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PLAC8 inhibits oral squamous cell carcinogenesis and epithelial-mesenchymal transition via the Wnt/β-catenin and PI3K/Akt/GSK3β signaling pathways

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Abstract. Placenta-specific 8 (PLAC8) is closely associated with the proliferation, apoptosis and autophagy of several tumor cells. However, the expression and function of PLAC8 in oral squamous cell carcinoma (OSCC) remain unknown. Therefore, the present study investigated the function and mechanism of PLAC8 in OSCC. Reverse transcription-quantitative PCR and western blot analyses were performed to quantify the expression of PLAC8 in OSCC cell lines. The function of PLAC8 in OSCC was investigated via transfection, the Transwell and Cell Counting Kit-8 assays, immunofluorescence staining and western blotting. The results demonstrated that PLAC8 exspression was downregulated in OSCC cell lines. PLAC8 inhibited the cell proliferation in OSCC. In addition, PLAC8 restrained invasion and epithelial-mesenchymal transition of OSCC cells. Furthermore, β-catenin helped to repress PLAC8 expression by regulating the Wnt/β-catenin and PI3K/Akt/GSK3β signaling pathways in OSCC cells. Collectively, the results of the present study suggest that PLAC8 acts as a tumor suppressor in OSCC by downregulating β-catenin.

Introduction

Oral cancer is mainly represented by epithelial-derived oral squamous cell carcinoma (OSCC) (1). Over the past two decades, the incidence of OSCC has increased to ~40%, particularly in women aged 15-49 years (2). The increasing incidence and younger onset of OSCC have become global healthcare issues (3). Furthermore, the 5-year survival rate of patients with OSCC remains at <60%, without major improvements over the last three decades (4,5). The high prevalence of death is mainly due to late diagnoses, poor prognosis and absence of effective treatments (6). Therefore, the study of the biochemical pathways and the potential molecules involved in OSCC could provide valuable evidence for the development of further preventive strategies and treatments.

Placenta-specific 8 (PLAC8) is a 12.4-kDa protein that was first identified in human dendritic cells (7). PLAC8 has been demonstrated to participate in immunity, adipocyte differentiation and embryo development (8-10). Previous studies have revealed that PLAC8 is closely associated with the proliferation, apoptosis and autophagy of tumor cells (11-14). Some studies have reported that high PLAC8 expression is positively associated with progression of malignancy and that it promotes the proliferation of cancer cells in the colon and lungs, as well as in renal cell carcinoma (12,15,16). Others studies have demonstrated that downregulation of PLAC8 expression may promote the growth and viability of hepatocellular cancer cells (17). Therefore, PLAC8 may be considered as a novel biomarker of cancer. However, to the best of our knowledge, the expression and function of PLAC8 in OSCC remain unknown.

The present study aimed to reveal the role of PLAC8 in OSCC progression and investigated the function and the potential mechanism of action of PLAC8 by overexpressing and silencing it in OSCC cell lines.

Materials and methods

Cell lines and culture. Human oral epithelial cells (HOECs) were purchased from the Cell Bank of Suzhou University (Suzhou, China). Three OSCC cell lines (HN4, HN30 and HN6) were provided by the Shanghai Key Laboratory of Stomatology (School of Medicine, Ninth People's Hospital, Shanghai

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Jiaotong University, Shanghai, China). Cells were seeded in Dulbecco's modified Eagle's medium (cat. no. 21013024; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (cat. no. 35-010-CV; Corning, Inc.) at 37˚C in 5% CO_2. XAV939 (100 μM; cat. no. X3004; Sigma-Aldrich; Merck KGaA), a potent small-molecule inhibitor of β-catenin (18), was used to treat HN4 cells for 72 h.

Western blotting. Western blotting was performed as previously described (19). Total protein was analyzed by 10% SDS-PAGE and blotted on a nitrocellulose filter membrane (cat. no. 10401196; Whatman plc; GE Healthcare Life Sciences). The primary antibodies (all used at 1:1,000) were all from Cell Signaling Technology, Inc., and were as follows: PLAC8 (cat. no. 13885), proliferating cell nuclear antigen (PCNA; cat. no. 13110), cyclin D1 (cat. no. 2978), vimentin (cat. no. 5741), c-Myc (cat. no. 5605), E-cadherin (cat. no. 14472), total-β-catenin (cat. no. 9587), glycogen synthase kinase 3β (GSK3β; cat. no. 12456), non-phospho (Active) β-catenin (Ser33/37/Thr41; cat. no. 8814S), phosphorylated-GSK3β (cat. no. 5558), Akt (cat. no. 4685) and phosphorylated-Akt (cat. no. 4060). β-actin (1:2,000; cat. no. 3700) was used as the control standard. Horseradish peroxidase-conjugated rabbit anti-mouse (1:2,000; cat. no. p0161; Dako; Agilent Technologies, Inc.) or goat anti-rabbit (1:5,000; cat. no. sc2357; Santa Cruz Biotechnology, Inc.) were used as secondary antibodies. The blots were probed with primary antibodies overnight at 4˚C and then incubated with the secondary antibody for 1 h at room temperature. An electrochemiluminescence western-blotting system (cat. no. 7003; Cell Signaling Technology, Inc.) was used to detect protein bands (Tanon 4600SF; Tanon Science and Techonlogy Co., Ltd.).

RNA extraction and reverse transcription-quantitative PCR. Total RNA was extracted from HOEC, HN4, HN30 or HN6 cells using TRIzol® reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.). Complementary DNA was synthesized using the RNA reverse transcriptase kit (cat. no. RR036A; Takara Bio, Inc.) according to the manufacturer's protocol. The temperature protocol was as follows: 37˚C for 15 min and 85˚C for 5 sec. Quantitative PCR was performed using SYBR™ Premix Ex Taq™ II (cat. no. RR820Q; Takara Bio, Inc.) in an ABI 7300 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: 94˚C for 5 min; 40 cycles at 94˚C for 30 sec, 58˚C for 45 sec and 70˚C for 50 sec. The expression of mRNA was assessed by evaluating the threshold cycle (CT) values, and GAPDH was used as the internal reference gene. Relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method (20). The following primers were used: GAPDH forward, 5'-AGGTCGGAGTCAACGGATTTGGT-3' and reverse, 5'-GTGCAGGAGGCATTGCTGATGAT-3'; and PLAC8 forward, 5'-CTGTCTGTGTGGAACAAGC-3' and reverse, 5'-GAGGACAGCAAAGAGTTGCC-3'.

