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# Correlation of 3'-phosphoadenosine-5'-phosphosulfate synthase 1 (PAPSS1) expression with clinical parameters and prognosis in esophageal squamous cell carcinoma

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**Summary.** Background. In recent years, 3'-phosphoadenosine-5'-phosphosulfate synthase 1 (PAPSS1) has been found to be highly expressed in some cancers and significantly associated with prognosis. Nevertheless, the role of PAPSS1 in esophageal squamous cell carcinoma (ESCC) is poorly understood.

Methods. In this study, PAPSS1 expression in ESCC samples was researched through real-time quantitative polymerase chain reaction (qPCR), immunohistochemistry (IHC), and western blot (WB) techniques. siRNA technology was then used to inhibit PAPSS1 expression in ESCC cells, and cytologic tests were conducted to research gene affection on cell apoptosis, proliferation, and migration. Then, the expression of Bcl2, Ki67, and Snail was detected using qPCR and WB tests. These experimental data were analyzed by GraphPad software, where the P-value <0.05 was statistically significant.

Results. The results showed that PAPSS1 expression level in ESCC tissues was higher than in the adjacent tissues. The data also showed that PAPSS1 was significantly correlated with N stage, and that the patients with high expressions had longer survival time. After transfection for 48 hours, the cell apoptosis rate of siRNA-PAPSS1 transfected groups decreased significantly, whereas the cell proliferation rate and migration ability increased relative to the control. At the same time, the expression levels of Bcl2, Ki67 and Snail were all upregulated by siRNA-PAPSS1. PAPSS1, however, was suppressed.

Conclusions. PAPSS1 may be an ESCC suppressor gene, and its specific molecular mechanism in ESCC needs to be further studied.

**Key words:** 3'-phosphoadenosine-5'-phosphosulfate synthase 1 (PAPSS1), Esophageal squamous cell carcinoma (ESCC), Clinical information, siRNA transfection

## Introduction

Esophageal cancer ranks 7th in terms of incidence and 6th in mortality overall, the latter signifying that esophageal cancer is responsible for 1 in every 18 cancer deaths in 2020 (Sung et al., 2021). It is worth noting that the incidence rate of esophageal cancer varies in different regions (Pickens and Orringer, 2003) with a higher incidence rate of esophageal squamous cell carcinoma (ESCC) in East Asia, and eastern and southern Africa (Jemal et al., 2011). Although great progress has been made in the diagnosis and treatment of esophageal cancer, there is no particularly effective treatment for this fatal disease (Allemann et al., 2016; Patti, 2016).

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**List of abbreviations**. cDNA, complementary deoxyribonucleic acid; DMEM, Dulbecco's modification of Eagle's medium Dulbecco; DAB, diaminobenzidine; ESCC, esophageal squamous cell carcinoma; IHC, immunohistochemistry; mRNA, messenger ribonucleic acid; OS, overall survival; PAPSS, 3'-phosphoadenosine-5'-phosphatesulfate synthase; PBS, phosphate balanced solution; qPCR, quantitative polymerase chain reaction; WB, western blot; OD, optical density

3'-Phosphoadenosine-5'-phosphate sulfate synthase (PAPSS) is an essential enzyme that can produce 3'-phosphoadenosine-5'-phosphatesulfate (PAPS) as a sulfate donor (Schröder et al., 2012). Two independent subtypes, PAPSS1 and PAPSS2, have been found in human tissues. The total length of PAPSS1, which is located on human chromosome 4q24-25, is approximately 70 kb. PAPSS2 is approximately 37 kb in length and located at 10q22-23 (Xu et al., 2000). In recent years, PAPSS has attracted more attention in human physiology. For example, studies have confirmed that PAPSS1 is involved in hepatitis B infection (Shih et al., 2009) and plays an unclear role in retroviral infection (Bruce et al., 2008). PAPSS2 has been confirmed to be related to cartilage deformity (Faiyaz ul Haque et al., 1998; Kurima et al., 1998) and might be involved in steroid metabolism (Noordam et al., 2009).

There have been few studies on the relationship between PAPSS1 and tumors, which suggests that PAPSS1 may have various functions in different cancers. For example, studies on PAPSS1 in breast cancer show that the expression of PAPSS1 in cancer tissues was significantly higher than that in adjacent tissues, and overexpression of PAPSS1 may delay the growth of breast cancer cell MCF-7 by preventing the cell cycle and inducing apoptosis (Xu et al., 2012). In contrast, patients with high PAPSS1 in ovarian cancer had worse survival rates, and this trend became more significant when only patients receiving cisplatin drugs were included in the analysis (Leung et al., 2017). However, studies on PAPSS1 and ESCC have not been reported.

In this research, real-time fluorescent qPCR, immunohistochemistry, and western blot were used to study the correlation between PAPSS1 expression and clinical indicators as well as the prognosis of ESCC. At the same time, the effects of PAPSS1 on cell apoptosis, proliferation and migration were also studied by siRNA transfection.

