

Original Article

PDGF-BB secreted by OTSCC orchestrates CAF-driven tumor progression through regulating the LINC00886/hsa-miR-15b-5p/AKT axis

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Abstract: Under the induction of factors derived from tumor cells, fibroblasts can transform into cancer-associated fibroblasts (CAFs), in which non-coding RNAs play a key role. This study aims to reveal the regulatory mechanisms of long non-coding RNAs (lncRNAs) in CAFs induced by PDGF-BB from oral tongue squamous carcinoma cells. This study found that PDGF-BB effectively induces the transformation of fibroblasts into cancer-associated fibroblasts (CAFs). Through focused analysis of transcriptional results, it was discovered that LINC00886 is significantly over-expressed in CAFs (PDGF-BB). Furthermore, knocking down LINC00886 in CAFs (PDGF-BB) resulted in a reversal of related CAF markers, including downregulation of CAF marker proteins, reduced lactate secretion, and inhibition of aerobic glycolysis. Additionally, as a long non-coding RNA, LINC00886 regulates cellular biological behaviors through interactions with miRNAs. Predictions from the ENCORI online database revealed that LINC00886 can interact with hsa-miR-15b-5p. Further analysis indicated that the AKT-mTOR signaling pathway is inhibited in the transcriptome data of CAFs with knocked-down LINC00886. When an inhibitor of hsa-miR-15b-5p was added to CAFs (sh-LINC00886), the total AKT protein level was significantly upregulated, and the phosphorylation level of mTOR was also upregulated. This result was confirmed in a xenograft model. Our study demonstrates that PDGF-BB induces the transformation of fibroblasts into CAFs and thereby promotes tumor progression by regulating the LINC00886/hsa-miR-15b-5p/AKT signaling axis, providing new potential targets for cancer therapy.

Keywords: Tongue squamous cell carcinoma, PDGF-BB, LINC00886, hsa-miR-15b-5p, cancer-associated fibroblasts

Introduction

Oral Squamous Cell Carcinoma (OSCC) is the most common subtype of oral cancer [1]. Its incidence rate has been increasing year by year, and it is not only one of the cancers with relatively high mortality but also exhibits strong local invasiveness and distant metastatic potential [2]. Epidemiological surveys have indicated that the incidence of tongue squamous cell carcinoma tends to be younger. Due to the indistinct early clinical symptoms, most patients are already at the middle or advanced stage when diagnosed [3]. Despite advances in therapeutic modalities for tongue squamous cell carcinoma in recent years, postoperative recurrence and distant metastasis remain major factors contributing to poor prognosis in

OSCC patients [4]. This is due to the complexity of the tumor microenvironment and its resistance to treatment which are likely to result in suboptimal therapeutic outcomes [5]. Thus, comprehensive studies of the molecular pathways underlying the OSCC, especially how cancer-associated fibroblasts (CAFs) contribute to the tumor microenvironment are of high importance in regards to the investigation of new targeted therapeutic approaches [6].

Being an inevitable stromal element of the tumor microenvironment, CAFs are very heterogeneous in their origin [7], primarily comprising tissue-resident fibroblasts in response to tumor signals, precursor cells, including mesenchymal stem cells recruited by the bone marrow, and tumor-associated epithelial or endothelial

cells transdifferentiated by pathways such as epithelial-mesenchymal transition (EMT) [8]. These recruited and activated CAFs are not passive structural supporters but active regulatory hubs for tumor progression [9]. They are involved in various biological functions of tumors, such as growth, invasion, metastasis, immune escape, angiogenesis, and chemoresistance, by secretion of continuous amounts of growth factors, chemokines, and chemoresistance as well as remodelling the extracellular matrix [10, 11]. The close and dynamic bidirectional crosstalk between CAFs and tumor cells is established in oral tongue squamous cell carcinoma (OTSCC) [12], among which platelet-derived growth factor-BB (PDGF-BB) secreted by tumor cells acts as a vital signaling molecule [13]. As a key pro-growth factor, PDGF-BB exerts its biological functions by activating multiple signaling pathways through its receptor PDGFR- β , thereby modulating the activity and functional properties of CAFs [14]. As one of the most active members of the PDGF family, PDGF-BB plays an indispensable role in the tumor microenvironment and serves as a core signaling hub connecting tumor cells with their surrounding stroma [15], constructing and maintaining a tumor-promoting ecosystem [16]. The binding of PDGF-BB to its specific high-affinity receptor PDGFR- β activates signaling pathways such as PI3K/AKT and MAPK [17], thereby promoting various tumor-related malignant behaviors, including cell proliferation, survival, migration, and angiogenesis [18, 19]. In the tumor microenvironment, PDGF-BB secreted by tumor cells themselves acts on the tumor stroma in a paracrine manner and is one of the potent factors inducing the transformation of quiescent fibroblasts into activated CAFs [20]. It endows CAFs with high contractility and strong secretory capacity by upregulating CAF markers such as α -smooth muscle actin (α -SMA) and fibroblast activation protein (FAP) [21]. Driven continuously by PDGF-BB signaling, activated CAFs secrete large amounts of other growth factors and chemokines, including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and CXCL12, which collectively promote tumor angiogenesis, tumor cell invasion, and immune cell recruitment [22]. Importantly, PDGF-BB is capable of causing metabolic changes in CAFs, turning their metabolism to oxidative phosphorylation into aerobic glycolysis. This metabolic rear-

angement augments the glucose uptake ability of CAFs, with enormous production and extracellular release of lactic acid that stimulates tumor development [23]. Taken together, there is growing evidence that the PDGF-BB/PDGFR axis coordinates various pro-tumorigenic functions: direct activation of stromal cells, metabolic symbiosis, formation of malignant signaling feedback loops, and regulation of tumor angiogenesis, to systematically mediate tumor progression [24].

Long non-coding RNAs (lncRNAs) can be defined as a type of RNA molecules that have a length of more than 200 nucleotides [25]. As knowledge of non-coding RNAs has increased, lncRNAs have been identified to be involved in a variety of cellular biological events [26] and play a central role, especially in the etiology and development of cancer [27]. In cancer initiation and progression, lncRNAs regulate the growth, metastasis, chemoresistance, and immune escape of tumors by altering the crosstalk between tumor cells and immune cells, fibroblasts, and endothelial cells in the tumor microenvironment [28]. They are also able to control other procedures like angiogenesis and degradation of the matrix and hence increase tumor invasiveness [29]. Moreover, lncRNAs have the potential to serve as competing endogenous RNAs (ceRNAs) targeting microRNAs (miRNAs), and thus regulate the inhibitory activity of miRNAs on the target messenger RNAs (mRNAs) [30]. This is a post-transcriptional gene regulation mechanism that is facilitated by the miRNA “sponge effect” and forms a major functional paradigm of lncRNAs [31]. miRNAs are a group of small non-coding RNAs which generally regulate their targets by binding to their 3' untranslated region (3' UTR) of target mRNAs, preventing their translation or enhancing their degradation, to control their gene expression [32]. Their low expression is strongly linked with malignant progression, metastasis, and chemoresistance of tumors [33].