Cell transfection. Transfection experiments were performed using Lipofectamine® 2000 (cat. no. 11668030; Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Briefly, HN4 and HN30 cells were transfected with PLAC8 plasmid (Sangon Biotech, Co., Ltd.) and control vector (cat. no. 631070; Takara Bio Inc.). HN6 cells were transfected with PLAC8 small interfering (si)RNA and negative control (Hippobiotec, Inc., http://www.hippobiotec.com). The OSCC cell lines were seeded into 6-well plates ($2.5x10^5$ cells/well) and treated with 10 ug plasmid or 20 μM siRNA after 24 h of culture. Cells were harvested after 72 h of culture for western blot analysis. Cells were regularly passaged for the Cell Counting Kit-8 (CCK-8) assay. Targeted sequences of PLAC8 siRNAs were: siRNA#1 sense, 5'-CUUUGCCAAAUCAAGAGAGAUdTdT-3' and antisense, 5'-AUCUCUCUUGAUUUGGCAAAGdTdT-3'; siRNA#2 sense, 5'-GCUGAUAUGAAUGAAUGCUGUdTdT-3' and antisense, 5'-ACAGCAUUCAU UCAUAUCAGCdTdT-3'; and negative control siRNA (siNC) sense, 5'-UUCUCCGAACGUGUCACGUTT-3'. Altered expression of PLAC8 was verified by western blotting.

CCK-8 assay. Cells were seeded at $5x10^3$ cells/well in a 96-well plate for 1-5 days. Cell proliferation was measured using CCK-8 (CK04; Dojindo Molecular Technologies, Inc.) according to the manufacturer's protocol. The absorbance was measured at a wavelength of 450 nm using a spectrophotometer (Omega Bio-Tek, Inc.).

Transwell invasion assay. Invasion of OSCC cells was measured using Matrigel®-coated Transwell chambers (cat. no. 354480; Corning Inc.). Briefly, Matrigel-coated Transwell chambers were rehydrated at 37˚C in 5% CO_2 for 2 h. Subsequently, $2.5x10^4$ cells were seeded in the Matrigel-coated Transwell chambers and culture medium without serum was placed in the lower compartment and incubated for 24 h at 37˚C in 5% CO_2. Non-invasive cells in the upper membrane were removed using a cotton swab, whilst the invasive cells were stained at room temperature for 2 min using the Differential Quik Stain kit (cat. no. B4132-1A; Allegiance Chemicals LLC), and manually counted in five pre-determined fields under a light microscope (magnifications, x10 and x40; BX51, Olympus Corporation).

Immunofluorescence staining. Immunofluorescence staining was performed as previously described (21). Briefly, adherent cells grown on coverslips were washed thrice with PBS and incubated in PBS containing 0.2-0.3% Triton X-100. Subsequently, E-cadherin (cat. no. 14472) and vimentin (cat. no. 5741) antibodies (both Cell Signaling Technology, Inc.) were diluted to 1:100 and incubated overnight at 4˚C with cells. Next, Alexa Fluor 555 anti-mouse IgG (H+L) (cat. no. 4409) and Alexa Fluor 488 anti-rabbit IgG (H+L) (cat. no. 4412) (both Cell Signaling Technology, Inc.) were incubated for 1 h at room temperature using a 1:200 dilution. Subsequently, DAPI (cat. no. H-1200; Vector Laboratories, Inc.) was used for nuclear staining for 1 min at room temperature. Images were captured using a fluorescence microscope (BX51; Olympus Corporation; magnification, x200).

Statistical analysis. Statistical analyses were conducted using GraphPad Prism 5.0 (GraphPad Software, Inc.). Paired Student's t-test and one-way ANOVA followed by Tukey's post hoc test were used for comparisons of two or multiple groups, respectively. All experiments were performed in triplicate and

