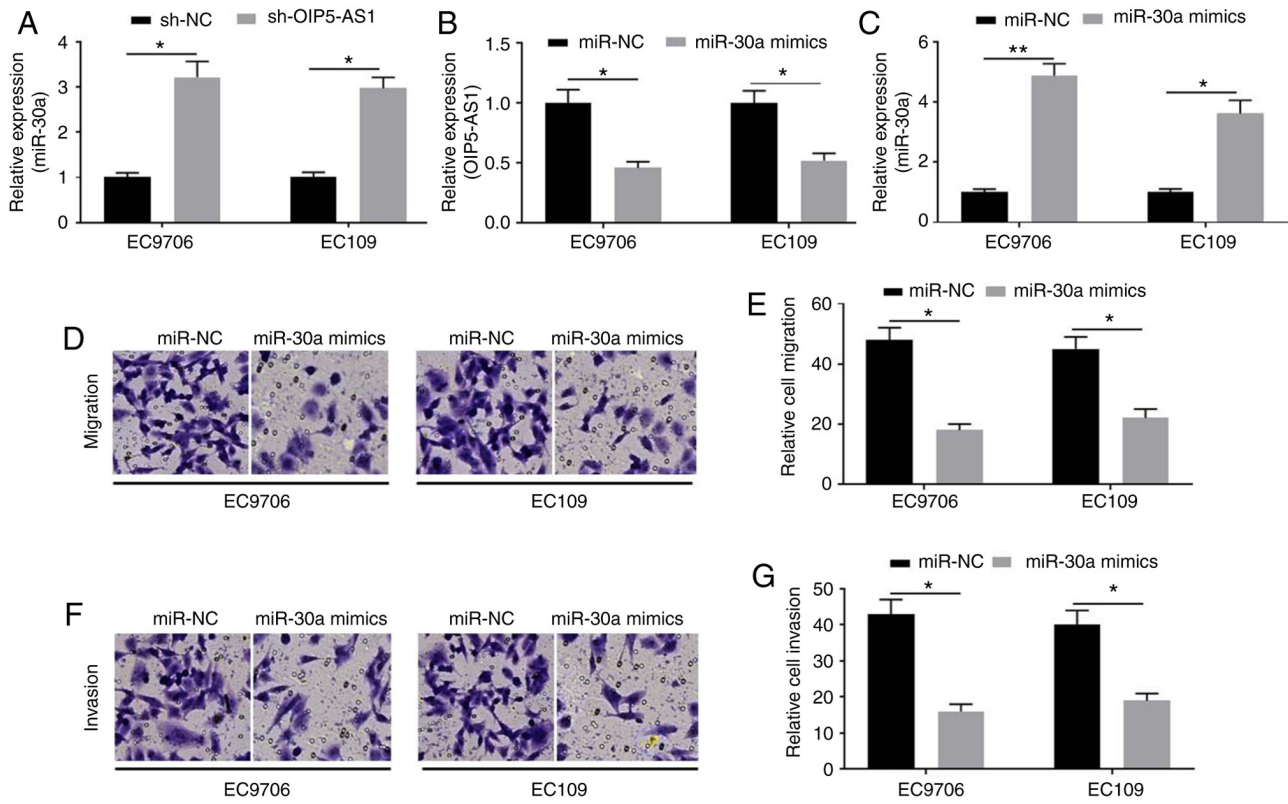


Figure 4. miR-30a overexpression ameliorates the migration and invasiveness of esophageal cancer cells. (A) miR-30a expression in sh-OIP5-AS1-transfected EC9706 and EC109 cells. (B) OIP5-AS1 expression in miR-30a mimics-transfected EC9706 and EC109 cells. (C) miR-30a expression in miR-30a mimics-transfected EC9706 and EC109 cells was detected by reverse transcription-quantitative PCR. Transwell assays were used to determine the (D and E) migration and (F and G) invasion in miR-30a mimics-transfected EC9706 and EC109 cells; magnification, x400. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$. OIP5-AS1, opa-interacting protein 5 antisense transcript 1; sh, short hairpin RNA; NC, negative control; miR-30a, microRNA-30a.