## Materials and methods

### *Patients and tissue samples*

Human tissue cDNA microarray (cDNA-HEsoS030PG01), tissue microarray (HEso-Squ180Sur-04), and frozen samples of ESCC were all obtained from Shanghai OUTDO Biotech Co., Ltd. (Shanghai, China). The tissue cDNA microarray, including 30 cDNA spots of tumor and peritumoral tissue from 15 ESCC samples, was used to detect the mRNA level of PAPSS1 by qPCR test. PAPSS1 protein expression was evaluated in the tissue microarray, which included 100 cases of ESCC tissues and matched 80 cases of paracancerous tissues, via immunohistochemical (IHC) technique. All patients with tissues included in the tissue array received surgical esophageal resections from January 2006 to October 2008 and were followed until September 2014. Total follow-up time ranged from 71 months to 104 months, and one case was lost to follow up. Most patients provided clinical information such as sex, age, tumor size, pathological grade, and TNM (Table 1). The six frozen samples were then used in western blot assay. All the samples in the experiments were ESCCs and were obtained from surgery without preoperative radiotherapy and chemotherapy. This study was approved by the ethics committee of Shanghai OUTDO Biotech Co., Ltd. The patient verbally provided consent for us to study the molecular biological mechanism and to use the prognostic biomarkers in ESCC by using these samples.

### *IHC staining*

IHC technique was used to detect the protein expression of PAPSS1 in the tissue microarray. The section was incubated with primary antibody PAPSS1 (1:1500, Proteintech, Wuhan, China, Cat. 14708-1-ap) at 4°C overnight, after dewaxing and antigen repairing. Goat anti-rabbit and mouse IgG antibodies against PAPSS1 (ready-to-use, EnVision FLEX/HRP, DAKO,

**Table 1.** Chi-square tests were used to analyze the correction between PAPSS1 expression and the clinical data of ESCC patients.

| Clinical factors | Number in low-PAPSS1 group | Number in high-PAPSS1 group | P-value | Chi-square value |
|---|---|---|---|---|
| Sex | | | 0.600 | 0.274 |
| Male | 20 | 42 | | |
| Female | 7 | 11 | | |
| Age | | | 0.149 | 2.083 |
| ≤60 | 5 | 18 | | |
| >60 | 22 | 35 | | |
| Tumor dimeter | | | 0.206 | 1.598 |
| ≤5 cm | 15 | 37 | | |
| >5 cm | 12 | 16 | | |
| Pathological grade | | | 0.410 | 1.785 |
| Grade 1 | 2 | 2 | | |
| Grade 2 | 16 | 39 | | |
| Grade 3 | 9 | 12 | | |
| T stage | | | 0.244 | 4.163 |
| T1 | 0 | 4 | | |
| T2 | 2 | 7 | | |
| T3 | 23 | 41 | | |
| T4 | 2 | 1 | | |
| N stage | | | 0.049* | 3.890 |
| No lymph node metastatic | 8 | 28 | | |
| Lymph node metastatic | 19 | 25 | | |
| M stage | | | NA | NA |
| M0 | 27 | 53 | | |
| M1 | 0 | 0 | | |
| TNM stage | | | 0.047* | 6.105 |
| TNM 1 | 0 | 4 | | |
| TNM 2 | 8 | 26 | | |
| TNM 3 | 19 | 23 | | |
| TNM 4 | 0 | 0 | | |

*, P<0.05. PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1; ESCC, esophageal squamous cell carcinoma. Grade 1, good differentiation; Grade 2, moderate differentiation; Grade 3, poor differentiation. NA, no answer.

Copenhagen, Denmark, Cat. SM802) were used as second antibodies. Then, the tissue array was washed with phosphate buffered solution (PBST) (DAKO, Copenhagen, Denmark, Cat. DM831) and stained with diaminobenzidine (DAB) (DAKO, Copenhagen, Cat. DM827).

IHC scoring was used to evaluate PAPSS1 expression in the tissues. Three visual fields were randomly selected from different regions of each tissue point, which included 100 cells in each field. The average staining intensity of each point was recorded as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The average staining percentage of each point was recorded as follows: 0 (no staining), 1 (1-20% staining), 2 (21-40% staining), 3 (41-60% staining), 4 (61-80% staining), and 5 (81-100% staining). The scores for intensity and percentage were then multiplied to obtain the total IHC score which ranged from 0 to 15, and the patients were grouped according to the average of the score as PAPSS1 low expression (≤7) and PAPSS1 high expression (>7) in the study.

### *Cell culture*

Human ESCC cell lines, TE-1 and KYSE150, were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China, Cat. TCHu89, Cat. TCHu236) and were both identified by karyotype analysis. The cells were inoculated in DMEM (Gibco, NY, USA, Cat. 12430054) containing 10% fetal bovine serum (Gibco, NY, USA, Cat. 10099141C) and cultured in an incubator with 5% $CO_2$ at 37°C.