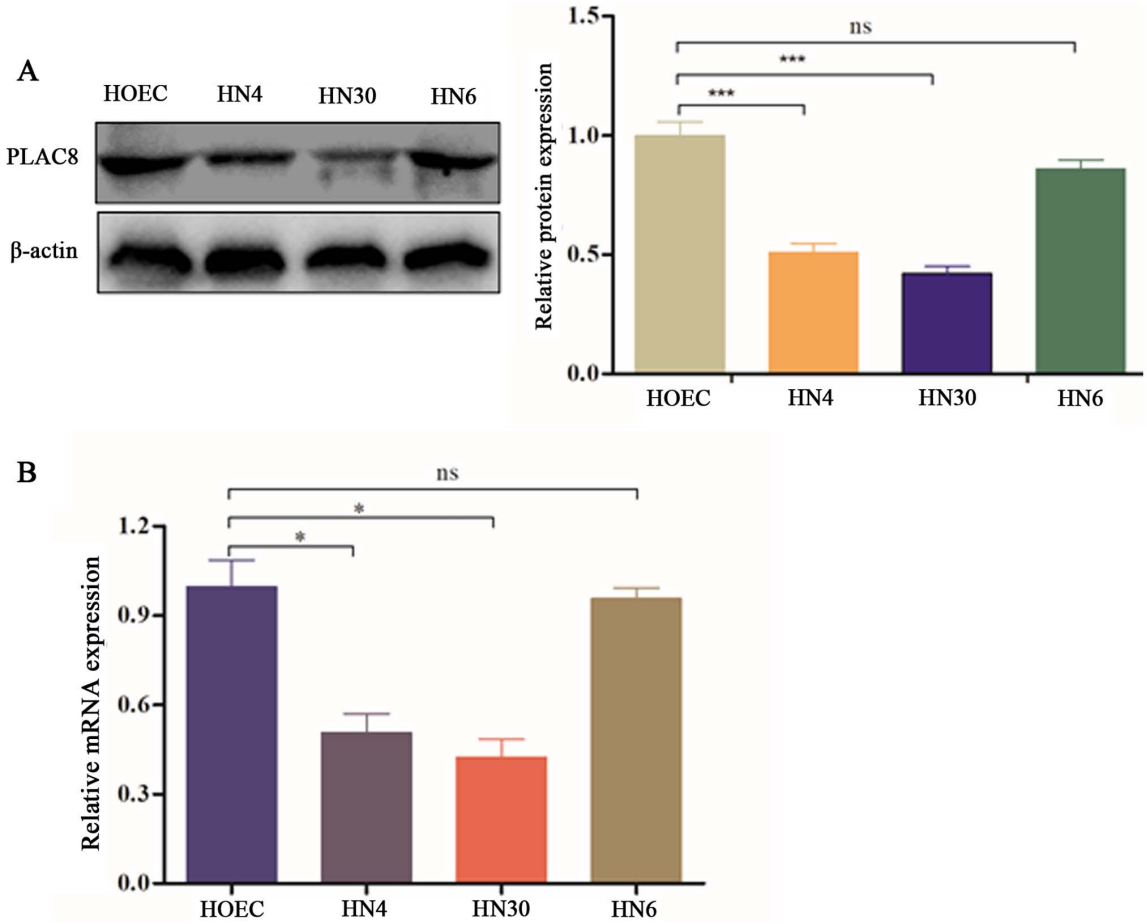

Figure 1. PLAC8 expression in HOECs and oral squamous cell carcinoma cell lines. (A) Western blot analysis of PLAC8 protein expression and (B) relative PLAC8 mRNA expression in HOECs, HN4, HN30 and HN6 cells. Data are presented as the mean ± SD. *P<0.05, ***P<0.001 according to one-way ANOVA with Tukey's post hoc test. HOEC, human oral epithelial cell; PLAC8, placenta-specific 8; ns, not significant.

data are presented as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

PLAC8 expression is downregulated in OSCC cells. To assess whether PLAC8 is expressed in OSCC, three OSCC cell lines and primary HOECs were selected to measure PLAC8 expression. Relative expression levels of the PLAC8 protein were decreased in HN4, HN30 and HN6 cells compared with in HOECs, with statistically significant differences in HN4 and HN30 cells (P<0.001; Fig. 1A). Additionally, PLAC8 mRNA expression was significantly downregulated in HN4 and HN30 cells compared with that in HOECs (P<0.05); however, HN6 cells exhibited no significant difference in PLAC8 mRNA expression compared with HOECs (Fig. 1B). Overall, the present findings suggested that OSCC cells had a low PLAC8 expression.

PLAC8 overexpression inhibits the proliferation of OSCC cells. In order to understand the function of PLAC8 in OSCC, HN4 and HN30 cells were selected for the overexpression of PLAC8, since PLAC8 expression was lower in these two cell lines compared with that in HN6 cells (Fig. 1). Western blot analysis was performed to confirm that PLAC8 was significantly increased following transfection with PLAC8 plasmid compared with non-transfected cells and cells transfected with empty vector (Fig. 2A). PLAC8 overexpression significantly suppressed cells proliferation from day 3 according to the CCK-8 assay, compared with non-transfected HN4 and HN30 cells (P<0.05; Fig. 2B. In additional experiments, PLAC8 overexpression significantly decreased the expression levels of PCNA, c-Myc and cyclin D1 in HN4 and HN30 cells (P<0.001; Fig. 2C and D, respectively). The present results suggested that PLAC8 overexpression suppressed the proliferation of OSCC cells.

PLAC8-silencing promotes the proliferation of OSCC cells. Two siRNAs were used to silence PLAC8 expression for further evaluation of the function of PLAC8 in OSCC. Expression levels of the PLAC8 protein were significantly decreased by transfection with siRNA#1 (si-#1) and siRNA#2 (si-#2) compared with those transfected with the negative control, therefore, si-#1 was randomly selected for further experimentation (P<0.001; Fig. 3A). The proliferation of HN6 cells was significantly increased from day 2 after silencing of PLAC8 expression (P<0.05; Fig. 3B). Western blotting revealed that protein levels of PCNA, c-Myc and cyclin D1 were significantly increased following PLAC8-knockdown (P<0.001; Fig. 3C). The present findings further indicated that PLAC8 repressed the proliferation of OSCC cells.