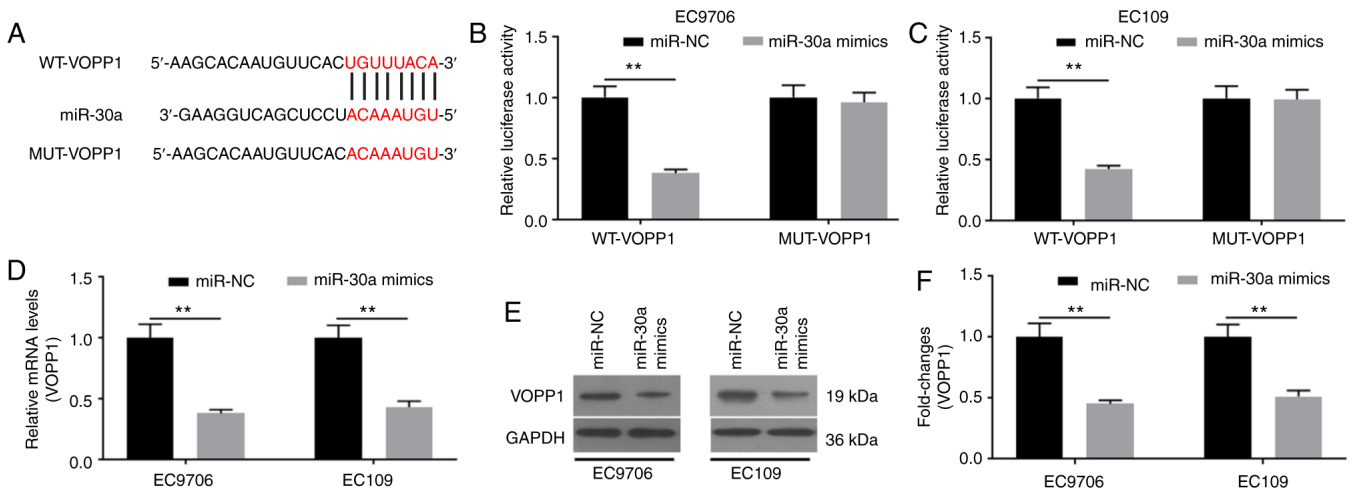


Figure 5. VOPPI is a direct target of miR-30a. (A) Potential interaction between miR-30a and VOPPI was predicted by TargetScan7.2 software. Relative luciferase activity was detected in (B) EC9706 and (C) EC109 cells co-transfected with both WT/MUT-VOPPI and miR-30a mimics/miR-NC. (D) VOPPI mRNA expression was detected in miR-30a/miR-NC-transfected EC9706 and EC109 cells. (E) VOPPI protein expression was detected in miR-30a/miR-NC-transfected EC9706 and EC109 cells. (F) The fold-changes of VOPPI protein expression were indicated. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. ** $P < 0.01$. miR-30a, microRNA-30a; NC, negative control; WT, wild-type; MUT, mutant; VOPPI, vesicular overexpressed in cancer prosurvival protein 1.

development. Numerous advanced technologies, such high-throughput sequencing and proteomics, have been employed to explore the critical factors associated with the pathological development of EC (19). In the present study, it

was revealed that OIP5-AS1 expression was upregulated in EC tissues and cultured EC cells. Knockdown of OIP5-AS1 attenuated the proliferation, migration and invasion of EC cells. miR-30a was identified to be a target of OIP5-AS1, and