One of the most critical pathways of signaling that controls cell survival and proliferation in tumor cells is the AKT signaling pathway [34]. Upon activation, AKT modulates a spectrum of biological processes, including cell proliferation, survival, migration, and immune escape, through a cascade of downstream effector mol-

ecules [35, 36]. Aberrant activation of the AKT pathway has been identified as a key contributor to tumor growth, invasion, and metastasis in OTSCC [37]. By activating the PDGFR- β receptor, PDGF-BB can initiate the PI3K/AKT signaling pathway, thereby promoting cell proliferation and survival [38]. Activation of AKT not only enhances the migratory and invasive abilities of CAFs but also promotes tumor angiogenesis and infiltration by regulating matrix metalloproteinases (MMPs), angiogenic factors, and other downstream effectors [39]. Thus, the AKT signaling pathway can be considered an important molecular intermediary that links the interaction between OTSCC cells and CAFs, regulates the malignant behavior of CAFs, and thus plays a key role in tumor development [40].

Since tumor-derived factors contributing to fibroblast to CAF transformation are complex, our findings reveal that PDGF-BB increases the expression of LINC00886 in CAFs. This upregulation, in turn, promotes the activation of the AKT-mTOR signaling pathway, thereby enhancing the functional interplay between TSCC cells and CAFs within the tumor microenvironment [41]. Using the ENCORI online database, we predicted that LINC00886 directly interacts with hsa-miR-15b-5p. Consistent with this prediction, knockdown of hsa-miR-15b-5p in LINC00886-knockdown CAFs resulted in upregulation of AKT and increased phosphorylation of mTOR, which further enhanced the malignant phenotype of CAFs. Additionally, exogenous administration of PDGF-BB to LINC00886-knockdown CAFs increased AKT expression and promoted mTOR phosphorylation. By elucidating the mechanistic role of the LINC00886/hsa-miR-15b-5p/AKT axis in tongue squamous cell carcinoma, this study advances our understanding of the functional contributions of CAFs within the OTSCC tumor microenvironment and identifies potential therapeutic targets for the treatment of this disease.

Material and methods

Reagents

α -SMA antibody (Proteintech, 14395-1-AP), PDGF Receptor beta antibody (Bioss, bsm-61037R), FAP antibody (Bioss, bs-5758R), and β -Tubulin antibody (Abcam, ab179513) were

used in this study. AKT antibody (ServiceBio, GB15689-50), p-AKT antibody (ServiceBio, GB150002-50), and mTOR antibody (HUABIO, HA500126) were purchased from HuaAn Biotech. The Phospho-mTOR (S2448) Mouse Monoclonal Antibody (HUABIO, HA600094) was also obtained from HuaAn Biotech. Ki67 antibody (ServiceBio, GB111499-50) and HE staining kit (ServiceBio, G1005) were purchased from ServiceBio. The platelet-derived growth factor receptor (PDGFR) inhibitor (CP73451) was purchased from MCE. Pentobarbital sodium was purchased from Sigma-Aldrich (Sigma-Aldrich, P-5178-100 mg).

Cell culture

Normal human oral mucosal fibroblasts (hOMF) were obtained from CellResearch Corp (Singapore), and Cal-27 cells were obtained from BioVector NTCC Inc. (Beijing, China). All cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂ in DMEM (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 U/mL penicillin, and 100 μ g/mL streptomycin (Gibco). CAF (Cal27-CM) cells were generated by treating hOMF with Cal-27 conditioned medium (3/4 Cal27-CM) for 48 hours. CAF (PDGF-BB) cells were generated by treating hOMF with 30 ng/mL PDGF-BB for 48 hours.

Establishment of stable LINC00886-knockdown cell lines

Cells were seeded into 24-well plates (Corning) and cultured until reaching 50-60% confluence prior to transfection. CAF cells were transfected with Lipofectamine LTX reagent (Invitrogen) in accordance with the manufacturer's protocols. Eighteen hours post-transfection, the medium containing transfection reagents was replaced with DMEM supplemented with 10% fetal bovine serum (FBS). At 48 hours post-transfection, cells were harvested and processed, and the silencing efficiency of LINC00886 ([Table S1](#)) was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR).

Use of the hsa-miR-15b-5p inhibitor

hsa-miR-15b-5p inhibitor was added to CAF (PDGF-BB) and CAF (sh-LINC00886) cells, and the inhibitory effect on hsa-miR-15b-5p ([Table](#)

S2) was detected by qPCR after 48 hours of treatment.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde at room temperature for 10-15 minutes, followed by permeabilization with 0.1% Triton X-100 for 5-10 minutes. After blocking with 5% bovine serum albumin (BSA) for 30-60 minutes, cells were incubated with primary antibodies at 4°C overnight (α -SMA antibody (100:1), and AKT antibody (100:1), The Phospho-mTOR (100:1), Ki67 antibody (100:1)). The secondary antibodies were then fluorescently labelled (200:1) and allowed to interact with the sample after 1 hour, in the dark. Photographs were taken by the use of a fluorescence microscope.

Western blotting

Cells were lysed in RIPA buffer (Kangwei, Beijing, China) supplemented with a protease and phosphatase inhibitor cocktail (Sigma). Equal amounts of protein were separated by 10% SDS-PAGE and transferred onto 0.22 μ m PVDF membranes (Millipore, MA, USA). Membranes were incubated with primary antibodies overnight at 4°C (α -SMA antibody (1000:1), PDGF Receptor beta antibody (1000:1), FAP antibody (1000:1), and β -Tubulin antibody 1000:1), AKT antibody (1000:1), p-AKT antibody (1000:1), and mTOR antibody (1000:1), The Phospho-mTOR (1000:1), followed by incubation with HRP-conjugated secondary antibodies (2000:1). Protein bands were visualized using an ECL chemiluminescence kit (Millipore, MA, USA).

Cell invasion assay

The upper surface of Transwell chambers was coated with Matrigel to simulate the extracellular matrix. Cells were seeded into the upper compartment, and culture medium supplemented with chemical factors was added to the lower compartment. The chambers were incubated at 37°C in a 5% CO₂ atmosphere for 12-48 hours. After fixation with tissue fixative for 30 minutes, the cells were stained with crystal violet for 30 minutes. The number of cells that had invaded the lower chamber was then observed and counted under a microscope.

Cell migration assay

Cells were cultured to 80%-90% confluence to ensure they were in a good state of growth. A sterile cell scraper was used to create a scratch in the cell monolayer. The migration of cells into the wounded area was then observed.

Cell proliferation

Cell proliferation was measured using the Cell Counting Kit-8 (CCK-8 Kit, Beyotime Institute of Biotechnology, China). Cells were seeded in 96-well plates and treated under different conditions for 12, 24, and 48 hours. Subsequently, 10 μ l of CCK-8 solution was added to each well, and the plates were incubated at 37°C for an additional 1-2 hours. Absorbance at 450 nm was measured using a BioTek Cytation 3 imaging microplate reader. EdU staining was performed to assess cell proliferation. Cells were seeded in 6-well plates and incubated with 10 μ M EdU working solution for 2 hours. Cells were then incubated and fixed with 1 mL of 4% paraformaldehyde in 20 minutes, permeabilized with 1 mL of 0.1% Triton X-100 in 10 minutes and blocked with 1 mL of 5% BSA in 60 minutes. The blocking solution was subsequently cleared, and a newly prepared Click reaction solution (980 μ L buffer + 10 μ L 100 mM CuSO₄ + 10 μ L fluorescent dye) was added and incubated with the solution (30 minutes in the dark). The 1 mL of 1 μ g/mL DAPI was added to the cells and after 5 minutes, the cells were seen and photographed under a microscope with fluorescence.

