Original Article

Study on the expression of lncRNA *PRKCA-AS1* in oral squamous cell carcinoma

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Background: Oral squamous cell carcinoma (OSCC) is one of the most malignant tumors in the oral and maxillofacial region, with a poor prognosis. Previous studies have shown that long non-coding RNAs (lncRNAs) play a crucial role in tumor development by regulating the biological behavior of various cancer cells. The aim of this study is to explore the role and potential mechanisms of lncRNA *PRKCA-AS1* in OSCC.

Methods: Real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) was used to detect the expression levels of lncRNA *PRKCA-AS1* in OSCC tissues and cell lines. Cell proliferation, migration and invasion were conducted to assess the biological functions of OSCC cell lines.

Results: The expression of lncRNA *PRKCA-AS1* in OSCC tissues was higher compared to that of adjacent non-cancerous tissues, and its expression level was associated with the depth of tumor infiltration, lymph node metastasis, and tumor node metastasis (TNM) staging. Compared to the control group of normal human oral keratinocytes (HOK), the expression of lncRNA *PRKCA-AS1* was also elevated in OSCC cell lines. Knockdown of lncRNA *PRKCA-AS1* significantly affected the proliferation, migration, and invasion ability of OSCC cells. However, when lncRNA *PRKCA-AS1* was further overexpressed, changes in cell proliferation and migration ability did not show statistical differences.

Conclusions: LncRNA *PRKCA-AS1* is highly expressed in OSCC, and its expression level is positively correlated with the depth of tumor infiltration, lymph node metastasis, and TNM staging. LncRNA *PRKCA-AS1* is involved in regulating the proliferation, migration, and invasion of OSCC cells.

Keywords: Long non-coding RNA (lncRNA); *PRKCA-AS1*; oral squamous cell carcinoma (OSCC); diagnosis; treatment

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Introduction

Oral squamous cell carcinoma (OSCC) is a common malignant tumor in the head and neck region, predominantly affecting individuals aged 40 to 70 years who have a history of smoking and alcohol consumption (1,2). OSCC is highly invasive, with a high rate of postoperative recurrence and lymph node metastasis, leading to a high mortality rate (3). In recent years, despite some progress in the research and treatment of OSCC, the diagnosis often occurs at an advanced stage, resulting in a 5-year survival rate of only around 50% (4,5). Currently, treatment methods for OSCC mainly include surgical resection, radiotherapy, chemotherapy, epidermal growth factor receptor (EGFR) inhibitors, cyclooxygenase-2 (COX-2) inhibitors, and photodynamic therapy. However, these approaches have shown limited success in improving the 5-year survival rate (6,7). The aggressive and metastatic nature of the tumor is considered the main reason for the poor prognosis of OSCC patients. Studying the occurrence and development of OSCC at the cellular and molecular levels may provide breakthroughs for early diagnosis and clinical treatment.

PRKCA is a subtype of protein kinase C (PKC) family, closely associated with the MAPK signaling pathway. PRKCA participates in various cellular processes including cell proliferation, differentiation, apoptosis, cell migration, cell transformation, tumor formation, and inflammation by activating signal cascades (8). Its regulatory effect on tumor cells is cell-type dependent, exhibiting different roles in different cell types. Research has demonstrated that PRKCA significantly inhibits the proliferation of various types of cancer cells, including those associated with colorectal, pancreatic, and breast cancer. Additionally, research has demonstrated that knocking down *PRKCA* expression in the liver cancer cell line *HA22T* slows down cell proliferation rate and significantly reduces cell migration ability. There has been notable progress in the study of *PRKCA* in recent years, revealing a strong association between abnormal *PRKCA* expression and the occurrence and progression of various cancers such as lung adenocarcinoma and esophageal squamous cell carcinoma (9,10). However, there is still a lack of research on *PRKCA* in OSCC.

Long non-coding RNA (lncRNA) is a group of non-coding RNAs with a length exceeding 200 nucleotides, which mediate epigenetic modifications of DNA and participate in various biological activities such as X chromosome inactivation in mammals (11). With the development of sequencing technology, the association between lncRNA and many human diseases is becoming increasingly apparent. Currently, there is growing evidence indicating that lncRNA plays an extremely important role in the occurrence and development of various human tumors. Certain lncRNAs and their related pathways are shown to regulate the functions of tumor cells in controlling the proliferation, apoptosis, invasion, and metastasis of tumor cells (12-15). The expression of lncRNA-*CCHE1* is higher in cervical cancer tissues than in adjacent tissues. The high expression of lncRNA-*CCHE1* is correlated with tumor quantity, extraglandular infiltration, and tumor staging. In addition, downregulation of lncRNA-*CCHE1* reduces the proliferation and invasion ability of cervical cancer cell lines (16). Research by Tong *et al.* suggests that *IGF2BP2-AS1* has higher expression in OSCC compared to normal tissue samples, and its expression is significantly correlated with patient age and T staging of OSCC, showing certain correlation with the survival rate of OSCC patients (17). Inhibition of *IGF2BP2-AS1* expression in OSCC cell lines significantly reduces cell proliferation and invasion. Increasing evidence suggests that lncRNA plays an important role in diagnosis, and treatment of oral cancer (18). *PRKCA-AS1*, as an antisense lncRNA transcribed from the second intron of *PRKCA*, its expression and biological functions in OSCC are not yet clear (19). Therefore, in-depth study of the molecular mechanism of lncRNA *PRKCA-AS1* is important in identifying diagnostic markers for early diagnosis and targeted therapy of OSCC. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-467/rc).

Highlight box

Key findings

- Long non-coding RNA (lncRNA) *PRKCA-AS1* plays a promoting role in the progression of oral squamous cell carcinoma (OSCC).

What is known and what is new?

- LncRNA plays an extremely important role in the occurrence and development of various human tumors.
- This study focuses on exploring the role of lncRNA *PRKCA-AS1* in OSCC.

What is the implication, and what should change now?

- LncRNA *PRKCA-AS1* is highly expressed in OSCC tissues and can affect the growth and invasion of OSCC cells by altering the expression of lncRNA *PRKCA-AS1*.