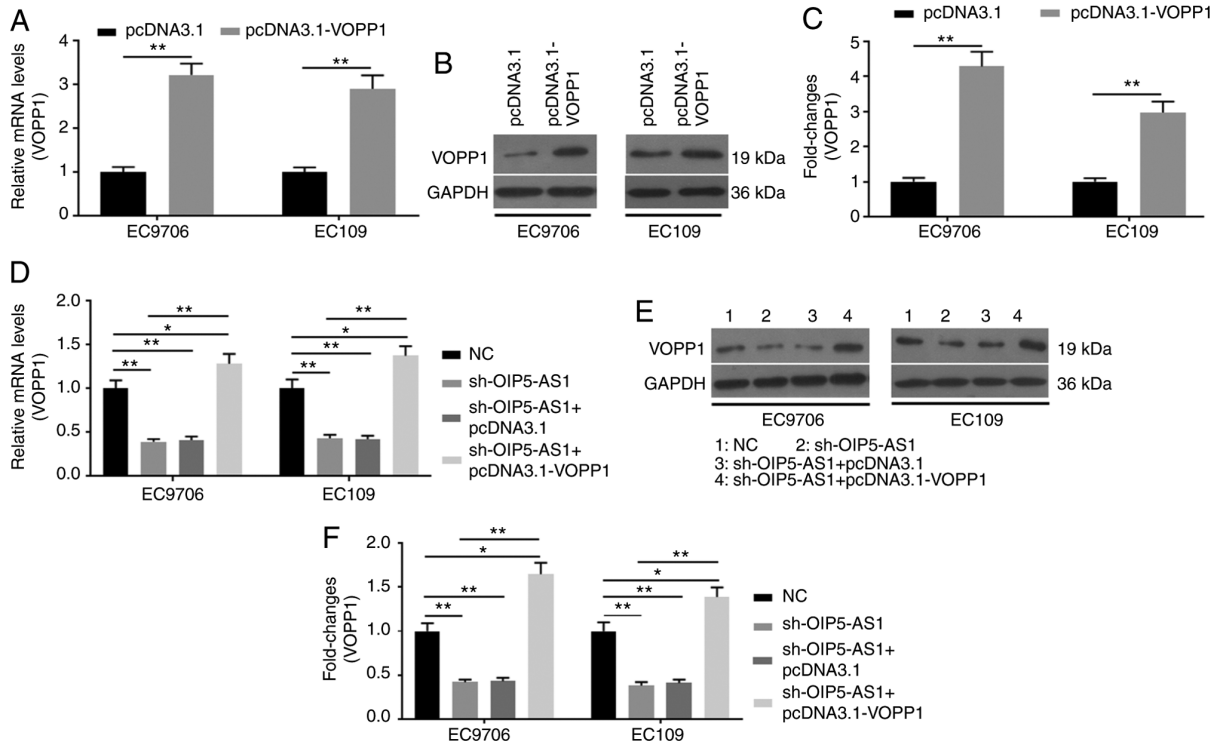


Figure 6. Overexpression of VOPPI in EC cells. (A) VOPPI mRNA expression was detected in EC cells. (B and C) VOPPI protein expression was determined in EC cells. (D) VOPPI mRNA expression was detected in NC, sh-OIP5-AS1-, sh-OIP5-AS1+pcDNA3.1- and sh-OIP5-AS1+pcDNA3.1-VOPPI-transfected EC cells. (E and F) VOPPI protein expression was determined. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$. EC, esophageal cancer; OIP5-AS1, opa-interacting protein 5 antisense transcript 1; sh, short hairpin RNA; NC, negative control; VOPPI, vesicular overexpressed in cancer prosurvival protein 1.

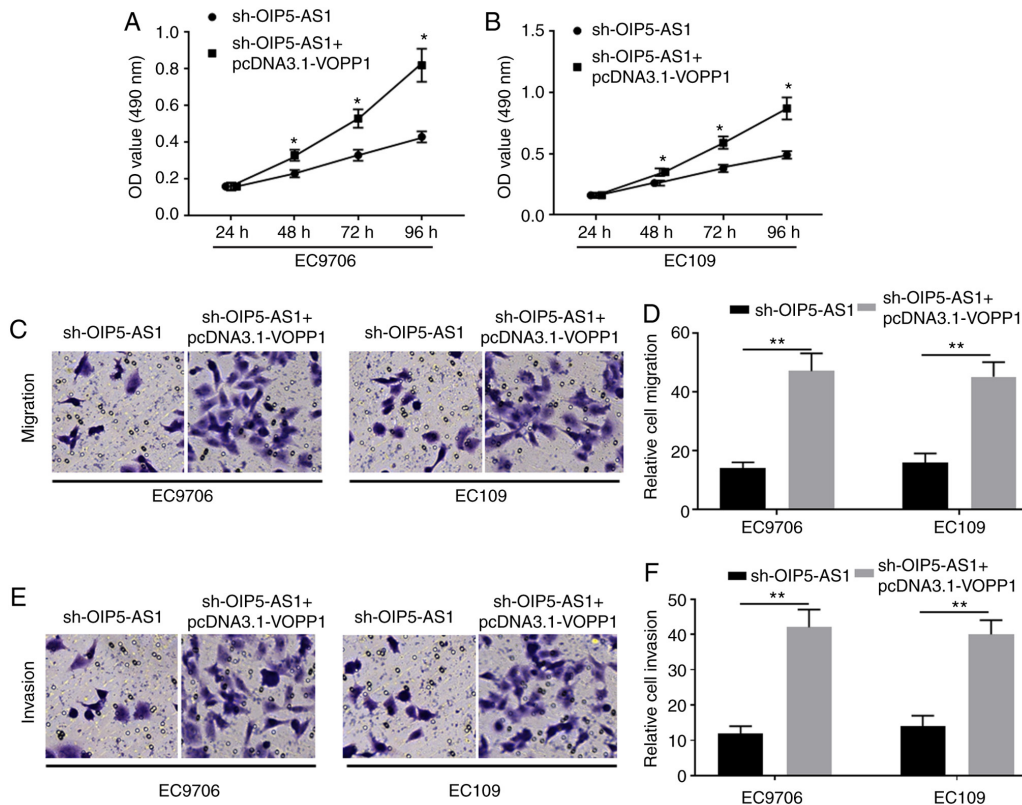


Figure 7. VOPPI overexpression improves the negative effects of OIP5-AS1-knockdown on EC cells. Proliferative activity was detected by MTT in sh-OIP5-AS1- and sh-OIP5-AS1+pcDNA3.1-VOPPI-transfected (A) EC9706 and (B) EC109 cells. (C and D) Migration (magnification, x200) and (E and F) invasion (magnification, x400) were determined in sh-OIP5-AS1- and sh-OIP5-AS1+pcDNA3.1-VOPPI-transfected EC cells. All experiments were performed in triplicate and data are presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$. OD, optical density; EC, esophageal cancer; OIP5-AS1, opa-interacting protein 5 antisense transcript 1; sh, short hairpin RNA; NC, negative control; VOPPI, vesicular overexpressed in cancer prosurvival protein 1.

miR-30a-mimics transfection produced similar effects as those of OIP5-AS1-knockdown. VOPPI was a direct target of miR-30a, and overexpression of VOPPI rescued the negative effects of OIP5-AS1-knockdown in EC cells. Collectively, OIP5-AS1 may promote EC progression via mediating the miR-30a/VOPPI axis.