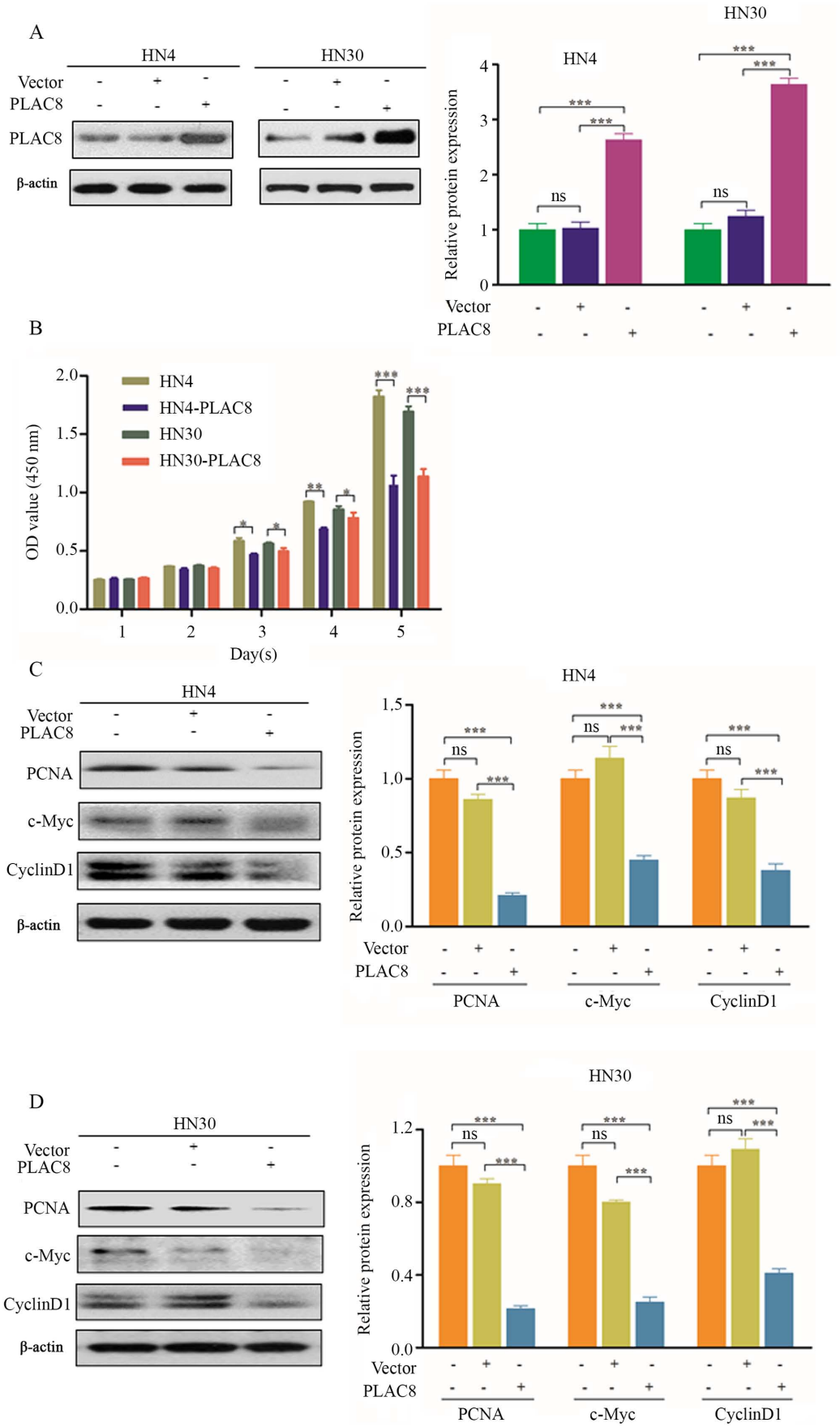

Figure 2. Effect of PLAC8 overexpression on the proliferation of oral squamous cell carcinoma cells. (A) PLAC8 overexpression in HN4 and HN30 cells analyzed via western blotting. (B) Proliferation of control and PLAC8-overexpressed cells at 1-5 days measured via the Cell Counting Kit-8 assay. Data are presented as the mean ± SD (n=3). Western blot analysis of PCNA, c-Myc and cyclin D1 expression in PLAC8-overexpressed (C) HN4 and (D) HN30 cell lines. Data are presented as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 according to one-way ANOVA with Tukey's post hoc test. PLAC8, placenta-specific 8; OD, optical density; PCNA, proliferating cell nuclear antigen; ns, not significant.

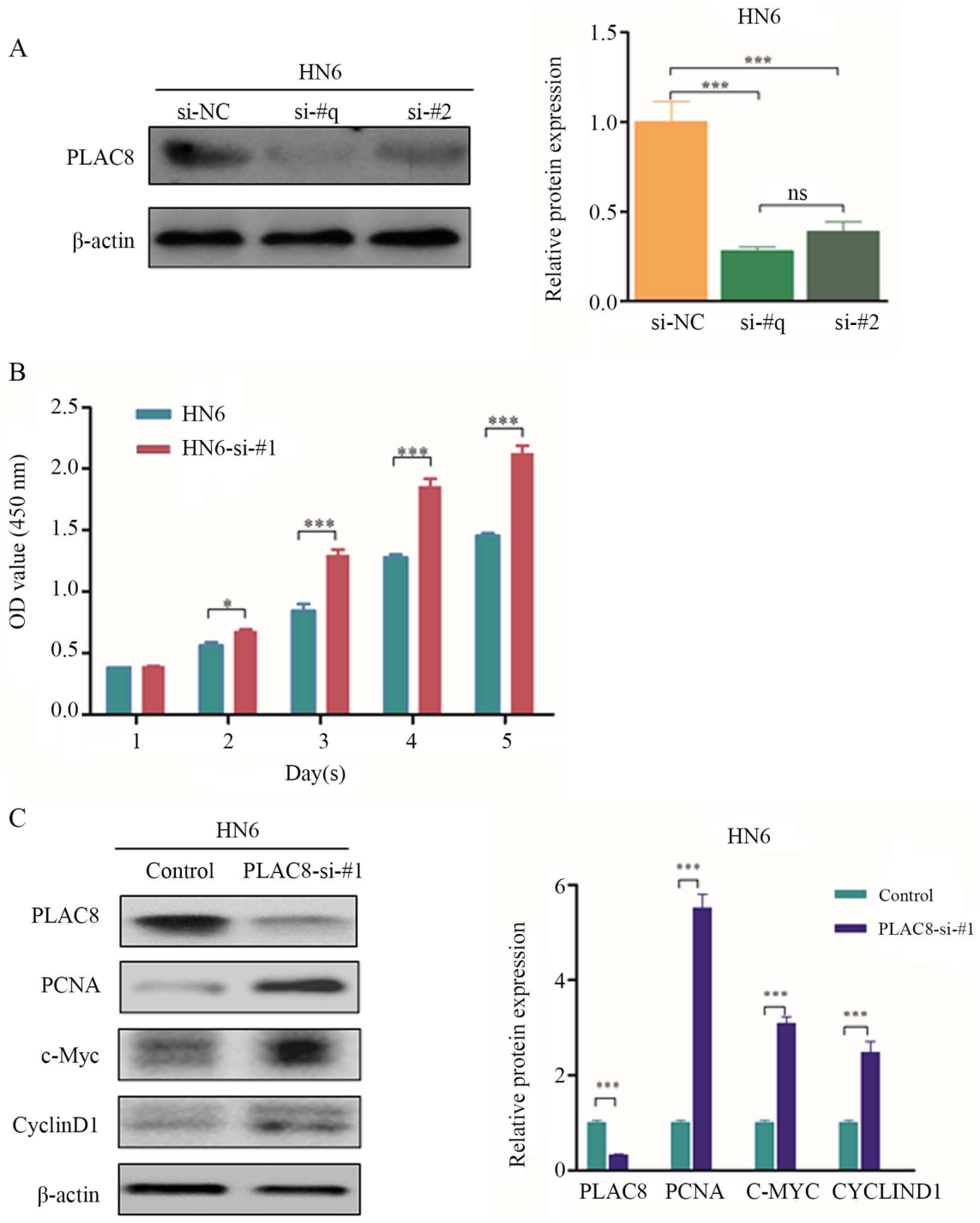

Figure 3. Effect of PLAC8 silencing on the proliferation of oral squamous cell carcinoma cells. (A) Western blot analysis of PLAC8 expression in HN6 cells after transfection with two PLAC8 siRNAs. Data are presented as the mean ± SD and analyzed by one-way ANOVA with Tukey's post hoc test. (B) Proliferation of control and PLAC8 siRNA#1-transfected cells at 1, 2, 3, 4 and 5 days measured via the Cell Counting Kit-8 assay. Data are presented as the mean ± SD. (C) Western blot analysis of PCNA, c-Myc and cyclin D1 expression in PLAC8-knockdown HN6 cells. Data are presented as the mean ± SD. *P<0.05, ***P<0.001. PLAC8, placenta-specific 8; OD, optical density; PCNA, proliferating cell nuclear antigen; si, small interfering; NC, negative control; ns, not significant.