Table 1 RT-qPCR primers and RNA sequences

Target	Sequence
GAPDH	F: GAAGGTGAAGGTCGGAGTC
	R: GAAGATGGTGATGGGATTTC
PRKCA-AS 1	F: CCAGAAACCAACAGACCCCA
	R: CGGCCAGATTTCTAAGCGCA
PRKCA-AS 1-OE	F: GTAGACATAATAGCAACAGACATAC
	R: CGCAAATGGGCGGTAGGCGTG
PRKCA-AS 1-Sh	CTTTCTCTAACATCGATTTAA
PRKCA-AS 1-NC	CCTAAGGTTAAGTCGCCCTCG

RT-qPCR, real-time fluorescent quantitative polymerase chain reaction; F, forward; R, reverse.

Methods

Data collection and preprocessing

Enrichment analysis of differentially expressed lncRNAs was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG). The Database for Annotation, Visualization and Integrated Discovery (DAVID) database was utilized for performing KEGG analysis. RNA sequencing (RNA-seq) data from 370 cases (including 338 OSCC samples and 32 normal controls) were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). Subsequently, differential expression analysis was conducted in the R software.

Human OSCC samples

A total of 33 cases of OSCC cancer and adjacent tissue samples diagnosed and treated from December 2021 to December 2023 at the First Affiliated Hospital of Wannan Medical College (Wuhu, China) were collected. The adjacent tissue samples were collected at the locations at least 5 cm away from the edge of the primary lesion. The inclusion criteria for this study were: (I) pathologically confirmed OSCC; (II) patients who had not undergone radiotherapy or chemotherapy before surgery; (III) no serious systemic diseases; (IV) no history of long-term medication; (V) eligible for surgical treatment; (VI) patients who had signed informed consent forms. All samples were placed in RNA Store solution (Solarbio, Beijing, China) immediately after being removed in the operating room, and total RNA was rapidly extracted. Clinical pathological and medical records of patients with coding were also collected. This study was conducted in accordance with the Helsinki Declaration (revised in 2013). The study was approved by the Research Ethics Committee of Wannan Medical College {No. 2022: [9], March 3, 2022}, and informed consent were obtained from all participants or their legal guardians.

RNA extraction and real-time fluorescent quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from cultured normal human oral keratinocytes (HOK), OSCC cell lines (HN30, SCC4, CAL27), and cancer and adjacent tissue samples using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific™, Massachusetts, USA). The expression of the lncRNA *PRKCA-AS1* gene in cells and tissues was detected using the Super Real PreMix Plus SYBR Green PCR Kit (Tiangen Biotech, Beijing, China) via RT-qPCR. The reaction system was 20 μL, and the two-step reaction program was as follows: pre-denaturation: 95 ℃ for 15 minutes; denaturation: 95 ℃ for 10 seconds; annealing: 62 ℃ for 30 seconds, with a total of 45 cycles in the PCR reaction. The RT-qPCR reaction was performed in triplicate. Gene primers were designed and synthesized by OBiO Technology (Shanghai, China) (*Table 1*).

GAPDH was used as an internal reference in qPCR reactions, and the ΔCT value (ΔCT = CT value of *PRKCA-AS1* – CT value of GAPDH gene); the relative expression level of cancer tissue was determined as $2^{-\Delta\Delta CT}$, where the ΔΔCT value (ΔΔCT = ΔCT value of cancer tissue *PRKCA-AS1* – ΔCT value of adjacent tissue *PRKCA-AS1*) (20).

Cell culture

Human normal oral epithelial cell line (HOK) and human OSCC cell lines (HN30, SCC4) were provided by the Key Laboratory of Oral Medicine, Nanjing Medical University (Nanjing, China), and the human OSCC cell line (CAL27) was purchased from Guangzhou Saike Biotechnology Co., Ltd. (Guangzhou, China). All cells are free from mycoplasma contamination. Cells were tested for mycoplasma using Mycoplasma Detection Kit from Solarbio Biotech Co., Ltd. All cells were cultured in

DMEM medium (Gibco™, Beijing, China) supplemented with 10% fetal bovine serum (Lonsera, Shanghai, China), 100 U/mL penicillin, and 100 µg/mL streptomycin, in a 5% CO_2, 37 ℃ humidified incubator.

Cell transfection

PRKCA-AS1 knockdown lentivirus, overexpression lentivirus [short hairpin-*PRKCA AS1* (Sh-*PRKCA-AS1*), overexpression-*PRKCA AS1* (OE-*PRKCA-AS1*)], and corresponding control lentivirus [short hairpin-negative control (Sh-NC), overexpression-negative control (OE-NC)] were constructed by Obio (Shanghai, China). Following the manufacturer's protocol, interference fragments were transfected into cells using lentiviral vector pSLenti with the assistance of polybrene as a transfection enhancer. Lentiviral overexpression vector (OE-lncRNA *PRKCA-AS1*), lentiviral empty vector (OE-NC), lentiviral knockdown vector (Sh-lncRNA *PRKCA-AS1*), lentiviral empty vector (Sh-NC), after reaching 60% confluency in the flask, puromycin (Solarbio) was added to select for transduced cells and remove untransfected cells. The cells were then cultured for an additional 5 days, total RNA was extracted, and RT-qPCR was used to detect the expression level of the target RNA, constructing stable *PRKCA-AS1* knockdown cell lines.

Cell proliferation assay

Cells were seeded in a 96-well plate with HN30 cell density adjusted to 1×10^4 cells/well, SCC4 cell density adjusted to 1×10^4 cells/well, CAL27 cell density adjusted to 1×10^4 cells/well, with a volume of 100 µL per well. After 24, 48, 72, and 96 hours, cell counting kit-8 (CCK-8) reagent (10 µL/well) was added to the 96-well plate. After incubation in a 5% CO_2, 37 ℃ incubator for 40 minutes, absorbance optical density (OD) was measured at a wavelength of 450 nm using a microplate reader (BioTek Instruments, Winooski, USA).

Cell scratch assay

Stably transfected cells were seeded in a 6-well plate at 70% confluence and cultured in a 5% CO_2, 37 ℃ incubator until a monolayer of cells covering 100% of the surface area was formed. A sterile 200 µL pipette tip was used to scratch the cell layer, floating cells were carefully removed with phosphate buffer solution (PBS) (Gibco™), and then replaced with serum-free Dulbecco's Modified Eagle's medium (DMEM) medium (Gibco™) for continued culture. The scratch width was observed and photographed using an inverted microscope (Olympus, Tokyo, Japan) after 0 and 24 hours, and measurements were recorded.