### *siRNA transfection*

The cells were harvested at the logarithmic growth stage, and then digested and resuspended in medium with the density adjusted to 50,000/ml. Two milliliters of cell suspension were added into a 6-well plate and cultured at 37°C. After 24 hours, the original medium was removed, the cells were gently cleaned with PBS (Thermo Fisher, MA, USA, Cat. 10010049), and, finally, washed with serum-free medium (Gibco, NY, USA, Cat. 12430054). The transfection complex, including Lipofectamine 2000 (Thermo Fisher, MA, USA, Cat. 11668019) and siRNA, was added into each well; the complete medium was replaced after six hours of transfection. Both TE-1 and KYSE150 cells were divided into a control group and an experimental group, which were transfected with siRNA-NC and siRNA-PAPSS1 (Genepharma, Shanghai, China. Cat. A10001), respectively.

### *Cell apoptosis experiment*

The cells transfected for 24 hours were adjusted to 50 000/ml and were seeded in a 96-well plate with 100 µl cell suspension in each well. After 24 hours of culture, 100 µl of staining solution (Hochest 10 ug/ml, PI 20 µM) was added to perform Hochest/PI staining at 37°C for 20 minutes in the dark. Software from Acumen Instruments (TTP Labtech, Cambridge, UK, Cat. Acumen eX3) was used to scan the cell staining results and output the experimental data. Hochest and PI were purchased from Beyotime Biotechnology (Beyotime, Nanjing, China, Cat. C1052).

### *Cell proliferation assay*

After transfection for 48 hours, cell proliferation analysis was performed by the Cell Counting Kit-8 (Vazyme, Nanjing, China, Cat. A311-01). The optical density (OD) value of each cell group was detected, and cell proliferation rate was calculated according to the following formula: cell proliferation rate = (OD value of experimental group - OD value of blank group)/(OD value of control group - OD value of blank group) × 100%.

### *Cell transwell test*

At first, Matrigel (Corning, NY, USA, Cat. 356234) was diluted with 8:1 in a serum-free medium (Gibco, NY, USA, Cat. 12430054) on ice and coated on the upper surface of the bottom membrane of a Transwell chamber at 37°C for 30 minutes to polymerize Matrigel into gels. Next, cells transfected for 6 hours were digested with trypsin, washed with PBS (Thermo Fisher, MA, USA, Cat. 10010049), and resuspended in serum-free medium for $5\times10^6$/ml suspension. Then, 100 µl cell suspension was added to the upper chamber of the Transwell and 600 µl complete medium was added to the lower chamber. After culturing at 37°C for 48 hours,

**Table 2.** Gene primer sequence for qPCR experiment.

| Gene | Forward primer sequence | Reverse primer sequence |
|---|---|---|
| PAPSS1 | 5'-GACTACTATGACTCTGAACACC-3' | 5'-TCCAGACAGACACCACAAAG-3' |
| Bcl2 | 5'-TGGAGAGCGTCAACCG-3' | 5'-CTTCAGAGACAGCCAGGAG-3' |
| Ki67 | 5'- CCTGCTCGACCCTACAGAGTG-3' | 5'- GTTGCTCCTTCACTGGGGTC-3' |
| Snail | 5'-AACCTCAAGATGCACATCCGAAGC -3' | 5'- CGGCACTGGTACTTCTTGACATCTG -3' |
| Beta-Actin | 5'-GAAGAGCTACGAGCTGCCTGA-3 | 5'-CAGACAGCACTGTGTTGGCG-3' |

PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1; qPCR, real-time quantitative polymerase chain reaction.

medium was removed, the chambers were washed with PBS 2-3 times, and 4% paraformaldehyde was added and fixed at room temperature for 20 minutes. Paraformaldehyde was removed, the cells not passing through the upper chamber were gently wiped off with a wet cotton swab, and the chambers were all cleaned with PBS. Finally, Hoechst dye (Beyotime, Shanghai, China, Cat. C0121) was added and dyed away from light for 15 minutes. Three visual fields were randomly selected under the microscope so the number of stained cells could be counted.

### *RNA extraction and reverse transcription*

Cells were harvested and collected after transfection for 48 hours. Total RNA was extracted by TRIzol (Sigma, St. Louis, MO, USA, Cat. T9424-200 ml) according to the instruction manual and quantified using the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA, Cat. ND-1000). The SuperScript IV reverse transcriptase (Thermo Fisher, MA, USA, Cat. 18090010) was used to synthesize cDNA from the total RNA.