Construction of co cultured 3D tumor spheres

Cells in logarithmic growth phase including Cal27, hOMF and CAF were digested with trypsin and centrifuged. Single cell suspensions were prepared with serum free stem cell medium and counted. Low adhesion culture plates were used for seeding. Each well was seeded with a mixture of 2000 Cal27 cells and 1000 corresponding fibroblasts or CAF cells, followed by the addition of serum free stem cell medium. The plates were incubated at 37 degrees Celsius with 5 percent carbon dioxide. Bright field microscope was used to capture images of tumor spheres on day 1 and day 5. Image software was applied to randomly measure sphere diameters for statistical analysis, so as to com-

pare the regulatory effects of different fibroblasts on the growth of Cal27 3D tumor spheres.

Cellular ATP assay

The cell lines were seeded in culture plates and exposed to various treatments in line with the experimental design. The equilibrating of the reagents was done using the Beyotime ATP Assay Kit at the room temperature over a period of 30 minutes. Cell culture medium was added and the mixture was gently swirled and allowed to incubate to allow full reaction. A luminescence intensity was then measured with a luminometer, which indicates the concentration of intracellular ATP. Lastly, ATP was determined by comparing to a standard curve, and statistical analysis was done on the differences between treatment groups.

Analysis of lactic acid secretion by cells

The hOMF cells under various conditions were grown in 6-well plates with the same density and left to adhere. Following 48 hours of additional culture, the culture supernatant of each well was removed and clarified by centrifugation at 2000×g during 5 minutes. The level of lactic acid in the supernatant was then determined as recommended by the manufacturer of the Lactic Acid Assay Kit (Nanjing Jiancheng Bioengineering Institute).

Cellular glucose uptake

The glucose-sensing fluorescent probe was a fluorescent analog of glucose called Glucose Uptake Probe Red, which simulates the glucose uptake into the cell and releases a red fluorescence. The labeled glucose was added and incubated after cell seeding (30-60 minutes). Glucose uptake was evaluated by the intensity of the intracellular fluorescence which was observed under inverted fluorescence microscope (Olympus Beijing Co., Ltd.), and the intensity of the fluorescence.

Transcriptome sequencing and analysis

Total RNA was extracted from treated cells using TRIzol reagent (Servicebio). Sequencing data were analyzed by alignment with the known reference genome and corresponding annotation files. The expression abundance of

each protein-coding gene in different samples was quantified as raw counts. The FPKM (Fragments Per Kilobase of transcript per Million mapped reads) method was used to eliminate the influence of gene length and sequencing depth on expression level calculations. Subsequently, gene counts from each sample were normalized using the DESeq2 software package. Normalization was performed using the BaseMean value to estimate expression levels, and the fold change for each gene was calculated. Differential expression significance was tested using the negative binomial (NB) test. Finally, significantly differentially expressed protein-coding genes were identified based on both the fold change and the results of the significance test. All sequencing data have been submitted to the GEO public database and are accessible under the accession number GSE294841 (pending upload).

Animal origin and housing

All BALB/c nude mice used in this study were purchased from Beijing SPF Biotechnology Co., Ltd. (Animal Production License: SCXK-2024-0001). The mice tested negative for 21 common pathogens, including hepatitis viruses, parasites, and ectromelia virus. The SPF mice were kept in a standardized animal house at Kunming Medical University. The humidity was kept at 40%-60% and the temperature was kept at 22-24°C. Mice were kept in a light/dark cycle of 12 hours light and 12 hours dark with a light intensity of about 200-300 lux. Sterile food and treated distilled water were provided and regularly replaced. All equipment and facilities in the animal housing environment were thoroughly cleaned and disinfected weekly to ensure a sterile environment.

Tumor xenograft model

Animal studies were sanctioned by the Ethics Review Committee for Animal Experiments at Kunming Medical University (kmmu20230673). Animals were obtained and housed as described above. We established a subcutaneous co-transplantation mouse model of hOMF/CAF and Cal-27 cells. A mixture of 5×10^6 hOMF/CAF cells and 5×10^6 Cal-27 cells was injected into the axillary region of each mouse. Six days after injection, the tumor size and body weight of tumor-bearing mice were recorded. Body weight and tumor size were measured every 3

days. Mice were sacrificed on day 27, and the final tumor weight was recorded. Tumor volume was calculated using the formula: $V = L \times W \times W/2$.

Euthanasia of mice

Mice were intraperitoneally injected with pento-barbital sodium at 50-70 mg/kg (body weight) and administered via intraperitoneal injection (IP). Evaluate the state of mice by assessing their muscle tone, external stimuli, and respiratory rate, and wait for the mice to enter an anesthetized state. Then hold onto the tail of the mouse and quickly pull it upwards to dislocate its neck. During the process, try to minimize noise and maintain fast and precise operation, avoid mouse stress, and reduce any potential pain.

Immunohistochemistry

Tumour tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned into 5 µm thick slices. Subsequently, the portions underwent dewaxing and rehydration. The tissue sections were then stained with antibodies against Ki67 (100:1), α-SMA (100:1), and p-mTOR (100:1). The morphology and architecture of the tissues were observed using hematoxylin and eosin staining. We conducted PAS and MASSON staining to investigate fibrosis further. The Olympus VS120 imaging system captured images of the stained sections. The ratio of Ki67-positive cells was employed to assess proliferative tumour cells. The ratios of α-SMA and p-mTOR positive areas were employed to evaluate the expression levels of α-SMA and p-mTOR proteins in cancer tissues. The percentages of PAS and MASSON positive regions were utilised to quantify the extent of fibrosis in tumour tissues.

Statistical analysis

All experimental data are presented as the mean ± standard deviation (Mean ± SD). Unpaired t-tests were used for comparisons between two groups. For multi group comparisons, one way ANOVA was applied for independent data, while repeated measures ANOVA was used for comparisons involving a time dependent variable (e.g., CCK 8 and EdU proliferation assays over multiple time points). Data analysis and graph generation were performed

using GraphPad Prism 9.5 software. Unless otherwise stated in the figure legends, each data point represents three biological replicates. Statistical significance was defined as follows: (ns) $P > 0.05$, (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$.

Results

PDGF-BB induced fibroblasts to acquire a CAF phenotype, which in turn promoted the malignant behavior of tumor cells

PDGF-BB is highly secreted by tumor cells. We treated hOMF cells with PDGF-BB (30 ng/ml) for 48 hours and observed that the cell morphology changed from a short spindle shape to a long spindle shape (**Figure 1A**). Furthermore, using immunofluorescence and Western blot analyses, we found that the expression levels of α-SMA, FAP, and PDGFR-β were significantly upregulated, indicating that the fibroblasts had acquired a CAF phenotype upon PDGF-BB treatment (**Figure 1B-I**). In addition, we found that PDGF-BB-treated fibroblasts exhibited significantly enhanced glucose uptake and markedly increased production of ATP and lactate (**Figure 1J-M**). These results indicate that glycolysis was significantly upregulated in PDGF-BB-treated fibroblasts. To further clarify this phenomenon, we observed that the expression levels of glycolysis-related proteins, including PKM2, GLUT1, and LDHA, were significantly increased in PDGF-BB-induced cells (**Figure 1N-Q**). We treated Cal27 cells with the supernatant from PDGF-BB-induced fibroblasts and found that it significantly promoted the proliferation, migration, and invasion of tumor cells (**Figure 1R-X**). To further mimic the interaction between CAFs and tumor cells in vivo, we co-cultured CAFs with Cal-27 cells to generate 3D tumor spheres. The results showed that the spheres in the CAF and Cal-27 group were significantly larger than those in the hOMF and Cal-27 group (**Figure 1Y, 1Z**). These results indicate that the cytokine PDGF-BB induces the transformation of fibroblasts into CAFs and promotes the proliferation, migration, and invasion of tumor cells.