Cell invasion assay

The invasive ability of OSCC cells after transfection was determined using Transwell chambers (Corning, USA) with a pore size of 8.0 µm. Matrigel (diluted 1:8 with DMEM) was added to the upper chamber and incubated in a CO_2 incubator for 3 hours. After removing the upper layer, 100 µL of DMEM was added and incubated for 30 minutes. Cells starved for 24 hours in serum-free medium were seeded in the upper chamber (HN30 cell density adjusted to 8×10^4 cells/well, SCC4 cell density adjusted to 1×10^5 cells/well, CAL27 cell density adjusted to 5×10^4 cells/well), with a total volume of 200 µL per well in the upper chamber. Complete culture medium containing serum was added to the lower chamber. After 24 hours of incubation, cells were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and photographed and counted in five random fields of view using an inverted microscope.

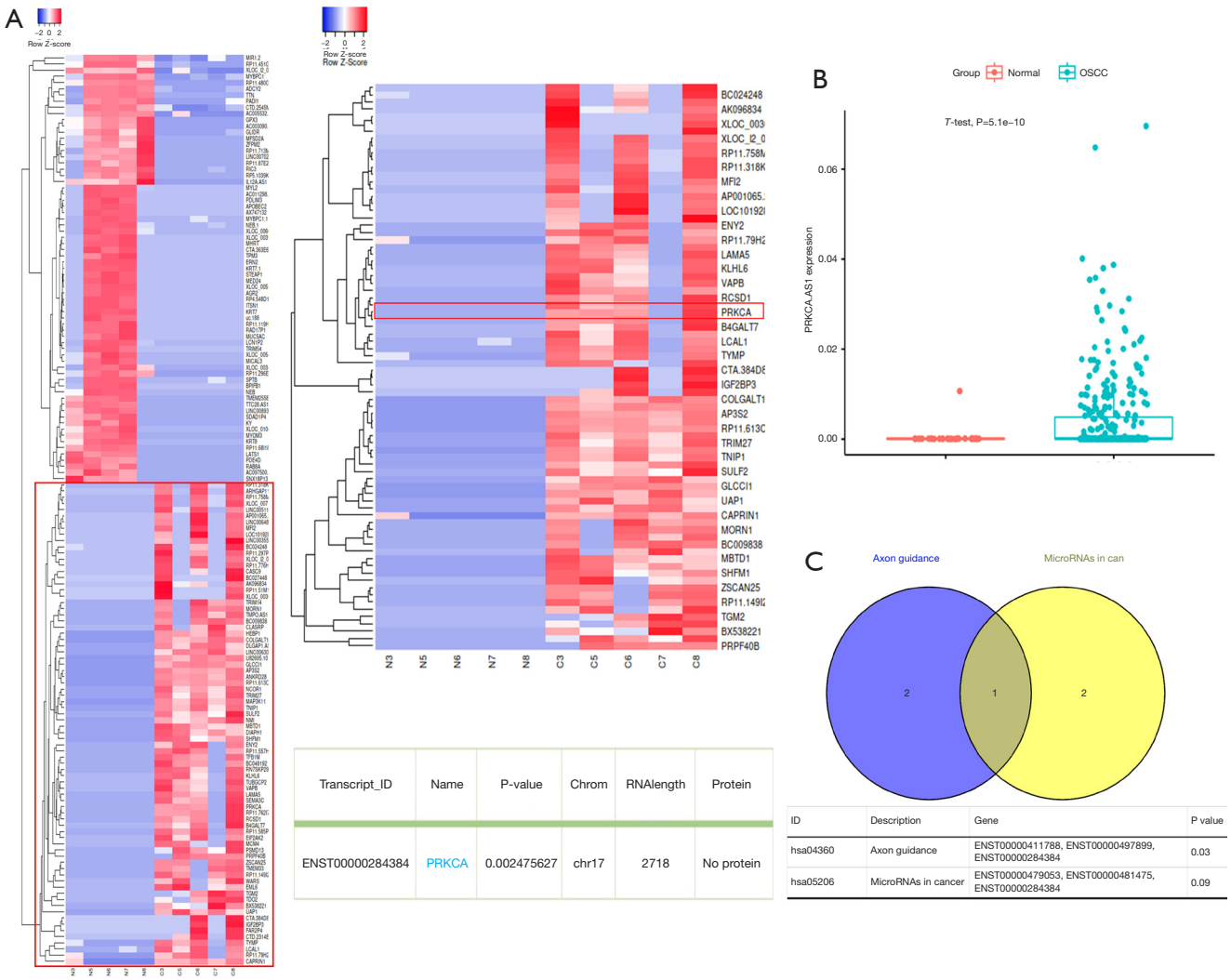
Statistical analysis

All statistical analyses were performed using SPSS 25.0 software. Graphpad Prism 9.5 and ImageJ software were used for data analysis and graph plotting. Gene heatmaps were generated using TBtools software. Comparisons between two or more groups were analyzed using *t*-tests or rank-sum tests. The expression difference of lncRNA *PRKCA-AS1* between cancer tissues and adjacent tissues was compared using paired *t*-tests; comparisons of lncRNA *PRKCA-AS1* expression levels among OSCC patients with different clinical features were performed using non-parametric tests (Mann-Whitney *U* test). All experiments were conducted at least three times, and results were considered statistically significant when $P<0.05$.