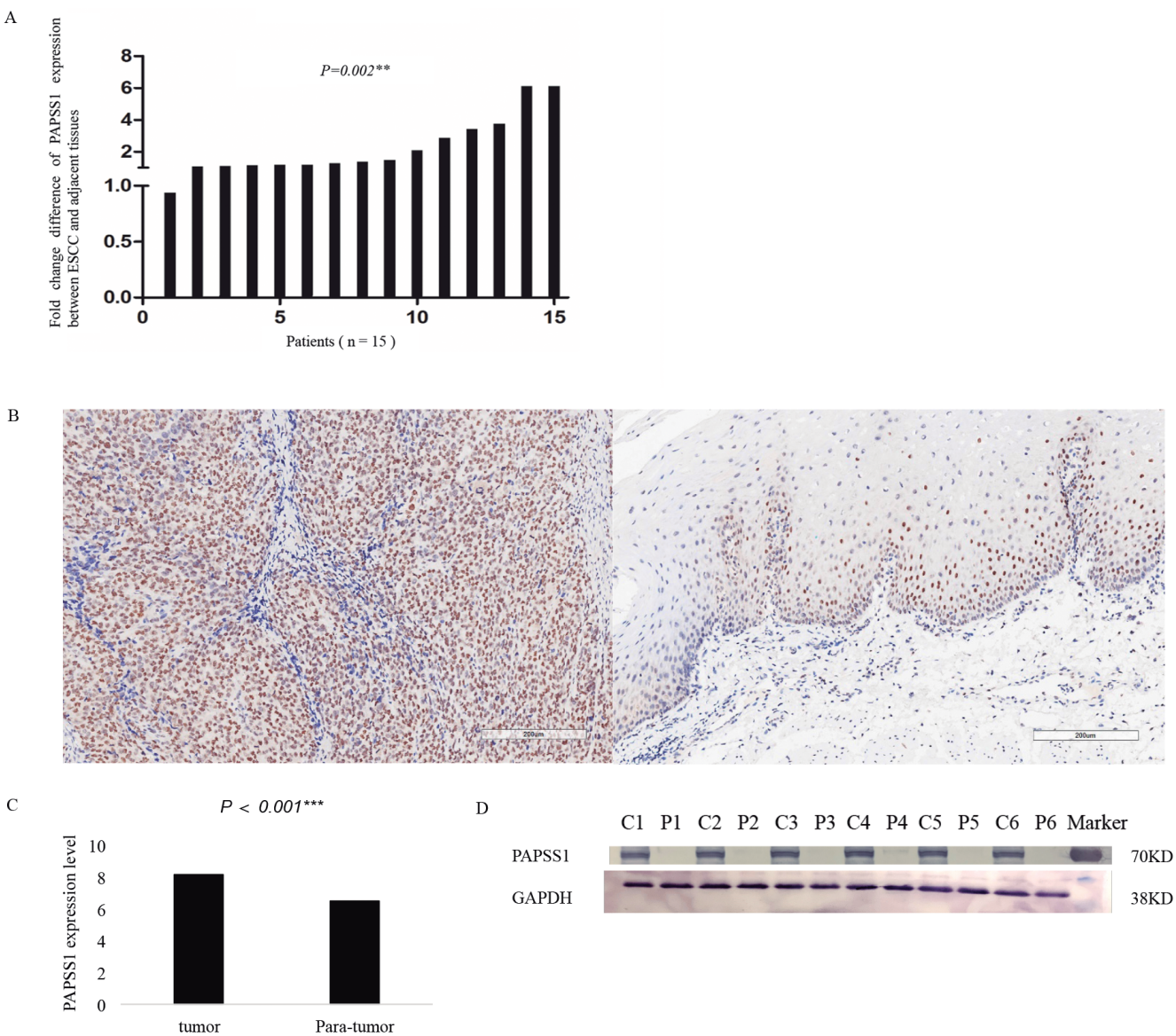


**Fig. 1.** Expressions of PAPSS1 in ESCC samples. **A.** The fold change differences of PAPSS1 mRNA expression between ESCC and the matched para-cancerous tissues were detected by qPCR test. **B.** PAPSS1 protein expression in ESCC and the adjacent normal tissues was detected using an IHC test. **C.** IHC score of PAPSS1 in ESCC and the para-tumor tissues. **D.** WB assay of PAPSS1 in ESCC (C) and the matched peri-carcinoma tissues (P). *, P<0.05; **, P<0.01. PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1; ESCC, esophageal squamous cell carcinoma; n, number; qPCR, real-time quantitative polymerase chain reaction; IHC, immunohistochemistry; WB, western blot.

*Real-time fluorescent quantitative PCR assay (qPCR)*

The mRNA expression of PAPSS1, Bcl2, Ki67, and Snail were detected by qPCR assay, and beta-actin was used as the control. The qPCR conditions were as follows: initial denaturation at 95°C for 30 seconds, denaturation at 95°C for 5 seconds, and then annealing at 60°C for 30 seconds for 40 cycles. The fluorescence signal was detected in the extension stage at 60°C. In the final step, denaturation was at 95°C for 5 seconds, annealing at 60°C for 60 seconds, and then annealing at 95°C. The fluorescence data were collected at the stage from 60°C to 90°C. SYBR Green Premix Ex Taq™ qPCR II (TAKARA, Osaka, Japan, Cat. RR820Q) and the qPCR system (Roche, Basel, Switzerland, Cat. LightCycler480) were used. The gene primers were synthesized by Shanghai Shenggong Company (Shanghai, China) (Table 2).