LINC00886 is significantly upregulated in CAFs (PDGF-BB/Cal27-CM)

Based on our previous findings that PDGF-BB induces the transformation of fibroblasts into a

CAF-driven pro-tumorigenic effects

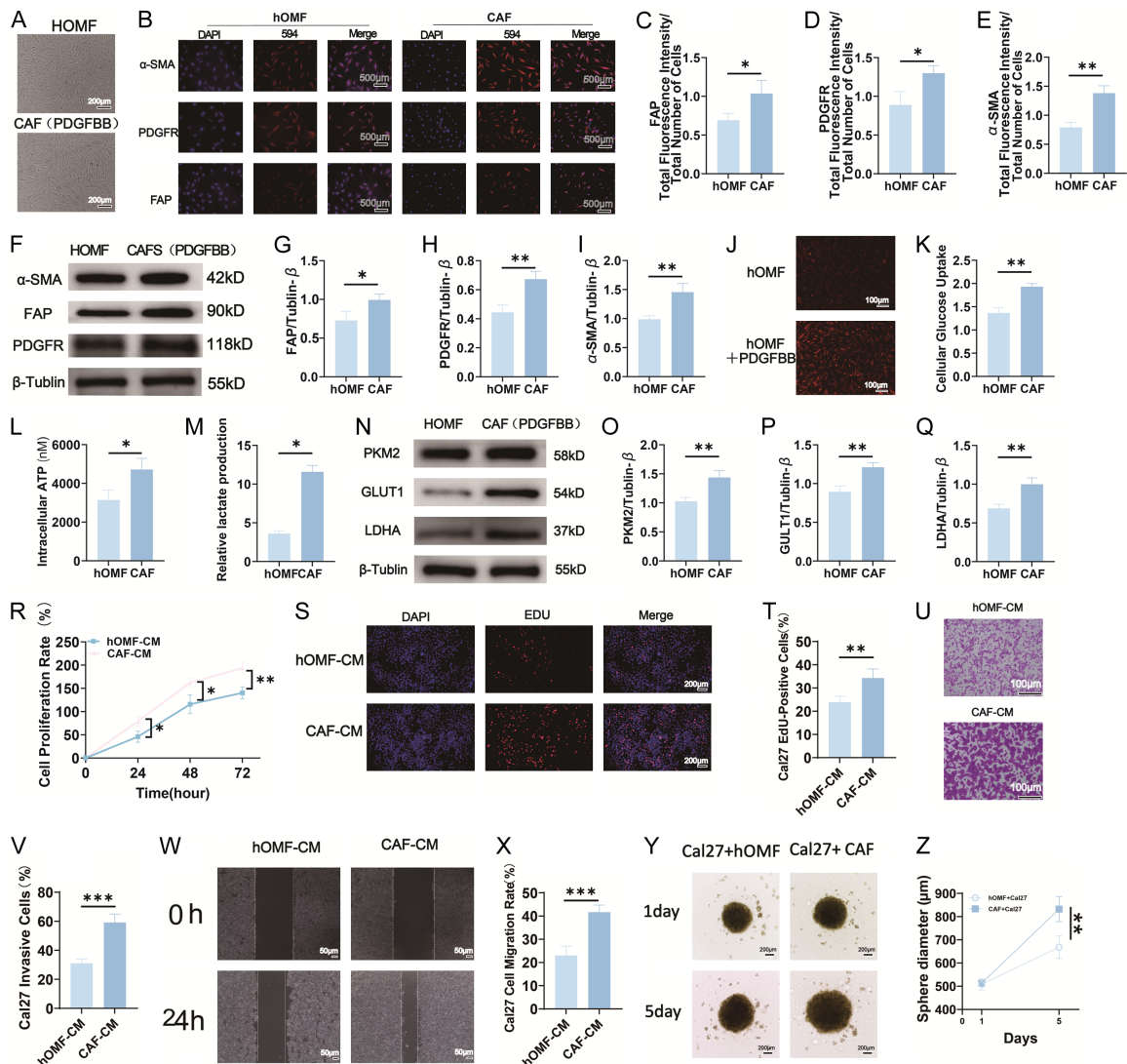


Figure 1. PDGF-BB induces the transformation of fibroblasts into CAFs and promotes the malignant behavior of tumor cells. **A**, Light microscopy images of hOMF cells after PDGF-BB treatment (scale bar, 200 μ m); **B**, Representative immunofluorescence images of α -SMA/FAP/PDGFR- β in hOMF cells after PDGF-BB treatment (scale bar, 500 μ m); **C-E**, Quantitative analysis of α -SMA/FAP/PDGFR- β immunofluorescence intensity in hOMF cells after PDGF-BB treatment; **F**, Western blot analysis of α -SMA/FAP/PDGFR- β in hOMF cells after PDGF-BB treatment; **G-I**, Quantitative analysis of α -SMA/FAP/PDGFR- β Western blot bands in hOMF cells after PDGF-BB treatment; **J**, Glucose uptake images of hOMF cells after PDGF-BB treatment (scale bar, 100 μ m); **K**, Quantitative analysis of glucose uptake in hOMF cells after PDGF-BB treatment; **L**, ATP levels in hOMF cells after PDGF-BB treatment; **M**, Lactate secretion by hOMF cells after PDGF-BB treatment; **N**, Western blot analysis of PKM2/GLUT1/LDHA in hOMF cells after PDGF-BB treatment; **O-Q**, Quantitative analysis of PKM2/GLUT1/LDHA Western blot bands in hOMF cells after PDGF-BB treatment; **R**, CCK-8 assay to evaluate the effect of CAF (PDGF-BB)-CM on Cal27 cell proliferation; **S**, EdU assay to evaluate the effect of CAF (PDGF-BB)-CM on Cal27 cell proliferation (scale bar, 200 μ m); **T**, Statistical analysis of EdU proliferation assay; **U**, Invasion assay to evaluate the effect of CAF (PDGF-BB)-CM on Cal27 cell invasion (scale bar, 100 μ m); **V**, Statistical analysis of Cal27 cell invasion upon CAF (PDGF-BB)-CM treatment; **W**, Migration assay to evaluate the effect of CAF (PDGF-BB)-CM on Cal27 cell migration (scale bar, 50 μ m); **X**, Statistical analysis of Cal27 cell migration upon CAF (PDGF-BB)-CM treatment; **Y**, Representative bright-field images of tumor spheres from Cal27 cells co-cultured with normal oral fibroblasts (hOMFs) or CAFs at day 1 and day 5 (scale bar, 200 μ m); **Z**, Quantitative statistics of tumor sphere diameters corresponding. The data were expressed as the mean $\pm$ standard deviation. *p* values were calculated using two-sided Student's *t*-test (^{ns}*P* > 0.05, **P* < 0.05, ***P* < 0.01, ****P* < 0.001).