Results

Analysis of lncRNA PRKCA-AS1 expression in gene sequencing

Differential expression of lncRNA genes in 5 pairs of OSCC tissues (T3, T5, T6, T7, T8) and adjacent tissues (N3, N5, N6, N7, N8) was visualized through a gene expression



Transcript_ID	Name	P-value	Chrom	RNAlength	Protein
ENST00000284384	PRKCA	0.002475627	chr17	2718	No protein

ID	Description	Gene	P value
hsa04360	Axon guidance	ENST00000411788, ENST00000497899, ENST00000284384	0.03
hsa05206	MicroRNAs in cancer	ENST00000479053, ENST00000481475, ENST00000284384	0.09

Figure 1 Analysis of lncRNA *PRKCA-AS1* expression in cancer and paracancerous tissues of patients with OSCC. (A) Heatmap of differential lncRNA gene expression between OSCC cancer tissues and adjacent tissues. (B) Comparison of *PRKCA-AS1* expression levels between normal tissues and OSCC tissues. (C) KEGG enrichment analysis and Venn diagram analysis. OSCC, oral squamous cell carcinoma; lncRNA, long non-coding RNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

heatmap (*Figure 1A*). ENST00000284384 (*PRKCA-AS1*) showed high expression in the 5 groups of cancer tissues and low expression in the 5 groups of adjacent tissues (*Figure 1A*). Expression data of OSCC and normal tissues downloaded from the TCGA database also showed higher expression of *PRKCA-AS1* in cancer tissues compared to normal tissues (*Figure 1B*). Subsequent KEGG enrichment analysis of differential lncRNAs revealed enrichment in the axon guidance and microRNAs in cancer pathways. Further intersection analysis of these two enriched pathways revealed *PRKCA-AS1* as the unique intersection (*Figure 1C*). These results suggest that the upregulation of *PRKCA-AS1* may be a risk factor for OSCC.

Expression of lncRNA PRKCA-AS1 in OSCC tissues and cell lines

Total RNA was extracted from 33 cases of OSCC tissues and their adjacent tissues, reverse transcribed into cDNA, and subjected to RT-qPCR analysis. Compared to the corresponding adjacent tissues, *PRKCA-AS1* was upregulated in 28 cases and downregulated in 5 cases of

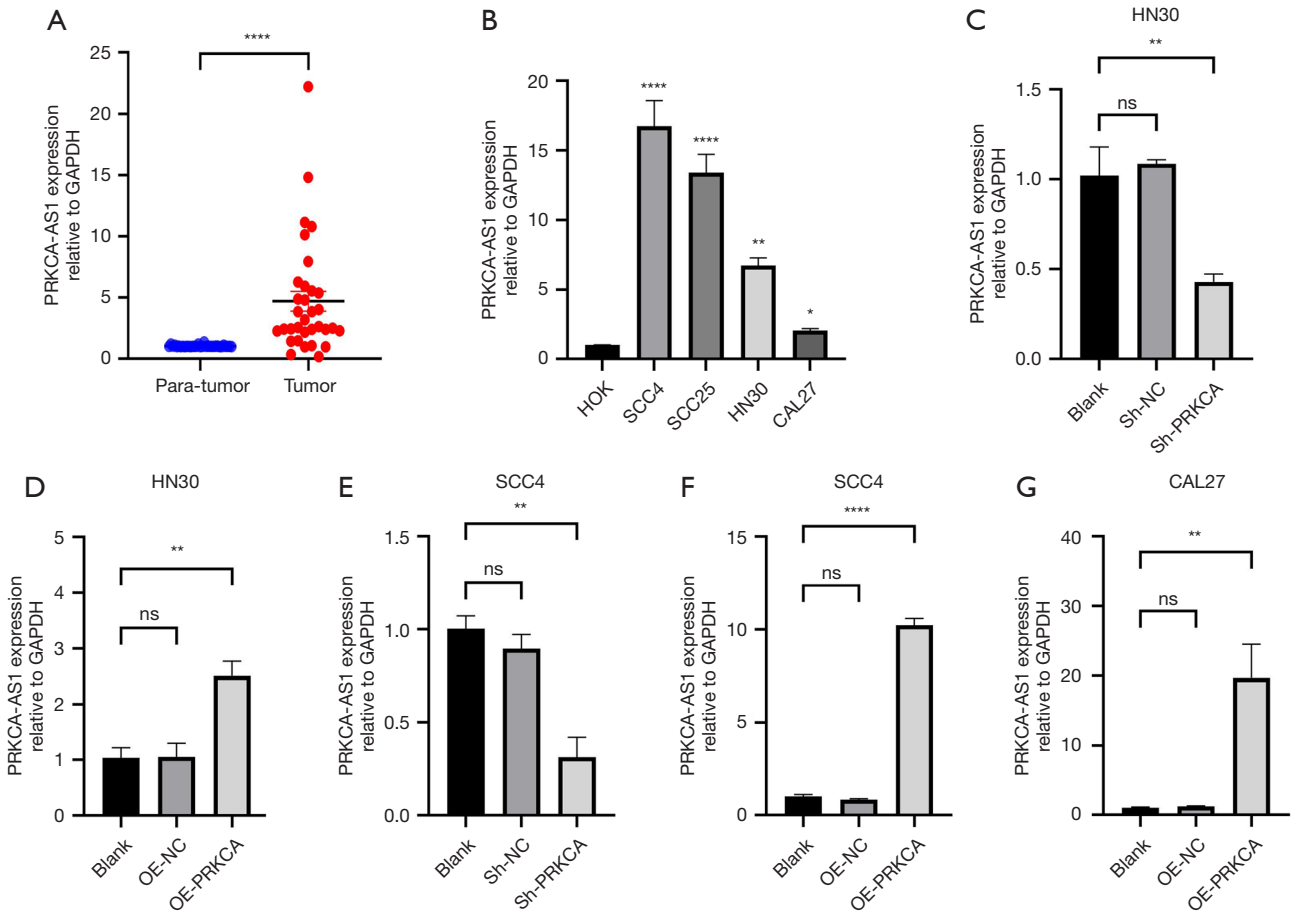


Figure 2 RT-qPCR detection of *PRKCA-AS1* expression in tissue and cell samples and validation of transfection efficiency. (A) Scatter plot showing the expression levels of *PRKCA-AS1* in 33 OSCC cancer tissue samples compared to adjacent tissue samples, as detected by RT-qPCR. (B) Differential expression of *PRKCA-AS1* in normal HOK and human OSCC cell lines (SCC4, SCC25, HN30, CAL27) detected by RT-qPCR. (C-G) Expression of *PRKCA-AS1* in OSCC cell lines after transfection. ns, P>0.05; *, P<0.05; **, P<0.01; ****, P<0.0001. HOK, human oral keratinocytes; sh-NC, short hairpin-negative control; sh-*PRKCA*, short hairpin-*PRKCA*; OE-NC, overexpression-negative control; OE-*PRKCA*, overexpression-*PRKCA*; RT-qPCR, real-time fluorescent quantitative polymerase chain reaction; OSCC, oral squamous cell carcinoma.

cancer tissues. LncRNA *PRKCA-AS1* was significantly overexpressed in cancer tissues (*Figure 2A*, P<0.001). Using RT-qPCR to detect *PRKCA-AS1* expression in four OSCC cell lines (HN30, SCC4, SCC25, CAL27) and normal HOK, the results showed that compared to HOK cells, lncRNA *PRKCA-AS1* was highly expressed in OSCC cell lines (*Figure 2B*, P<0.05).