PLAC8 inhibits the invasion and epithelial-mesenchymal transition (EMT) of OSCC cells. Transwell assays were performed to assess the role of PLAC8 on the invasive ability of OSCC cells. The invasive ability of HN6 cells was significantly increased after PLAC8 expression was silenced (P<0.001; Fig. 4A and B), which suggested that PLAC8 inhibited the invasion of OSCC cells.

To ascertain if EMT is involved in how PLAC8 influences the invasion of cells, western blotting was used to detect EMT-associated markers. E-cadherin expression was significantly increased, whereas vimentin expression was significantly decreased following PLAC8-overexpression in HN4 and HN30 cells (P<0.001; Fig. 4C). Knockdown of PLAC8 expression resulted in a significant downregulation of E-cadherin expression

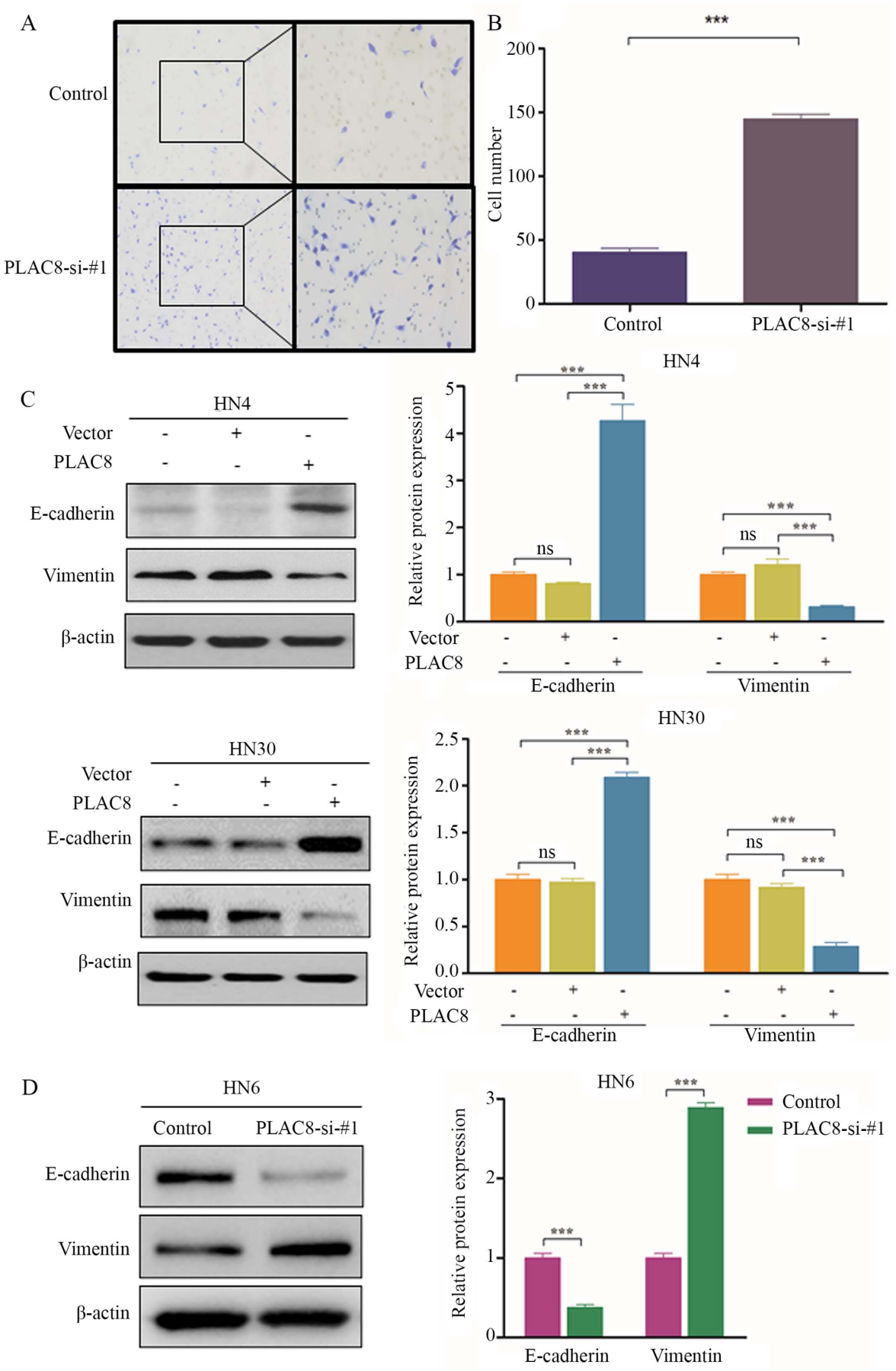

Figure 4. PLAC8 inhibits the invasion and EMT of OSCC cells. (A) Transwell assays were conducted using control or PLAC8-siRNA-tranfected HN6 cells. Magnification, x100 (left) and x400 (right). (B) Cell numbers were assessed via the Transwell assay. Western blot analysis was used to measure protein levels of EMT biomarkers (E-cadherin and vimentin) in OSCC cells after (C) PLAC8 overexpression or (D) knockdown. Data are presented as the mean ± SD. ***P<0.001 according to one-way ANOVA with Tukey's post hoc test.