*Western blot (WB) assay*

The frozen samples and transfected cells were added to pre-cooled RIPA lysis buffer (Beyotime, Shanghai, China, Cat. P0013B) and lysed on ice for 20 minutes. Then, the protein concentration in the supernatant was determined using the BCA method. After that, denatured proteins were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Beyotime, Nanjing, China, Cat. P0012A, P0014B) and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, MA, USA, Cat. IPFL00010). After immersion with 5% milk blocking solution, samples were incubated with the primary antibodies (PAPSS1, 1:500, Proteintech, Wuhan, China, Cat. 14708-1-ap) (Bcl2, 1:2000, Proteintech, Wuhan, China, Cat. 12789-1-AP) (Ki67, 1:1000, ABclonal, Wuhan, China, Cat. A2094) (Snail, 1:10 000, ABclonal, Wuhan, China, Cat. A5243) overnight at 4°C, and GAPDH was used as the control. Finally, the samples were incubated with the secondary antibodies for 1 hour in the dark, and alkaline phosphatase (AP) (Solarbio, Beijing, China, Cat. K0031G-AP) was used to develop color.

*Statistical analysis*

SPSS 17.0 software (IBM Corporation, Armonk, NY, USA) was used to analyze the IHC data and $P<0.05$ was considered statistically significant. The expression difference of PAPSS1 in tumor tissues and the adjacent tissues was analyzed by paired t-test. Then, the

**Table 3.** The Kaplan-Meier method and log-rank test were used in univariate analysis of the overall survival time (OS).

| Variables | Total sample (n=80) | Mean OS (months) | log-rank | P-value |
|---|---|---|---|---|
| PAPSS1 expression | | | 5.881 | 0.015* |
| Low-PAPSS1 | 27 | 20.19±4.36 | | |
| High-PAPSS1 | 53 | 35.17±3.95 | | |
| Sex | | | 4.842 | 0.028* |
| Male | 62 | 26.63±3.20 | | |
| Female | 18 | 42.11±7.72 | | |
| Age | | | 0.032 | 0.857 |
| ≤60 | 23 | 31.30±5.61 | | |
| >60 | 57 | 29.63±3.73 | | |
| Tumor dimeter | | | 3.163 | 0.075 |
| ≤5 cm | 52 | 33.63±3.86 | | |
| >5 cm | 28 | 23.57±5.03 | | |
| Pathological grade | | | 0.339 | 0.844 |
| Grade 1 | 4 | 33.50±17.33 | | |
| Grade 2 | 55 | 28.40±3.70 | | |
| Grade 3 | 21 | 33.95±6.11 | | |
| T stage | | | 10.737 | 0.013* |
| T1 | 4 | 67.75±4.27 | | |
| T2 | 9 | 44.22±10.35 | | |
| T3 | 64 | 26.64±3.27 | | |
| T4 | 3 | 11.67±2.85 | | |
| N stage | | | 10.131 | 0.001** |
| No lymph node metastatic | 36 | 41.36±5.16 | | |
| Lymph node metastatic | 44 | 20.91±3.13 | | |
| M stage | | | NA | NA |
| M0 | 80 | _ | | |
| M1 | 0 | _ | | |
| TNM stage | | | 24.038 | < 0.001*** |
| TNM 1 | 4 | 67.75±4.27 | | |
| TNM 2 | 34 | 42.00±5.45 | | |
| TNM 3 | 42 | 16.90±2.30 | | |
| TNM 4 | 0 | _ | | |

*, P<0.05; **, P<0.01, ***, P<0.001. PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1; OS, overall survival time; Grade 1, good differentiation; Grade 2, moderate differentiation; Grade 3, poor differentiation. NA, no answer.

**Table 4.** COX multivariate regression analysis.

| | B | SE | Wald | df | Sig. | Exp(B) | 95.0% CI for Exp(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| PAPSS1 expression | -0.493 | 0.268 | 3.396 | 1 | 0.065 | 0.611 | 0.361 | 1.032 |
| Sex | -0.582 | 0.368 | 2.505 | 1 | 0.113 | 0.559 | 0.272 | 1.149 |
| T stage | 0.860 | 0.295 | 8.521 | 1 | 0.004** | 2.363 | 1.326 | 4.208 |
| N stage | 0.532 | 0.286 | 3.456 | 1 | 0.063 | 1.702 | 0.972 | 2.980 |

**, P<0.01. PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1.

correlation between PAPSS1 expression and clinical indicators was analyzed by a chi-square test. The Kaplan-Meier method and log-rank test were used in a univariate analysis of survival. Variables with statistical significance in the univariate analysis were then included in a Cox multivariate regression survival analysis. GraphPad Prism 7.0 Software (GraphPad Software, Inc., La Jolla, CA, USA) was used to analyze the data of the cell apoptosis test, cell proliferation test, and qPCR experiment. A value of $P<0.05$ was statistically significant.

## Results

### *PAPSS1 was overexpressed in esophageal carcinoma tissues.*

The mRNA expression of PAPSS1 in tissue cDNA array was detected by qPCR technique. The results show that PAPSS1 was more expressed in 14 cases of ESCC tissues and less expressed only in 1 cancer sample. PAPSS1 was overexpressed in carcinoma tissues (P=0.002) (Fig. 1A).