Relationship between lncRNA PRKCA-AS1 expression in OSCC tissues and clinical features

Statistical analysis was conducted to assess the correlation between *PRKCA-AS1* expression and clinical pathological features [age, gender, smoking history, alcohol consumption history, tumor maximum diameter, tumor infiltration depth, lymph node metastasis, pathological grade, tumor node metastasis (TNM) stage] (*Table 2*). The expression level of *PRKCA-AS1* was correlated with tumor infiltration depth, lymph node metastasis, and TNM stage. *PRKCA-AS1* expression was higher in the lymph node metastasis group compared to the non-metastasis group (5.457 *vs.* 2.398, P<0.001), higher in TNM stage III–IV compared to stage I–II (5.734 *vs.* 2.267, P<0.001), and lower in the group with tumor infiltration depth less than 0.7 cm compared to

Table 2 Correlation analysis between lncRNA PRKCA-AS1 expression and clinical parameters of OSCC patients

Clinical indicators	Samples	Expression of PRKCA-AS1 [M (Q1, Q4)]	Z value	P value
Age (years)			0.795	0.43
<60	15	2.498 (2.178, 4.812)		
≥60	18	3.938 (2.080, 6.011)		
Sex			−0.336	0.74
Male	25	3.188 (2.231, 5.734)		
Female	8	2.483 (1.644, 8.565)		
Smoking history			−0.364	0.72
No	14	3.938 (2.257, 5.509)		
Yes	19	2.498 (1.470, 6.270)		
Drinking history			−1.722	0.09
No	12	4.841 (2.777, 6.184)		
Yes	21	2.413 (1.453, 4.786)		
Maximum tumor diameter (cm)			1.470	0.14
<2.0	8	2.231 (0.982, 5.405)		
≥2.0	25	3.188 (2.406, 5.907)		
Tumor infiltration depth (cm)			3.533	<0.001
<0.7	14	2.296 (0.984, 2.516)		
≥0.7	19	5.370 (2.639, 10.136)		
Lymph node metastasis			−3.742	<0.001
No	12	2.398 (1.259, 2.604)		
Yes	21	5.457 (4.225, 10.086)		
Pathological stage			1.566	0.12
I–II	30	2.001 (0.982, 2.533)		
III–IV	3	3.188 (3.188, 5.544)		
TNM			4.899	<0.001
I–II	17	2.267 (1.033, 2.431)		
III–IV	16	5.734 (4.225, 10.635)		

OSCC, oral squamous cell carcinoma; TNM, tumor node metastasis.

the group with depth greater than 0.7 cm (2.296 *vs.* 5.370, P<0.001). High expression of *PRKCA-AS1* was detected in 9 cases of TNM stage I–II and 8 cases of stage III–IV (40.9% *vs.* 72.7%, P<0.05). The presence of high *PRKCA-AS1* expression was detected in 2 patients without lymph node metastasis and 10 patients with lymph node metastasis (9.5% *vs.* 83.3%, P<0.01). Also, high expression of *PRKCA-AS1* was detected in 2 patients with cancer tissue infiltration depth less than 0.7 cm and 14 patients with cancer tissue infiltration depth greater than 0.7 cm (14.3% *vs.* 73.7%, P<0.01). The findings indicate that elevated expression of *PRKCA-AS1* is linked to the malignancy of OSCC.

Impact of lncRNA PRKCA-AS1 on OSCC cell proliferation

To investigate the effect of lncRNA *PRKCA-AS1* on OSCC cell proliferation, we chose OSCC cell lines HN30 and SCC4 for knocking down *PRKCA-AS1* and