Transcriptome sequencing has identified >58,000 lncRNAs in human cells (20). An integrative bioinformatics method has been used to identify the key functional lncRNAs involved in EC development (21). lncRNA625 has been found to promote cell proliferation, migration and invasion in EC cells, and it exhibits a specific prognostic value for clinical diagnosis (21). lncRNA CCAT1 expression has been reported to be upregulated in ESCC tissues, to promote cell proliferation and migration by mediating the SPRY4/HOXB13 axis via sponging miR-7, and to be associated with a poor survival in patients with ESCC (22). OIP5-AS1 has been reported to regulate carcinogenesis in various types of cancer (23). In cervical cancer, silencing of OIP5-AS1 markedly decreases cell proliferation, colony formation and invasion by increasing the expression of integrin subunit $\alpha 6$ through sponging miR-143-3 (24). Additionally, OIP5-AS1 expression is upregulated in trastuzumab-resistant breast cancer cells by mediating the miR381-3p/HMGB3 axis, and knockdown of OIP5-AS1 may rescue the sensitivity of cells to trastuzumab (25). The present study revealed that OIP5-AS1 expression was upregulated in EC tissues and cultured cells, and knockdown of OIP-AS1 expression decreased the proliferation, migration and invasion of EC9706 and EC109 cells.

Generally, lncRNAs show biological functions by sponging miRNAs, which normally degrade their target genes by binding to their 3'-UTR (26). miR-30a-5p expression is downregulated in renal cancer, and its overexpression ameliorates the malignant phenotypes of renal carcinoma cells by degrading SOX4 (27). miR-30a has been considered as a potential prognostic biomarker for clear cell renal cell carcinoma, and it inhibits cell migration and invasion of HCT116 cells (28). In the present study, it was revealed that miR-30a was downregulated in EC tissues and cultured cells, and miR-30a-mimics transfection significantly ameliorated cell migration and invasion. For further investigation of OIP5-AS1 biological functions in EC, the prediction by StarBase2.0 software was conducted, revealing that OIP5-AS1 interacted with miR-30a and inhibited its activity. Thus, it was suggested that OIP5-AS1 promoted the progression of EC by sponging miR-30a in EC9706 and EC109 cells.

To further investigate the molecular mechanism of OIP5-AS1/miR-30a in mediating EC progression, the target of miR-30a was predicted by TargetScan7.2. VOPPI was verified as the direct target of miR-30a. VOPPI expression has been reported to be upregulated in various types of cancer, including colorectal cancer, squamous cell carcinoma, gastric cancer and glioblastoma (29). VOPPI has been demonstrated to promote cell proliferation and migration, and inhibit apoptosis in SMMC-7721 and BEL-7404 cells (30) and human squamous cell carcinoma cell lines (31), and suppression of VOPPI expression attenuates EMT and the subsequent intrusion and metastasis of human lung adenocarcinoma cells (13). VOPPI acts as an essential gene for RPB3 for hepatocellular carcinoma proliferation (30). However, in breast cancer cells, VOPPI may promote cellular transformation and enhance the

growth of transplanted tumors by inhibiting the antitumor effect of WW domain-containing oxidoreductase (32). In the present study, VOPPI expression was upregulated in EC9706 and EC109 cells. VOPPI overexpression effectively rescued the effects of sh-OIP5-AS1 on EC cells. Thus, VOPPI acted as the downstream factor and the effector of OIP5-AS1. However, there are limitations in the present study that should be addressed in future studies. To further investigate the roles of OIP5-AS1/miR-30a/VOPPI in EC cells, OIP5-AS1-and/or VOPPI-knockout animals or miR-30a transgenic animals should be used.

In conclusion, OIP5-AS1 promoted the proliferation, migration and invasion of EC9706 and EC109 cells by enhancing VOPPI expression through sponging miR-30a.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SJC designed the study and wrote the manuscript. JX, ZC, ZF, SXC, YG, XL and KC performed the experiments, analyzed the data, revised and finalized the manuscript. SJC and JX confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All patients signed the consent forms prior to the use of their tissues in the present study. The experimental protocols have been approved by the Ethics Committee of The First Affiliated Jiujiang Hospital of Nanchang University (Jiujiang, China) according to the Declaration of Helsinki Principles.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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