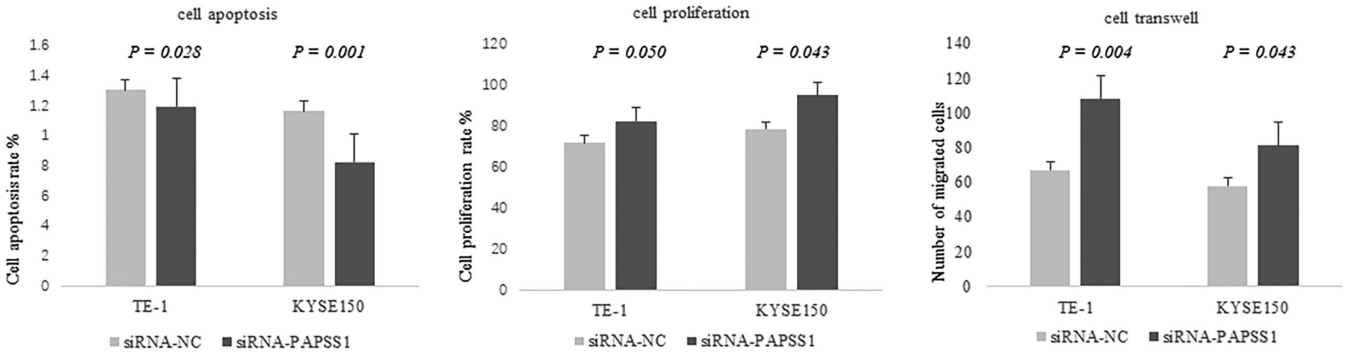


**Fig. 2.** The cell apoptosis rate of TE-1 and KYSE150 were both suppressed by siRNA-PAPSS1 **(A)**, while the cell proliferation rate of esophageal cancer cells was increased **(B)**. Transwell analysis showed that siRNA-PAPSS1 also increased the number of migrating cancer cells **(C)**. *, P<0.05; **, P<0.01. PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1.

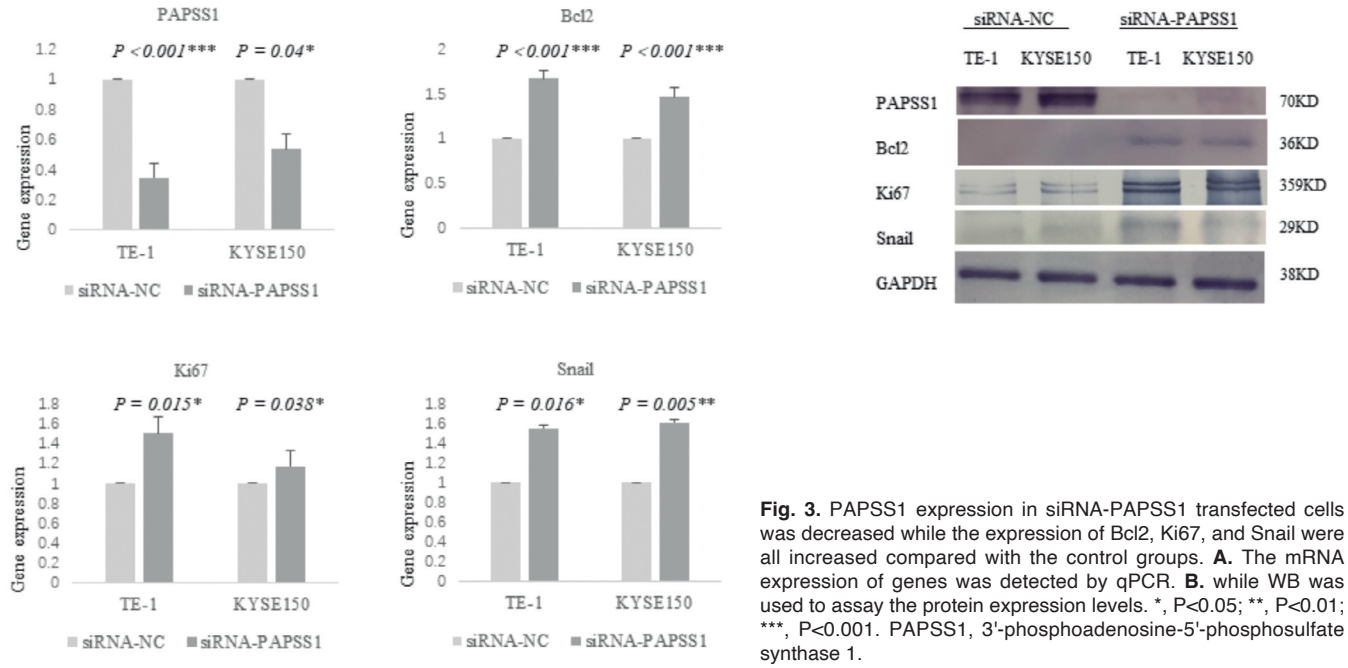


**Fig. 3.** PAPSS1 expression in siRNA-PAPSS1 transfected cells was decreased while the expression of Bcl2, Ki67, and Snail were all increased compared with the control groups. **A.** The mRNA expression of genes was detected by qPCR. **B.** while WB was used to assay the protein expression levels. *, P<0.05; **, P<0.01; ***, P<0.001. PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1.