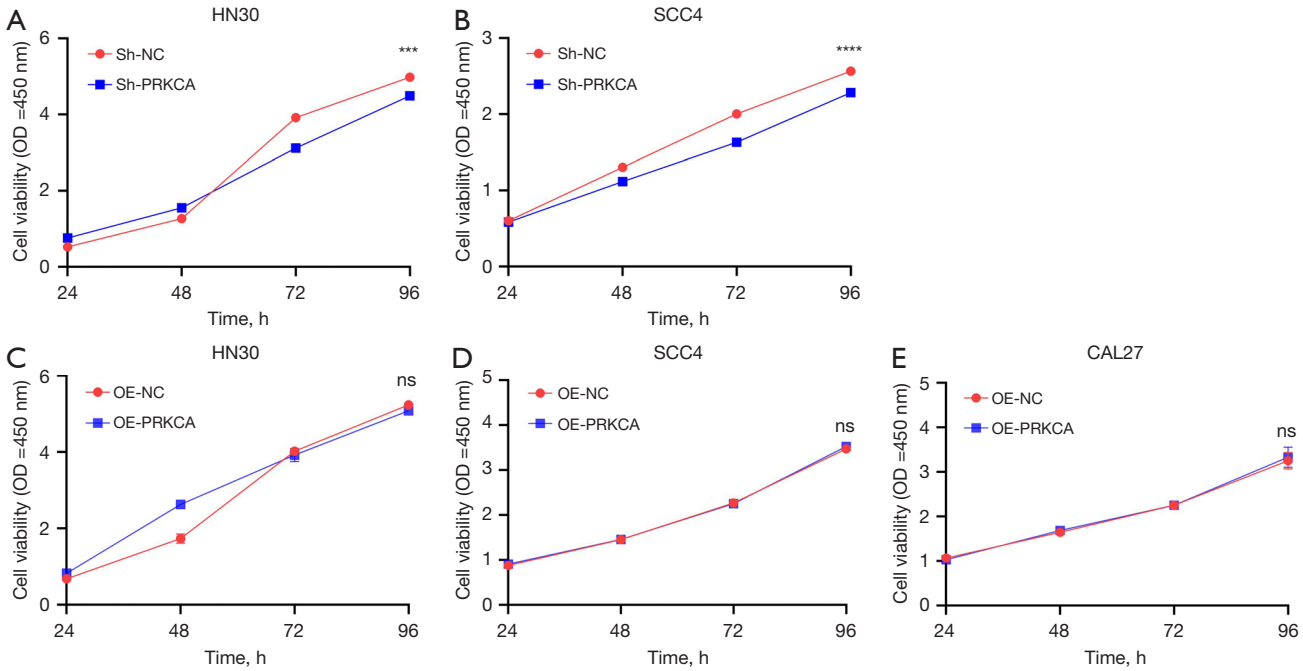


Figure 3 The effect of lncRNA *PRKCA-AS1* on the proliferation of HN30, SCC4, and CAL27 cells. (A,B) Analysis of the effect of knocking down lncRNA *PRKCA-AS1* on the proliferation of HN30, SCC4 through cells through CCK-8 experiments. (C-E) Analyze the effect of overexpression of lncRNA *PRKCA-AS1* on the proliferation of HN30, SCC4, and CAL27 cells through CCK-8 experiment. ns, P>0.05; ***, P<0.001; ****, P<0.0001. sh-NC, short hairpin-negative control; sh-*PRKCA*, short hairpin-*PRKCA*; OD, optical density; OE-NC, overexpression-negative control; OE-*PRKCA*, overexpression-*PRKCA*; lncRNA, long non-coding RNA; CCK-8, cell counting kit-8.

HN30, SCC4, CAL27 for overexpressing *PRKCA-AS1* (Sh-*PRKCA*, OE-*PRKCA*, *Figure 2C-2G*). CCK-8 results showed that compared to the negative control group (Sh-NC), the proliferation rate of HN30 and SCC4 cell lines transfected with Sh-*PRKCA* was inhibited (P<0.05, *Figure 3A,3B*). However, compared to the negative control group (OE-NC), there was no statistically significant difference in the proliferation rate of the three OSCC cell lines HN30, SCC4, and CAL27 transfected with OE-*PRKCA* (P>0.05, *Figure 3C-3E*). The above results indicate that *PRKCA-AS1*, which is highly expressed within a certain range, can promote the proliferation ability of OSCC cell lines.

Impact of lncRNA PRKCA-AS1 on OSCC cell migration

The effect of lncRNA *PRKCA-AS1* on OSCC cell migration was assessed using a scratch assay. Compared to the negative control group (Sh-NC), the migration area of HN30 and SCC4 cell lines transfected with Sh-*PRKCA* decreased at the same time point (P<0.05, *Figure 4A,4B*). However, compared to the negative control group (OE-NC), there was no statistically significant difference in the migration area of the three OSCC cell lines (HN30, SCC4, CAL27) transfected with OE-*PRKCA* (P>0.05, *Figure 4*). The above results indicate that lncRNA *PRKCA-AS1*, which is highly expressed within a certain range, can promotes OSCC cell migration.

Impact of lncRNA PRKCA-AS1 on OSCC cell invasion

The results indicate that compared to the negative control group (Sh-NC), the invasion ability of HN30 and SCC4 cells transfected with Sh-*PRKCA* was weakened (P<0.05, *Figure 5A,5B*). Compared to the negative control group (OE-NC), the invasion ability of HN30 cells transfected with OE-*PRKCA* was enhanced (P=0.001, *Figure 5C*), while there was no significant change in the invasion ability of SCC4 and CAL27 cells after overexpressing *PRKCA-AS1* (P>0.05, *Figure 5D,5E*). The above results indicate that *PRKCE-AS1*, which is highly expressed within a certain range, can promote the invasive ability of OSCC cell lines.