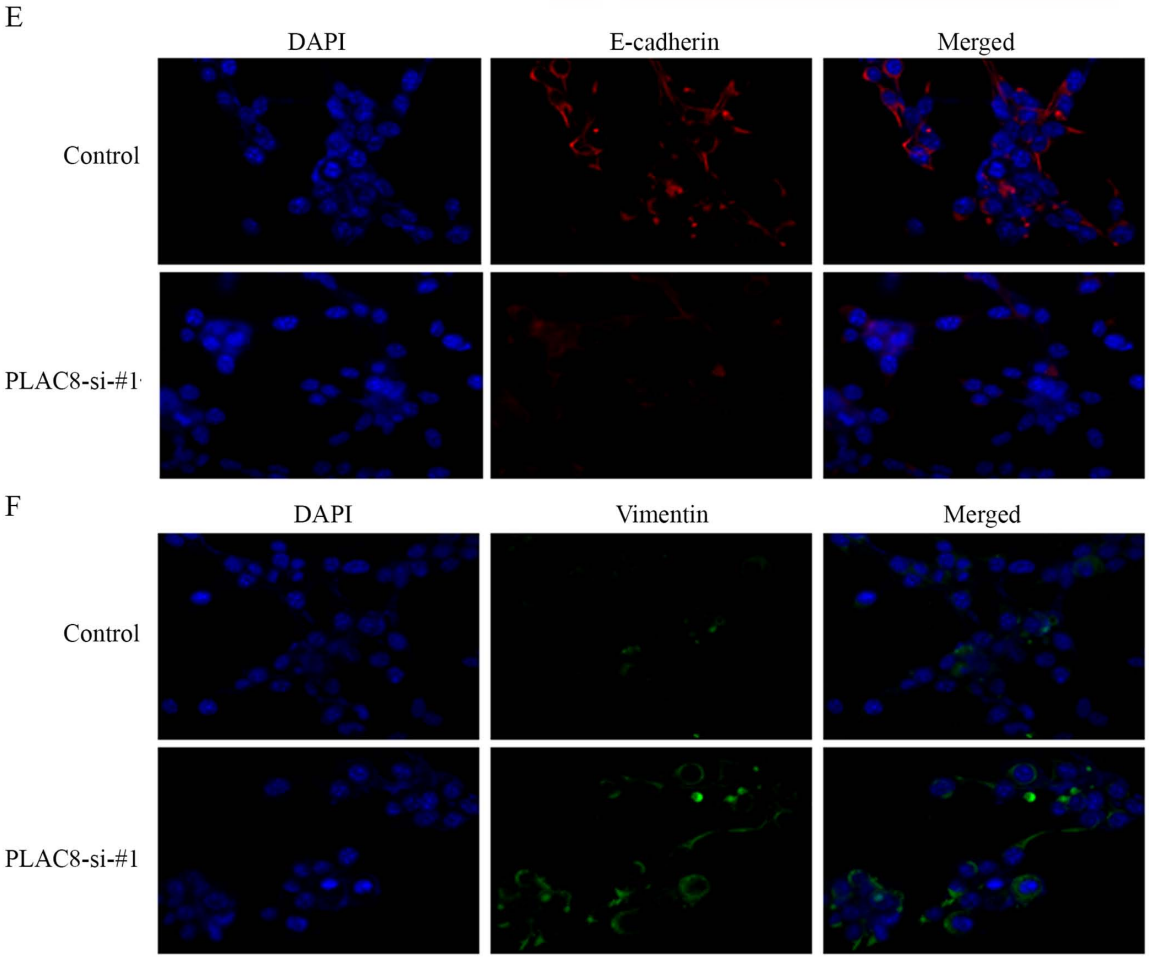

Figure 4. (Continued). (E and F) Immunofluorescence staining was used to measure protein levels of E-cadherin and vimentin in HN6 cells. Magnification, x200. EMT, epithelial-mesenchymal transition; OSCC, oral squamous cell carcinoma; PLAC8, placenta-specific 8; si, small interfering; DAPI, 4',6-diamidino-2-phenylindole; ns, not significant.

and significant upregulation of vimentin expression in HN6 cells (P<0.001; Fig. 4D). Immunofluorescence staining indicated higher EMT activity in HN6 cells in the silenced-PLAC8 group compared with in the control group (Fig. 4E and F). Overall, the present data suggested that PLAC8 may inhibit the invasive ability of OSCC cells via EMT suppression.

β-catenin helps to repress PLAC8 expression in OSCC cells. Overexpression or knockdown of PLAC8 affected c-Myc and cyclin D1 expression (Figs. 2D and 3D), which are target genes of the Wnt signaling pathway. β-catenin is a pivotal player of Wnt signaling and promotes EMT in cancer (22). Therefore, the role of PLAC8 in regulating β-catenin expression was analyzed. PLAC8-overexpression significantly decreased the protein levels of non-phospho β-catenin (Ser33/37/Thr41) compared with HN4 cells transfected with empty vector, whereas PLAC8 silencing performed after overexpression of PLAC8 reverted non-phospho β-catenin (Ser33/37/Thr41) expression in HN4 cells (P<0.001). Furthermore, PLAC8-overexpression slightly increased the protein levels of total β-catenin compared with HN4 cells transfected with empty vector, whereas PLAC8 silencing performed after overexpression of PLAC8 significantly decreased total β-catenin expression in HN4 cells (P<0.05; Fig. 5A).

Whether inhibition of β-catenin expression affected PLAC8 function was also evaluated. Expression levels of non-phospho β-catenin (Ser33/37/Thr41) were significantly decreased when XAV939 (a β-catenin inhibitor) was used to treat HN4 cells in which PLAC8 expression had been knocked down (Fig. 5B). Furthermore, protein levels of c-Myc, cyclin D1 and vimentin were significantly downregulated, while those of E-cadherin were significantly upregulated after XAV939 treatment (P<0.001; Fig. 5B). The present findings indicated that PLAC8 may inhibit the proliferation and EMT pathway of OSCC cells by inactivating the Wnt/β-catenin pathway.