Excluding cases that fell outside of the experiment and cases with incomplete data, the IHC staining data of PAPSS1 in samples of 80 ESCC and 53 matched adjacent tissues were collected, and they show that the protein is mainly located in the nucleus (Fig. 1B). The expression level of PAPSS1 was higher in 35 cancer tissues and lower in 10 cancer tissues and demonstrated no expression difference in tumor and peritumoral tissue of 8 patients. Paired t-test analysis showed that PAPSS1 expression in tumor samples was significantly higher than that in peritumoral tissue samples (8.15±0.32 vs 6.49±0.23, P<0.001) (Fig. 1C).

Western blot was used to detect the PAPSS1 protein level in six paired cancer samples. The protein was found to be higher expressed in cancer tissues of all six samples (Fig. 1D).

*The expression of PAPSS1 was significantly correlated with N stage of esophageal carcinoma.*

A chi-square test was used to analyze the relationship between PAPSS1 expression and the clinical information of patients with ESCC. The analysis demonstrated that PAPSS1 was significantly correlated with N stage (P=0.049), but not with other clinical parameters (P>0.05) (Table 1). The IHC score of PAPSS1 was lower in the lymph node metastatic group than that in the non-lymph node metastatic group (7.75±0.30 vs 8.07±0.41).

*The prognosis of ESCC patients with high PAPSS1 expression was significantly better.*

Univariate analysis of survival showed that the ESCC patients with a high PAPSS1 protein level had a significantly longer overall survival (OS) time (35.17±3.95 months vs. 20.19±4.36 months, P=0.015) (Fig. 4). In addition, sex, T stage, N stage, and TNM stage were also significantly associated with the prognosis of patients (P<0.05) (Table 3).

Next, variables with statistical significance in univariate analysis were included in a Cox multivariate regression survival analysis, including PAPSS1 expression, sex, T stage, and N stage. Considering the high correlation between T stage, N stage, and TNM stage, TNM stage was excluded from the analysis. The results demonstrated that only T stage was an independent predictor of survival (P=0.004) (Table 3).

*Reducing PAPSS1 expression decreased cell apoptosis of ESCC but increased cell proliferation and migration.*

To further understand the biological function of genes, siRNA technology was used to reduce PAPSS1 expression and cytological experiments were conducted. After transfection for 48 hours, the results showed that the cell apoptosis rates of TE-1 and KYSE-150 transfected with siRNA-PAPSS1 decreased by 8.37% and 29.32% (P=0.028, P=0.001), respectively, and the cell proliferation rates increased by 14.60% and 17.54% (P=0.050, P=0.043), compared with the controls (Fig. 2). siRNA-PAPSS1 also promoted the migration ability of cancer cells, which increased the number of TE-1 and KYSE-150 cells that migrated by 61.19% and 39.66%, respectively, over control values (P=0.004, P=0.043) (Fig. 2).

*Reducing PAPSS1 by siRNA-PAPSS1 also increased the expression level of Bcl2, Ki67, and Snail in the ESCC cells.*

After transfection for 48 hours, the expressions of PAPSS1, Bcl2, Ki67 and Snail were detected in the ESCC cells by qPCR and WB techniques. The mRNA level of PAPSS1 in siRNA-PAPSS1 transfected cells, TE-1 and KYSE150, was found to be reduced by 65.61% and 46.16%, respectively (P<0.001, P=0.004), when compared with the controls (Fig. 3A). However, the expression of Bcl2 in siRNA-PAPSS1 transfected cells increased by 67.02% and 46.700% (P<0.001, P<0.001), Ki67 expression level was upregulated by 50.94% and 16.93% (P=0.015, P=0.038), and Snail expression was also upregulated by 55.13% and 60.62% (P=0.016, P=0.005) over controls (Fig. 3A). The WB assay showed similar results to the qPCR test: PAPSS1 protein level was decreased in all siRNA-PAPSS1 transfected groups while Bcl2, Ki67, and Snail were all increased (Fig. 3B).

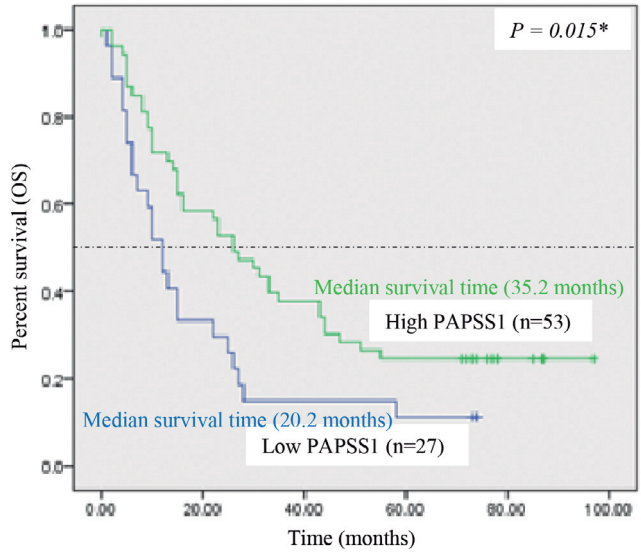


**Fig. 4.** Univariate analysis of survival showed that ESCC patients with high PAPSS1 had significantly better OS. **, P<0.01. ESCC, esophageal squamous cell carcinoma; PAPSS1, 3'-phosphoadenosine-5'-phosphosulfate synthase 1; OS, overall survival time (OS); n, number.