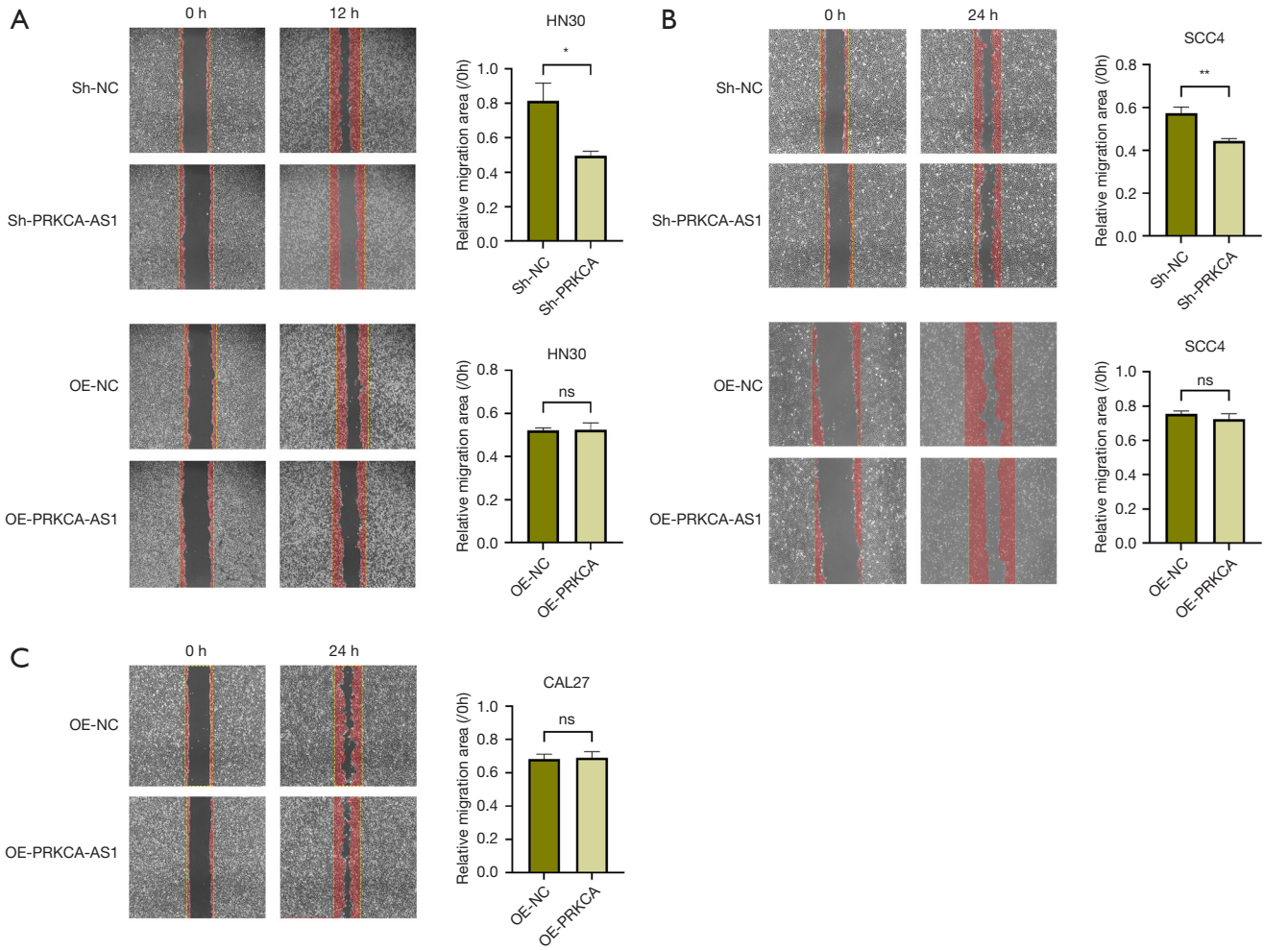


Figure 4 The effect of lncRNA *PRKCA-AS1* on the migration of HN30, SCC4, and CAL27 cells. (A) Analysis of the effect of lncRNA *PRKCA-AS1* on the migration of HN30 cells through scratch assay. (B) Analysis of the effect of lncRNA *PRKCA-AS1* on the migration of SCC4 cells through scratch assay. (C) Analysis of the effect of lncRNA *PRKCA-AS1* on the migration of CAL27 cells through scratch assay. ns, P>0.05; *, P<0.05; **, P<0.01. The six-well plate was placed under a ×40 microscope to calculate the cell healing area in each field of view. sh-NC, short hairpin-negative control; sh-*PRKCA*, short hairpin-*PRKCA*; OE-NC, overexpression-negative control; OE-*PRKCA*, overexpression-*PRKCA*; lncRNA, long non-coding RNA.

Discussion

Numerous studies have indicated the significant role of lncRNAs in the occurrence and development of various malignant tumors, including OSCC (12-15). Yang *et al.* conducted RT-qPCR analysis of *CASC9* expression using 35 OSCC tissues and corresponding adjacent tissues (21). The study results demonstrated that compared to adjacent normal tissues, *CASC9* was significantly upregulated in OSCC tissues and was significantly associated with tumor size, regional lymph node metastasis, and clinical staging of OSCC. Tan *et al.* investigated the impact of lncRNA *MEG3* on the biological activity of OSCC cell lines and its related mechanisms, demonstrating that *MEG3* can inhibit the progression of OSCC (22). *MEG3* acts as a sponge for mir-548D-3P, leading to decreased expression levels of mir-548D-3P, subsequently upregulating the target genes SOCS5 and SOCS6 to regulate the JAK-STAT pathway and the biological activity of OSCC cells. Our work demonstrates the partial role of lncRNA *PRKCA-AS1* in OSCC. The research indicates that lncRNA *PRKCA-AS1* is aberrantly expressed in OSCC tissues. Analysis of histological clinical cases reveals a positive correlation