Akt phosphorylation of GSK3β may lead to stabilization of β-catenin and accumulation of nuclear β-catenin (22). Numerous studies have suggested that Wnt signaling cross-talks with the PI3K/Akt signaling pathway (17,23). Therefore, the present study investigated whether PLAC8 could also contribute to the PI3K/Akt/GSK3β signaling pathway in OSCC. PLAC8 overexpression significantly decreased the ratio of phosphorylated/total protein of GSK3β and Akt (P<0.001), while silencing overexpressed-PLAC8 slightly increased the ratio of phosphorylated/total protein of GSK3β and Akt in HN4 cells (P<0.005; Fig. 5C). XAV939 treatment resulted in a significant decrease in the protein levels of phosphorylated-GSK3β and phosphorylated-Akt in PLAC8-silenced HN4

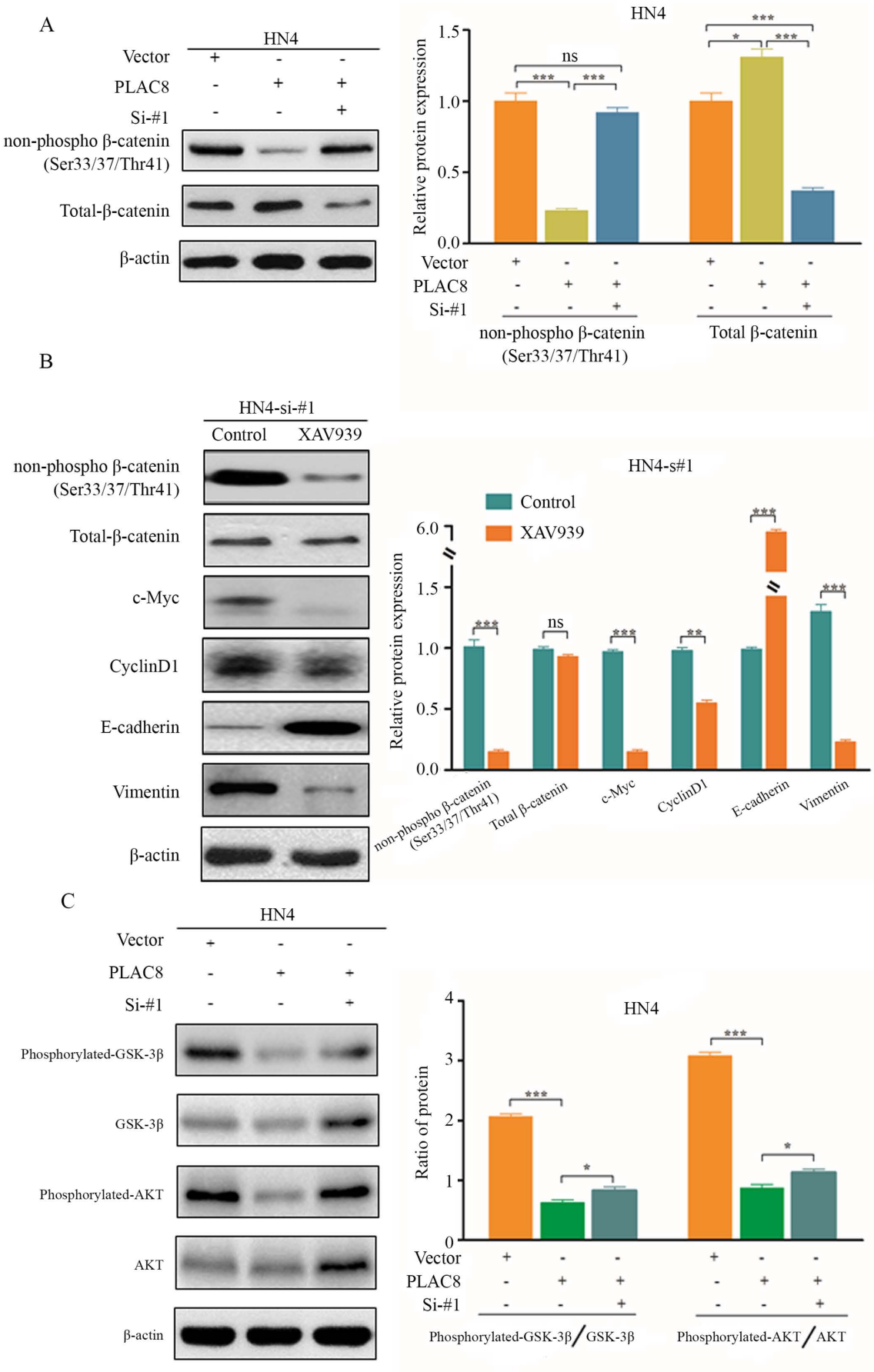

Figure 5. β-catenin contributes to the inhibitory effect of PLAC8 in HN4 cells. (A) Protein levels of non-phospho β-catenin (Ser33/37/Thr41) and total β-catenin in HN4 cells treated with PLAC8-overexpressed and/or PLAC8-siRNA. (B) Protein levels of non-phospho β-catenin (Ser33/37/Thr41), total β-catenin, c-Myc, cyclin D1, E-cadherin and vimentin in PLAC8-knockdown HN4 cells with or without XAV939 treatment. (C) Protein levels of phosphorylated-GSK3β, total GSK3β, phosphorylated-Akt and total Akt in HN4 cells treated with PLAC8-overexpressed and/or PLAC8-siRNA.

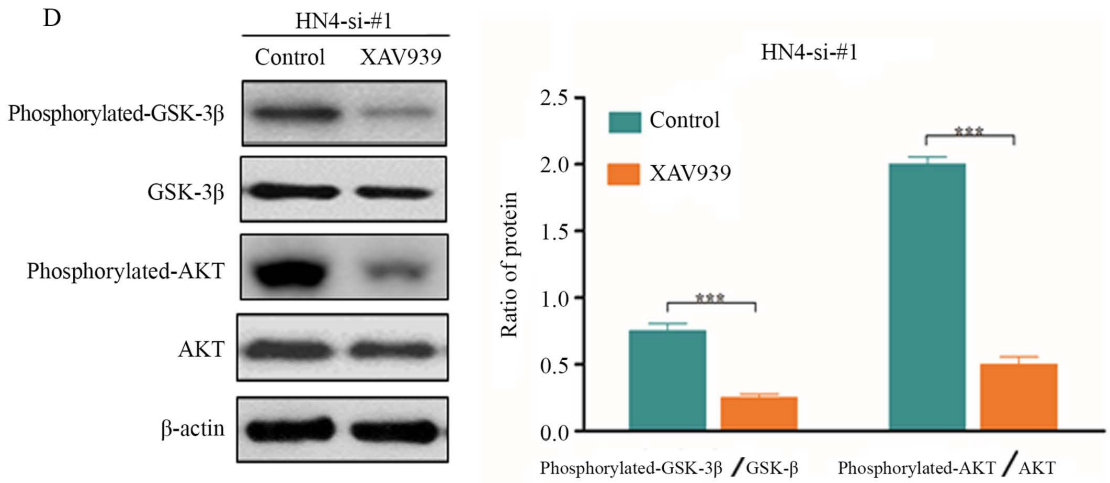

Figure 5. (Continued). (D) Protein levels of phosphorylated-GSK3β, total GSK3β, phosphorylated-Akt and total Akt in PLAC8-knockdown HN4 cells with or without XAV939 treatment. Data are presented as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 according to one-way ANOVA with Tukey's post hoc test. PLAC8, placenta-specific 8; si, small interfering; GSK3β, glycogen synthase kinase 3β; ns, not significant.

cells, which suggested that β-catenin contributed to the inhibitory effect of PLAC8 in the PI3K/Akt signaling pathway (Fig. 5D). Overall, these results suggested that PLAC8 may regulate the PI3K/Akt and Wnt/β-catenin signaling pathways via suppression of β-catenin expression in OSCC cell lines.