## Discussion

The PAPSS1 protein can produce bioactive sulfate substrate for all sulfonation reactions (Schröder et al., 2012). In recent years, studies have confirmed that PAPSS1 is involved in the progress of various cancers, including breast cancer, endometrial cancer, lung cancer, ovarian cancer, and glioma (Xu et al., 2012; Leung et al., 2015, 2017; Li et al., 2018).

In this study, the results showed that the mRNA and protein expressions of PAPSS1 in ESCC tissues were both higher than those in the matched adjacent tissues via qPCR (Fig. 1A) and WB test (Fig. 1D). In addition, IHC experiments showed that the PAPSS1 protein was mainly located in the nucleus of ESCC samples (Fig. 1B), which was consistent with the observation reported by Besset et al. (2000). The IHC score of PAPSS1 in cancer tissues was also higher (Fig. 1C). Further, a chi-square analysis found that the expression of PAPSS1 was significantly correlated with N stage (Table 1), and that the IHC score in patients with lymph node metastatic cancer was lower than that in the non-lymph node metastatic group. Survival analysis showed that ESCC patients with high PAPSS1 had significantly better overall survival (Fig. 4, Table 3), but the protein was not an independent predictor (Table 4). We speculated that PAPSS1 might prolong the overall survival of ESCC patients by inhibiting lymph node metastasis. Previous studies on PAPSS1 expression and the prognosis of lung cancer reached similar conclusions, which suggested that patients with high PAPSS1 had a longer overall survival rate when the treatment status was not specified (median survival 112.7 vs. 69 months for high vs. low PAPSS1, P=0.0019) (Leung et al., 2017). However, the research on ovarian cancer drew the opposite conclusion, demonstrating that ovarian cancer patients with high PAPSS1 had poorer disease-free survival rates (median survival 18.93 months vs. 22.5 months for high vs. low PAPSS1, P=0.012) (Leung et al., 2017).

To further understand gene function, siRNA technology was used to suppress PAPSS1 in ESCC cell lines and cytological experiments were conducted. The results demonstrated that the cell apoptosis rate in TE-1 and KYSE150 cells transfected with siRNA-PAPSS1 decreased significantly (Fig. 2A), but the cell proliferation rate and migration number increased (Fig. 2B,C) when compared with the controls. Meanwhile, the expression levels of the anti-apoptotic gene Bcl2, the cell proliferation promoting the Ki67 gene and the cell migration related Snail gene were all upregulated in the siRNA-PAPSS1 groups (Fig. 3A,B). These results confirmed that siRNA-PAPSS1 enhanced the expression of Bcl2, Ki67, and Snail while reducing PAPSS1, although the detailed molecular biological mechanism was not understood. We inferred that PAPSS1 might promote the apoptosis of ESCC cells and inhibit cell proliferation and migration by reducing the expression of Bcl2, Ki67, and Snail, and thus finally prolong the overall survival of the ESCC patients.

However, PAPSS1 was also found to be highly expressed in ESCC tissues, which seemed initially to contradict the above results. To solve the puzzle, we searched the available PAPSS1 literature and found a similar conclusion in an article on breast cancer published in 2012. The results of the article showed that the expression of PAPSS1 in ER-positive and ER-negative breast cancer tissues detected by IHC technique was both significantly higher than that in the matched adjacent tissues. The same conclusions were also confirmed in qPCR and WB experiments. However, cytological studies showed that the overexpression of SULT1E1 and PAPSS1 mediated by adenovirus could block the estrogen pro-proliferating effect, and promote the apoptosis induced by $H_2O_2$ in breast cancer cells when estrogen E2 was added, as well as downregulating the expression level of c-Myc, CyclinD1, and Bcl2. In contrast, however, no similar results were found in breast cancer cells without estrogen. It could be inferred from these results that PAPSS1 might be an anti-tumor gene in estrogen-dependent breast cancer and regulated by estrogen, although it was highly expressed in cancer tissues (Xu et al., 2012). Based on previous literature on lung cancer, ovarian cancer, and breast cancer (Xu et al., 2012; Leung et al., 2015, 2017), we concluded that PAPSS1 might have different regulatory mechanisms in different cancers, and its specific molecular biological mechanism in ESCC requires further research and discussion.

In conclusion, our study confirmed that the high expression of PAPSS1 is significantly closely associated with less lymph node metastasis and longer overall survival in ESCC patients. Reducing PAPSS1 expression can decrease the rate of apoptosis in ESCC cells while promoting cell proliferation and migration. At the same time, the levels of expression of Bcl2, Ki67, and Snail were all increased. PAPSS1 inhibited the progress of ESCC and might be a suitable biomarker for predicting esophageal cancer.

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