CAF phenotype and enhances their glycolytic metabolism, thereby promoting the malignant

behavior of tumor cells, we performed transcriptome sequencing and identified the long

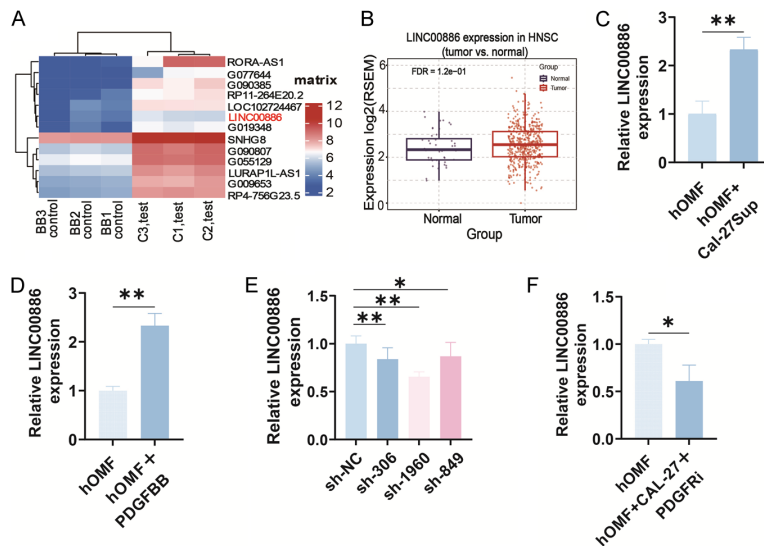


Figure 2. LINC00886 is upregulated in CAF (PDGF-BB/Cal27-CM) cells. A. Heatmap of differentially expressed genes in hOMF cells following PDGF-BB treatment. B. Expression difference of LINC00886 between tumor and normal tissues. C. LINC00886 expression in CAF (Cal27-CM) cells. D. LINC00886 expression in CAF (PDGF-BB) cells. E. Knockdown efficiency of different sh-LINC00886 sequences. F. LINC00886 expression in hOMF cells co-treated with Cal27-CM and a PDGFR inhibitor. The data were expressed as the mean $\pm$ standard deviation. p values were calculated using two-sided Student's t -test ($^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

non-coding RNA LINC00886 as being significantly upregulated in PDGF-BB-treated hOMF cells (Figure 2A). LINC00886 was also markedly overexpressed in tumor tissues compared with normal tissues (Figure 2B). Consistent with these results, qPCR analysis confirmed that PDGF-BB treatment significantly increased LINC00886 expression in hOMF cells (Figure 2D). In addition, treatment of hOMF cells with Cal27-CM also upregulated LINC00886 (Figure 2C). However, when a PDGFR inhibitor was added to Cal27-CM, the upregulation of LINC00886 in hOMF cells was significantly attenuated (Figure 2E). Collectively, these results indicate that LINC00886 is a key gene involved in the transformation of hOMF cells into CAFs. To identify the shRNA with optimal LINC00886 knockdown efficiency, qRT-PCR was performed to test all candidate sequences. Normal CAFs and PDGF-BB-treated CAFs expressing negative control shRNA served as controls. Statistical analysis of repeated experiments showed divergent silencing efficiencies across shRNAs; sh-1960 achieved the most robust, stable, and statistically significant reduction in LINC00886 transcription (Figure 2F).

LINC00886 is essential for CAF (PDGF-BB)-mediated promotion of tumor cell malignancy

Based on these findings, we generated a stable LINC00886-knockdown CAF (PDGF-BB) cell line (Figure 3A). Knockdown of LINC00886 significantly decreased the proliferation, migration, and invasion abilities of CAF (PDGF-BB) cells themselves (Figure 3B-H), and also markedly reduced glucose uptake (Figure 3I, 3J). In addition, ATP and lactate production were significantly attenuated in LINC00886-knockdown CAF (PDGF-BB) cells (Figure 3K, 3L). Western blot analysis showed that the expression of glycolysis-related proteins PKM2, GLUT1, and LDHA was significantly downregulated in LINC00886-depleted CAF (PDGF-

BB) cells (Figure 3M-P). These results indicate that the CAFs' phenotype and functions were reversed by LINC00886 knockdown. When tumor cells were treated with conditioned medium from LINC00886-knockdown CAF (PDGF-BB) cells, their proliferation, migration, and invasion abilities were significantly inhibited compared with those treated with conditioned medium from control CAF (PDGF-BB) cells (Figure 3Q-W). Furthermore, we co-cultured Cal 27 cells with LINC00886 knockout CAF (PDGF-BB) cells to form 3D tumor spheres. The results showed that the spheres in the group of Cal 27 cells co cultured with LINC00886 knockout CAF PDGF-BB cells were significantly smaller than those in the group of Cal 27 cells co-cultured with normal CAF (PDGF-BB) cells (Figure 3X, 3Y). Collectively, these results demonstrate that LINC00886 is a key mediator of the pro-tumorigenic effects of CAFs.

hsa-miR-15b-5p downregulation correlates with poor prognosis in patients with head and neck squamous cell carcinoma and may potentially bind to LINC00886

Accumulating evidence indicates that lncRNAs do not function independently but often regu-