Discussion

Oral cancer has become a global health problem due to its high incidence, high mortality and early onset. According to the GLOBOCAN, oral cancer may have caused 350,000 deaths with 710,000 new cases worldwide in 2018 (24,25). OSCC accounts for >90% of oral malignant tumors (26). In the present study, PLAC8 expression in OSCC cells. PLAC8 expression was lower in OSCC cell lines compared with primary HOECs. In addition, PLAC8 appeared to repress cell invasion and EMT features via downregulation of the expression levels of non-phospho β-catenin (Ser33/37/Thr41) in OSCC cells. Furthermore, protein levels of Wnt/β-catenin target genes and phosphorylated-Akt/GSK3β were inhibited in PLAC8-knockdown cells after treatment with a β-catenin inhibitor. The present results indicated that PLAC8 may inhibit the proliferation and EMT features of OSCC cells by repressing β-catenin expression, which regulates the Wnt/β-catenin and PI3K/Akt/GSK3β signaling pathways.

PLAC8 has been observed in several cell types, including dendritic, myeloid, lymphoid and epithelial cells, and its functions vary depending on cellular processes and human diseases (9,7). PLAC8 has been described as a tumor suppressor and a tumor promoter in different types of cancer. For example, PLAC8 overexpression promotes the tumorigenic transformation in clear-cell renal-cell carcinoma, lung cancer, colon cancer and breast cancer, but it also suppresses the proliferation of hepatocellular carcinoma cells (12-17). In accordance with the latter finding, the present study identified that PLAC8 expression was significantly lower in OSCC cell lines compared with human oral epithelial cells. Furthermore, PLAC8 expression inhibited the proliferation of OSCC cells, and downregulated the protein levels of PCNA, c-Myc and cyclin D1, which are transcriptional markers of tumor cell proliferation (21,27). The present results suggested that downregulated expression of PLAC8 may be an indicator of oral tumor progression, and may serve an important part in the inhibition of this malignancy.

OSCC cells often display EMT features in invasion and metastasis (28). In the present study, PLAC8-knockdown increased the invasion of OSCC cells, suggesting that PLAC8 may be involved in EMT regulation. EMT is characterized by epithelial cells acquiring a mesenchymal behavior, caused by loss of intercellular adhesions and apicobasal polarity, and gain of mesenchymal proteins, which promotes the proliferation of cancer cells (29,30). Data from western blotting and immunofluorescence experiments in the present study revealed that E-cadherin expression was increased, while vimentin expression was decreased after PLAC silencing. Conversely, PLAC8 overexpression upregulated E-cadherin expression and downregulated vimentin expression. The present results suggested that PLAC8 inhibited the invasion of OSCC cells accompanied by EMT progression.

The functions of β-catenin include the regulation of gene transcription and cell-cell adhesions (31). Nuclear expression of β-catenin inhibits the transcription of E-cadherin and induces EMT by binding to members of the T-cell factor/lymphoid enhancer factor family (32). The lower expression of E-cadherin causes β-catenin accumulation (33). Additionally, β-catenin, as the main component of the Wnt/β-catenin signaling pathway, induces the transcription of c-Myc and cyclin D1 that stimulates cancer cell proliferation (22). It has been previously demonstrated that the Wnt/β-catenin signaling pathway is important in cathelicidin-promoted growth of lung and colon cancer cells (19,23,34). In the present study, PLAC8 overexpression downregulated c-Myc and cyclin D1 expression. Overall, the present data indicated that β-catenin may be involved in the inhibitory effect of PLAC8 on OSCC cells. Therefore, β-catenin expression was measured in OSCC cells: PLAC8 overexpression inhibited the expression of non-phospho β-catenin (Ser33/37/Thr41), whereas PLAC8 knockdown promoted the expression of non-phospho

β-catenin (Ser33/37/Thr41). Additionally, the expression levels of c-Myc and cyclin D1 were significantly decreased, and EMT reversion was induced after XAV939 (a β-catenin inhibitor) treatment in HN4 cells in which PLAC8 expression had been knocked down. The present findings indicated that PLAC8 may attenuate EMT in OSCC cells by suppressing β-catenin expression and inactivating the Wnt/β-catenin signaling pathway.

GSK3b is a downstream target of Akt phosphorylation/activation (35). GSK3β is also a major component of the Wnt/β-catenin signaling pathway. PLAC8 has been reported to suppress cell growth by inhibiting phosphorylation of Akt and GSK3β in hepatocellular carcinoma (17). Similarly, the present study revealed a significant decrease in phosphorylated-GSK3β and phosphorylated-Akt expression in PLAC8-silenced HN4 cells after treatment with a β-catenin inhibitor. Therefore, it was suggested that β-catenin may serve an important role in inhibiting PLAC8 expression by suppressing the PI3K/Akt/GSK3β signaling pathway in OSCC cell lines.

The limitation of the present study is that only *in vitro* experiments were performed. Further studies with animal experiments and clinical samples are required to confirm the preliminary results, and thus better understand the clinical value.

In conclusion, PLAC8 may inhibit carcinogenesis and EMT via downregulation of β-catenin expression in the PI3K/Akt/GSK3β and Wnt/β-catenin signaling pathways in OSCC. The present results suggested that PLAC8 may serve a novel role in the inhibition of OSCC.

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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

LW, XTW, AQS, GV, ZJS, PJ, DYY, AMW YWY and DL conceived and designed the present study. JLW, XTW, AQS, PJ, DYY and AMW performed the experiments. ZJS provided the cell lines. YWY and JLW drafted the initial manuscript. YWY, GV, JLW and DL reviewed and edited the initial manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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