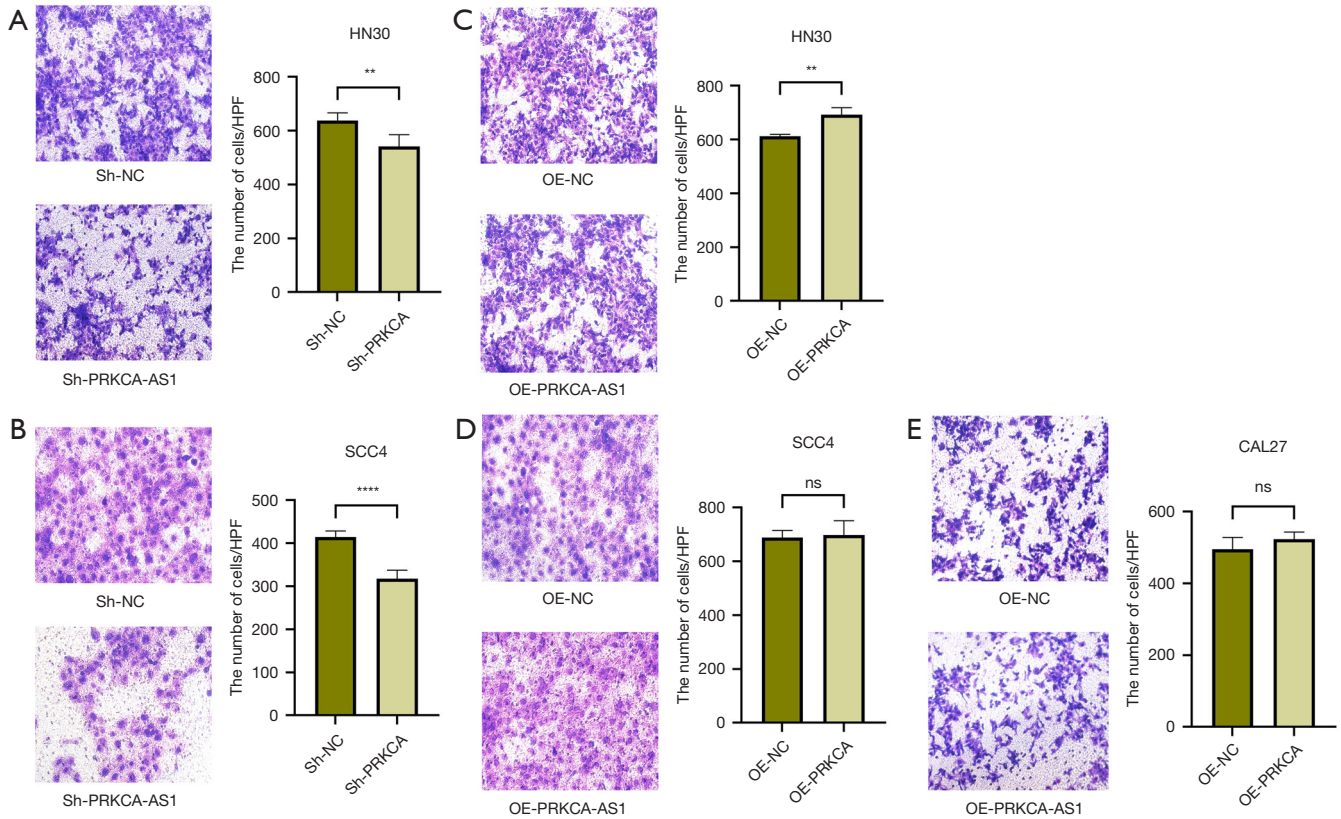


Figure 5 The effect of lncRNA *PRKCA-AS1* on the invasion of HN30, SCC4, and CAL27 cells. (A,B) Analysis of the effect of knocking down lncRNA *PRKCA-AS1* on the invasion of HN30, SCC4 cells through Transwell assay. (C-E) Analyze the effect of overexpression of lncRNA *PRKCA-AS1* on the invasion of HN30, SCC4, and CAL27 cells through Transwell assay. ns, P>0.05; **, P<0.01; ****, P<0.0001. Staining method: crystal violet staining. The Transwell chamber was placed under a ×200 microscope to count the number of cells in each field of view. sh-NC, short hairpin-negative control; sh-*PRKCA*, short hairpin-*PRKCA*; HPF, high-power field; OE-NC, overexpression-negative control; OE-*PRKCA*, overexpression-*PRKCA*; lncRNA, long non-coding RNA.

between the expression level of lncRNA *PRKCA-AS1* and the depth of tumor tissue infiltration, lymph node metastasis, and TNM stage. The results directly indicating differences in the expression level of lncRNA *PRKCA-AS1* among different clinical indicators, suggesting the potential of this gene for early diagnosis of OSCC. Cell genetic alteration experiments indirectly suggest that lncRNA *PRKCA-AS1* may promote OSCC progression.

Cell experimental results showed that in HN30 and SCC4 cell lines, compared to cells transfected with empty plasmid, the proliferation, migration, and invasion abilities of cells transfected with *PRKCA-AS1* knockdown plasmid were all inhibited. In the HN30 cell line, compared to cells transfected with empty plasmid, the invasion ability of cells transfected with overexpressed lncRNA *PRKCA-AS1* plasmid was enhanced. These findings may suggest that overexpressed lncRNA *PRKCA-AS1* plays a promoting role in the progression of OSCC. However, the changes in proliferation and migration abilities of cells overexpressing *PRKCA-AS1* in HN30 and SCC4 were not statistically significant. To further validate the effect of overexpressed *PRKCA-AS1* on OSCC, we selected CAL27 cells with relatively low expression of lncRNA *PRKCA-AS1* in OSCC cell lines for overexpression-related experiments, but the results still showed no statistical difference. This may be because compared to normal oral epithelial cells, the expression level of lncRNA *PRKCA-AS1* in OSCC cells has already reached a relatively high level, and overexpression of lncRNA *PRKCA-AS1* cannot further promote the proliferation and migration abilities of OSCC cell lines.

Despite our investigation into the impact of the lncRNA *PRKCA-AS1* on the proliferation, migration, its downstream

targets are still unclear. A previous study has shown that *PRKCA-AS1*, an antisense lncRNA transcribed from the second intron of *PRKCA*, is positively correlated with DNA methylation at the promoter in rheumatic heart disease (19). However, it is still unclear whether it can exert the same mechanism in cancer. Previous research has demonstrated that the Wnt/β-catenin pathway is closely associated with cell proliferation, invasion, migration, apoptosis, and participates in the occurrence and development of OSCC (9,23). Therefore, assessing whether *PRKCA-AS1* can activate the WNT/β-catenin pathway will be a key focus of research after fully verifying the carcinogenic effect of *PRKCA-AS1* in OSCC cell lines.

LncRNAs may regulate the expression of various pathways involved in various cancers. Regarding *PRKCA*, previous studies have shown that for mRNA *PRKCA*, circRNA PRKCA can participate in cancer regulation through related pathways (10,22). However, there have been fewer studies on lncRNA *PRKCA-AS1* in previous pathway research. The limited existing research results suggest that *PRKCA-AS1* has an inflammation-induced effect, where the activated p38/MAPK pathway can inhibit the expression of *PRKCA-AS1* in rheumatic heart disease, thereby suppressing *PRKCA* transcription (24,25). Bridging the inflammatory signaling pathway seems to be complex in different diseases, and its potential therapeutic mechanisms in cancer need further exploration. Therefore, we further investigated the gene expression levels in 33 pairs of OSCC tissues and their corresponding adjacent tissues through cancer and adjacent tissue gene sequencing and database data mining. We found that lncRNA *PRKCA-AS1* indeed showed a stable differential expression trend. Over all, we demonstrated that lncRNA *PRKCA-AS1* is highly expressed in OSCC tissues, thereby promoting the progression of OSCC. The epigenetic modification or selective inhibition of lncRNA *PRKCA-AS1* represents a highly promising therapeutic target for targeted therapy of OSCC, providing a research basis for subsequent early diagnosis and identification of new drug. LncRNA *PRKCA-AS1* has the potential to serve as a valuable biomarker for OSCC diagnosis, prognosis, and treatment.

Conclusions

In summary, our study revealed the overexpression of lncRNA *PRKCA-AS1* in OSCC tissue, correlating with unfavorable outcomes in patients with OSCC. The proliferation, migration, and invasion abilities of the OSCC cell line can be suppressed by reducing the expression of lncRNA *PRKCA-AS1*. These findings indicate that lncRNA *PRKCA-AS1* may act as a potential therapeutic agent for the diagnosis, prognosis, and treatment of OSCC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-467/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-467/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-467/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Helsinki Declaration (revised in 2013). The study was approved by the Research Ethics Committee of Wannan Medical College {No. 2022: [9], March 3, 2022}, and informed consent were obtained from all participants or their legal guardians.

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