CAF-driven pro-tumorigenic effects

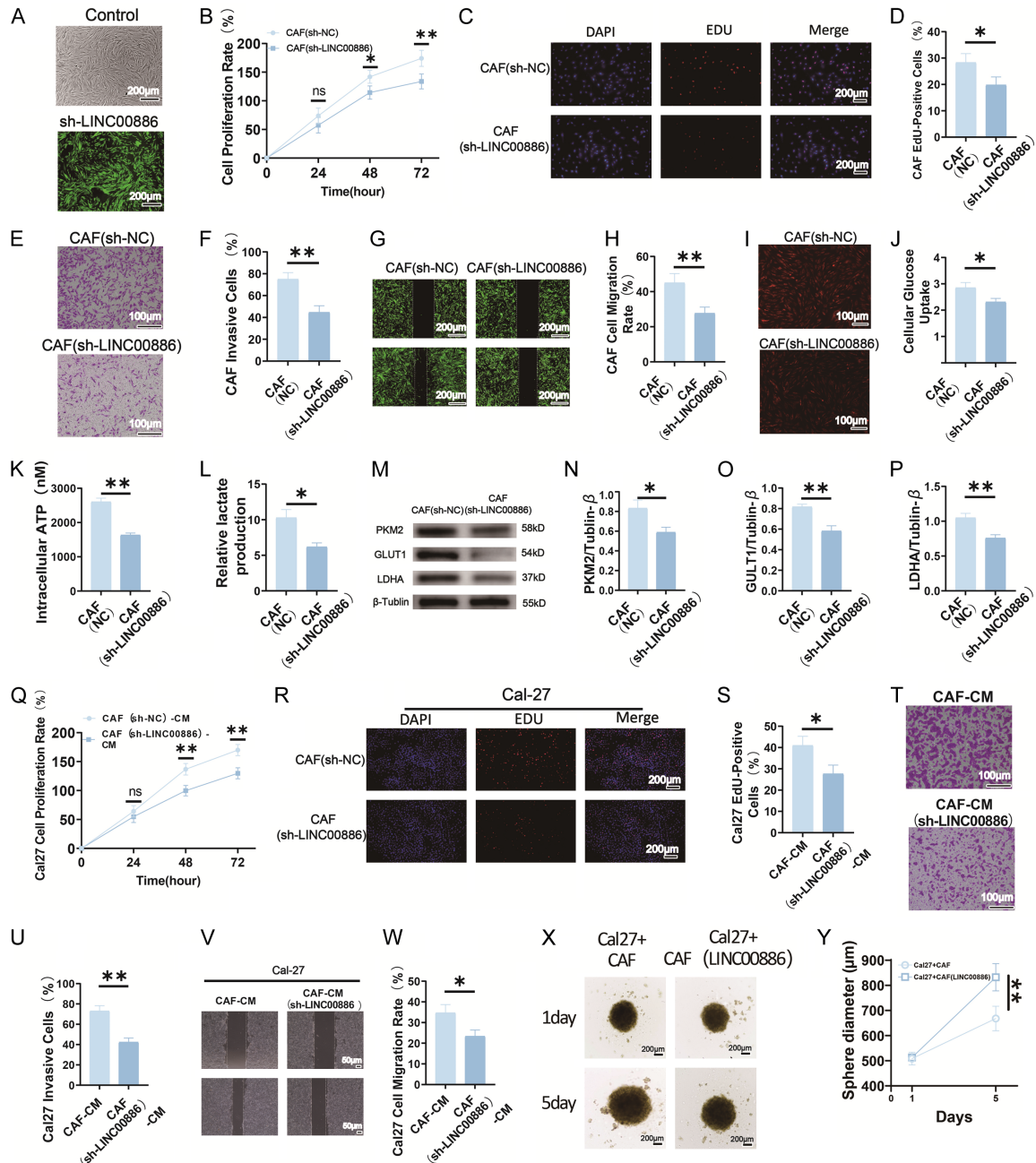


Figure 3. Knockdown of sh-LINC00886 impairs the pro-tumorigenic effects of CAFs. **A.** Representative images of CAFs after LINC00886 knockdown (scale bar, 200 μ m). **B.** CCK-8 assay showing the proliferation of CAF (sh-LINC00886) cells. **C.** EdU staining showing the proliferation of CAF (sh-LINC00886) cells (scale bar, 200 μ m). **D.** Quantitative analysis of EdU-positive cells. **E.** Invasion assay showing the invasive ability of CAF (sh-LINC00886) cells (scale bar, 100 μ m). **F.** Quantitative analysis of cell invasion. **G.** Migration assay showing the migratory ability of CAF (sh-LINC00886) cells (scale bar, 200 μ m). **H.** Quantitative analysis of cell migration. **I.** Representative images of cellular glucose uptake (scale bar, 100 μ m). **J.** Quantitative analysis of glucose uptake. **K.** ATP levels in CAF (sh-LINC00886) cells. **L.** Lactate secretion by CAF (sh-LINC00886) cells. **M.** Western blot analysis of PKM2, GLUT1, and LDHA in CAF (sh-LINC00886) cells. **N-P.** Densitometric quantification of PKM2/GLUT1/LDHA Western blot bands. **Q.** CCK-8 assay showing the effect of CAF (sh-LINC00886)-CM on Cal27 cell proliferation. **R.** EdU staining showing the effect of CAF (sh-LINC00886)-CM on Cal27 cell proliferation (scale bar, 200 μ m). **S.** Quantitative analysis of EdU-positive Cal27 cells. **T.** Invasion assay showing the effect of CAF (sh-LINC00886)-CM on Cal27 cell invasion (scale bar, 100 μ m). **U.** Quantitative analysis of Cal27 cell invasion. **V.** Migration assay showing the effect of CAF (sh-LINC00886)-CM on Cal27 cell migration (scale bar, 50 μ m). **W.** Quantitative analysis of Cal27 cell migration. **X.** Representative bright-field images of tumor spheres from Cal27 cells co-cultured with control CAFs or LINC00886-knockdown CAFs at day 1 and day 5 (scale bar, 200 μ m). **Y.** Quantitative statistics of tumor sphere diameters corresponding. The data were expressed as the mean $\pm$ standard deviation. p values were calculated using two-sided Student's t -test (ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

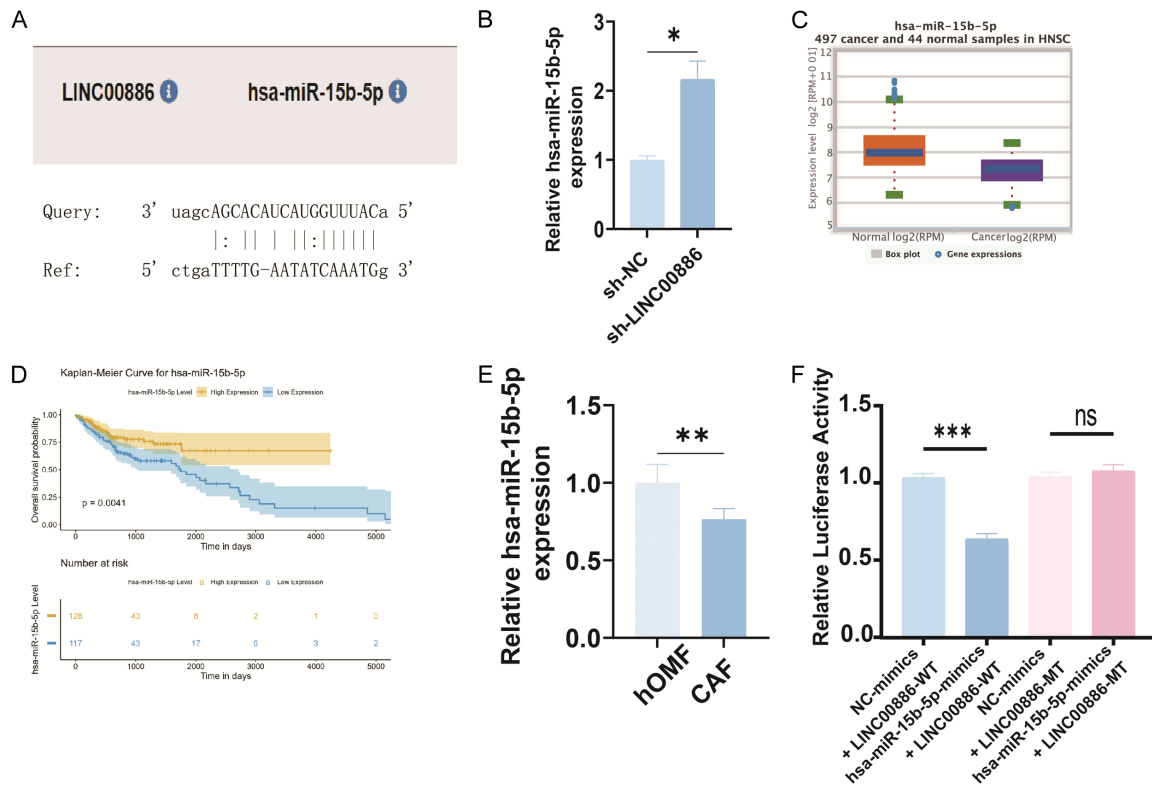


Figure 4. The miRNA has-miR-15b-5p potentially binds to LINC00886 and correlates with prognosis in patients with head and neck squamous cell carcinoma. A. Prediction by ENCORI (<https://rnasysu.com/encori/>) indicating potential binding between LINC00886 and has-miR-15b-5p. B. Expression of has-miR-15b-5p in CAF (sh-NC) and CAF (sh-LINC00886) cells. C. Expression of has-miR-15b-5p in head and neck squamous cell carcinoma (HNSC) tissues and normal tissues. D. Survival analysis of has-miR-15b-5p in HNSC patients (data from GEPIA2, <https://gepia2.cancer-pku.cn>). E. Expression difference of has-miR-15b-5p between hOMF and CAF (PDGF-BB) cells. F. Dual-Luciferase Reporter Assay Confirms the Direct Interaction between LINC00886 and has-miR-15b-5p. The data were expressed as the mean $\pm$ standard deviation. p values were calculated using two-sided Student's t -test ($^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

late cellular biological behaviors by acting as miRNA sponges. Using the online tool ENCORI, we predicted potential miRNAs that might bind to LINC00886 and identified has-miR-15b-5p as a candidate with potential binding capacity (**Figure 4A**). Consistent with this prediction, knockdown of LINC00886 in CAF (PDGF-BB) cells resulted in a significant upregulation of has-miR-15b-5p (**Figure 4B**). Furthermore, analysis using the online database GEPIA-2 revealed that has-miR-15b-5p expression was lower in head and neck squamous cell carcinoma (HNSC) tissues than in normal tissues (**Figure 4C**). In addition, HNSC patients with high has-miR-15b-5p expression exhibited better overall survival compared with those with low has-miR-15b-5p expression (**Figure 4D**). Based on these findings, we propose that has-miR-15b-5p may potentially bind to LINC00886.

Treatment of normal fibroblast cell lines with PDGF-BB resulted in a significant decrease in the expression of has-miR-15b-5p (**Figure 4E**). To validate whether LINC00886 functions as a molecular sponge for has-miR-15b-5p, we performed a dual-luciferase reporter assay using wild-type (WT) and mutant-type (MT) LINC00886 reporter constructs containing the predicted binding sites for has-miR-15b-5p co-transfection of has-miR-15b-5p mimics with the LINC00886-WT reporter construct significantly reduced the relative luciferase activity compared to the NC-mimics control group. In contrast, no significant difference in luciferase activity was observed between the NC-mimics and has-miR-15b-5p mimics groups when co-transfected with the LINC00886-MT reporter construct. These results indicate that LINC00886 directly interacts with has-miR-15b-5p

via the predicted binding sites, confirming that LINC00886 acts as a ceRNA to sponge hsa-miR-15b-5p in OSCC cells (**Figure 4F**).

LINC00886 interacts with hsa-miR-15b-5p to regulate the AKT-mTOR signaling pathway

We hypothesized that the interaction between LINC00886 and hsa-miR-15b-5p might influence the biological behavior of CAF (PDGF-BB) cells. Transcriptome sequencing analysis was performed on CAF and CAF (sh-LINC00886) cells, and the volcano plot demonstrated 149 upregulated genes and 289 downregulated genes (**Figure 5A**). Notably, knockdown of LINC00886 led to the significant enrichment of GO terms associated with energy metabolism (**Figure 5C**). Consistent with this finding, Western blot analysis of glycolysis-related proteins (PKM2, GLUT-1, LDHA), along with measurements of glucose uptake, lactate secretion, and ATP production, further confirmed that glycolytic activity was suppressed in LINC00886-depleted CAFs (**Figure 3M-P**). KEGG pathway enrichment analysis demonstrated that the PI3K-AKT signaling pathway was significantly downregulated in CAF (sh-LINC00886) cells (**Figure 5B**), which was further validated by Western blotting (**Figure 5G-I**). Interestingly, this pathway was activated in CAF (PDGF-BB) cells (**Figure 5D-F**). Transfection of CAF (PDGF-BB) cells with hsa-miR-15b-5p inhibitor increased total AKT protein levels and promoted mTOR phosphorylation (**Figure 5M-O**). Furthermore, re-treatment of LINC00886-knockdown CAFs with PDGF-BB restored AKT expression and enhanced mTOR phosphorylation (**Figure 5J-L**). Following transfection of CAF (sh-NC) and CAF (sh-LINC00886) cells with hsa-miR-15b-5p inhibitor, the expression levels of p-mTOR, mTOR, and AKT were reversed (**Figure 5P-R**). Furthermore, we treated Cal 27 cells with conditioned medium collected from hOMF and CAF respectively. The results showed that the levels of phosphorylated AKT and phosphorylated mTOR were obviously increased in Cal 27 cells treated with CAF conditioned medium (**Figure 5S-U**). Collectively, these results indicate that hsa-miR-15b-5p regulates AKT expression, thereby modulating the PDGF-BB/AKT/mTOR signaling pathway, and this process is itself controlled by LINC00886-mediated regulation of hsa-miR-15b-5p.

CAF (sh-LINC00886) affects tumor progression in a co-transplantation model

To further investigate whether LINC00886 plays an important role in CAF (PDGF-BB)-mediated promotion of tumor cell malignancy, we established a subcutaneous co-transplantation model containing hOMF/CAF and Cal-27 cells (**Figure 6A, 6M**). The results showed that tumor growth was significantly enhanced in the hOMF/Cal-27 co-transplantation group compared with the Cal-27 alone group (**Figure 6B-E**). PAS and MASSON staining revealed extensive fibrosis in the hOMF/Cal-27 co-transplantation group (**Figure 6F-H**). Immunohistochemical staining showed that the expression of α -SMA was significantly higher in the hOMF/Cal-27 co-transplantation group than in the Cal-27 alone group (**Figure 6F, 6J**), and the proportion of Ki67-positive cells was also increased (**Figure 6F, 6L**). These results indicate that fibroblasts are induced by tumor cells to transform into CAFs, thereby promoting tumor progression. In addition, tumor growth in the PDGF-BB-induced CAF/Cal-27 co-transplantation group was significantly greater than that in the Cal-27 alone group and the hOMF/Cal-27 co-transplantation group (**Figure 6B-E**). Furthermore, tumors in the CAF (sh-LINC00886)/Cal-27 co-transplantation group were significantly smaller than those in the CAF/Cal-27 co-transplantation group. PAS and MASSON staining showed that the fibrotic area was markedly reduced in the CAF (sh-LINC00886)/Cal-27 group compared with the CAF/Cal-27 group. Immunohistochemical staining revealed that the expression of α -SMA was significantly lower in the CAF (sh-LINC00886)/Cal-27 group than in the CAF/Cal-27 group, and the proportion of Ki67-positive cells was also decreased. In addition, the positive area of p-mTOR, AKT, was also reduced (**Figure 6F, 6I, 6K**). These results indicate that LINC00886 plays an important role in PDGF-BB-induced CAF-mediated tumor progression.

Discussion

Platelet-derived growth factor-BB (PDGF-BB) is a key profibrotic factor in the tumor microenvironment (TME) [42]. In various solid tumors such as breast cancer and pancreatic cancer, PDGF-BB has been shown to induce the transformation of fibroblasts into cancer-associated

CAF-driven pro-tumorigenic effects

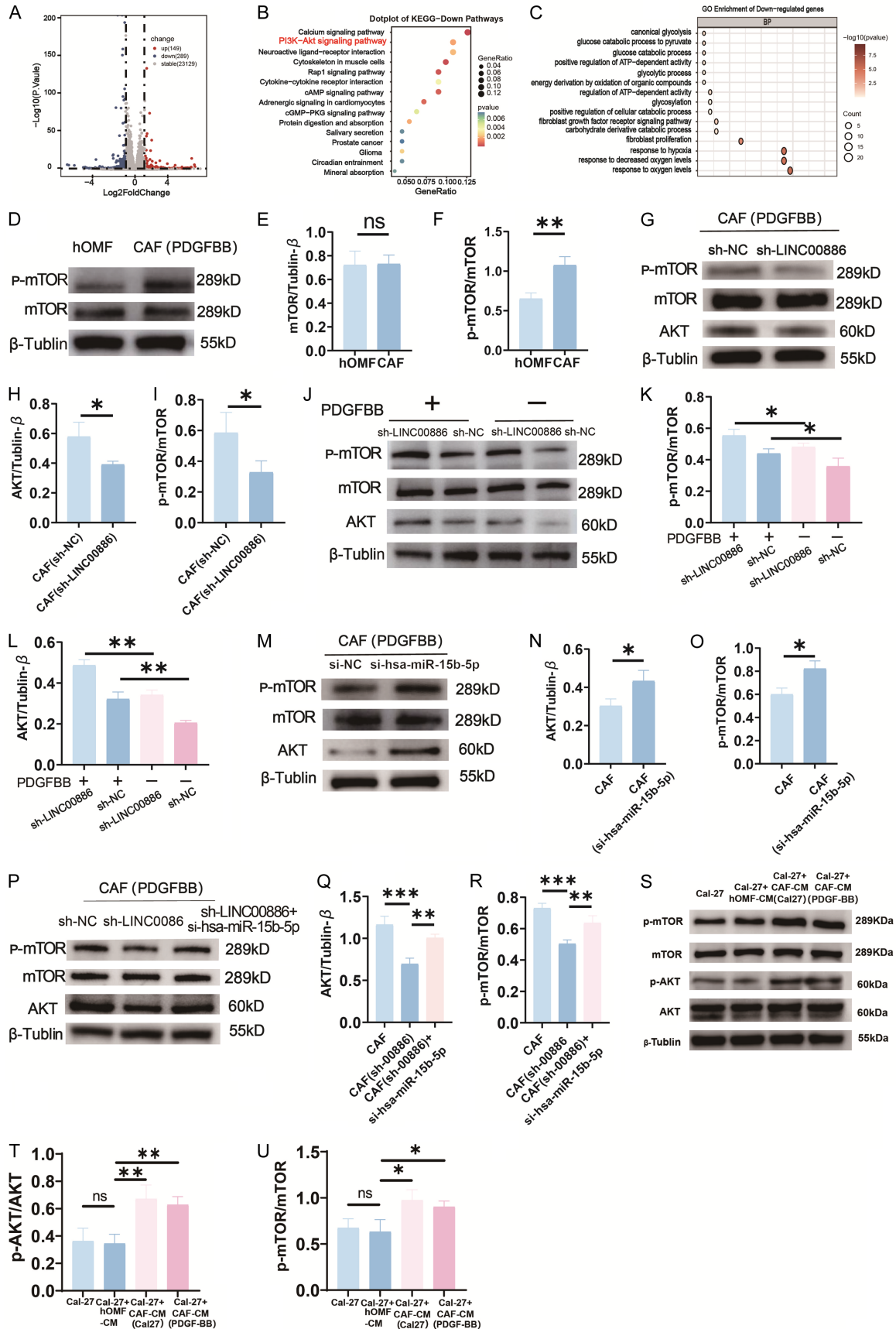


Figure 5. LINC00886 interacts with hsa-miR-15b-5p to regulate AKT expression and modulate the AKT-mTOR signaling pathway. A. Volcano plot of differentially expressed genes in CAF (PDGF-BB) cells after sh-LINC00886 knockdown. B. KEGG pathway enrichment analysis of CAF (PDGF-BB) cells after sh-LINC00886 knockdown. C. GO functional enrichment analysis of CAF (PDGF-BB) cells after sh-LINC00886 knockdown. D. Western blot analysis of p-mTOR and mTOR in hOMF and CAF (PDGF-BB) cells. E, F. Quantitative analysis of p-mTOR and mTOR Western blot bands. G. Western blot analysis of p-mTOR, mTOR, and AKT in CAF (sh-NC) and CAF (sh-LINC00886) cells. H, I. Quantitative analysis of p-mTOR, mTOR, and AKT Western blot bands. J. Western blot analysis of p-mTOR, mTOR, and AKT in CAF (sh-NC) and CAF (sh-LINC00886) cells following PDGF-BB treatment. K, L. Quantitative analysis of p-mTOR, mTOR, and AKT Western blot bands after PDGF-BB treatment. M. Western blot analysis of p-mTOR, mTOR, and AKT in CAF (PDGF-BB) cells transfected with hsa-miR-15b-5p inhibitor. N, O. Quantitative analysis of p-mTOR, mTOR, and AKT Western blot bands after hsa-miR-15b-5p inhibitor transfection. P. Western blot analysis of p-mTOR, mTOR, and AKT in CAF (sh-LINC-NC) and CAF (sh-LINC00886) cells transfected with hsa-miR-15b-5p inhibitor. Q, R. Quantitative analysis of p-mTOR, mTOR, and AKT Western blot bands in hsa-miR-15b-5p-transfected CAF (sh-NC) and CAF (sh-LINC00886) cells. S. Western blot detection of intracellular p-mTOR, mTOR, p-AKT and AKT proteins in Cal-27 cells treated with CAF conditioned medium. T. Quantitative statistics of relative p-AKT/AKT protein expression. U. Quantitative statistics of relative p-mTOR/mTOR protein expression. The data were expressed as the mean $\pm$ standard deviation. *p* values were calculated using two-sided Student's *t*-test (^{ns}*P* > 0.05, **P* < 0.05, ***P* < 0.01, ****P* < 0.001).

fibroblasts (CAFs) by activating the PDGFR- β signaling pathway [43]. However, whether and how PDGF-BB regulates CAF activation through non-coding RNAs in tongue squamous cell carcinoma remains unclear. In the present study, treatment of human normal oral fibroblasts (hOMFs) with 30 ng/mL PDGF-BB for 48 hours induced a morphological shift from a short spindle shape to the long spindle shape characteristic of CAFs [44], and also resulted in a marked upregulation of the long non-coding RNA LINC00886. This effect was abrogated by a PDGFR inhibitor, suggesting the existence of a PDGF-BB-PDGFR-LINC00886 regulatory axis in tongue squamous cell carcinoma. In oral cancer, PDGF-BB has also been reported to promote CAF activation by upregulating the lncRNA LURAP1L-AS1, further supporting the diverse lncRNA-mediated regulatory networks of PDGF-BB in stromal cell activation across malignancies [45]. This study is the first to demonstrate that PDGF-BB induces LINC00886 expression and promotes CAF activation in tongue squamous cell carcinoma, suggesting that PDGF-BB may drive CAF transformation by regulating distinct lncRNAs in different cancer types, reflecting the cancer-specific nature of tumor micro-environment regulation. Being a long non-coding RNA, LINC00886 is not a simple transcription product, but rather a regulatory molecule of significance that has a significant role in the TME. Knockdown of LINC00886 resulted in lowering CAF protein markers, lactate secretion, and aerobic glycolysis, which further confirms that LINC00886 plays a critical role in the CAF transformation and the metabolism of the tumor, as summarized in **Figure 7** [46, 47].

We predicted in this study the existence of potential binding sites in LINC00886 of hsa-miR-15b-5p using the ENCORI database. Experimental validation showed that knockdown of LINC00886 significantly increased hsa-miR-15b-5p expression in CAFs. Analysis of the head and neck squamous cell carcinoma (HNSC) dataset via the GEPIA-2 database revealed that hsa-miR-15b-5p is downregulated in tumor tissues, and its low expression is significantly associated with poor patient prognosis, indicating a tumor-suppressive role. Further mechanistic studies demonstrated that LINC00886 acts as a competing endogenous RNA (ceRNA) to sponge hsa-miR-15b-5p, thereby relieving its inhibitory effect on downstream targets.

miR-15b-5p is a well-known miRNA implicated in the progression of various cancers, and its low expression is closely associated with malignant transformation, metastasis, and drug resistance in tongue squamous cell carcinoma [48, 49]. Beyond PDGF-BB-related stromal regulation, miRNAs also play critical roles in tumor progression through targeted gene interactions: hsa-miR-15b-5p was previously shown to promote gastric cancer progression by directly targeting PAQR3 [50]. In the present study, we identified for the first time that hsa-miR-15b-5p regulates the AKT-mTOR pathway in tongue squamous cell carcinoma CAFs. When sponged by LINC00886, hsa-miR-15b-5p's inhibitory effect on AKT is relieved, leading to increased total AKT protein levels and activated mTOR phosphorylation, which ultimately enhances CAF proliferation, migration, and metabolic

CAF-driven pro-tumorigenic effects

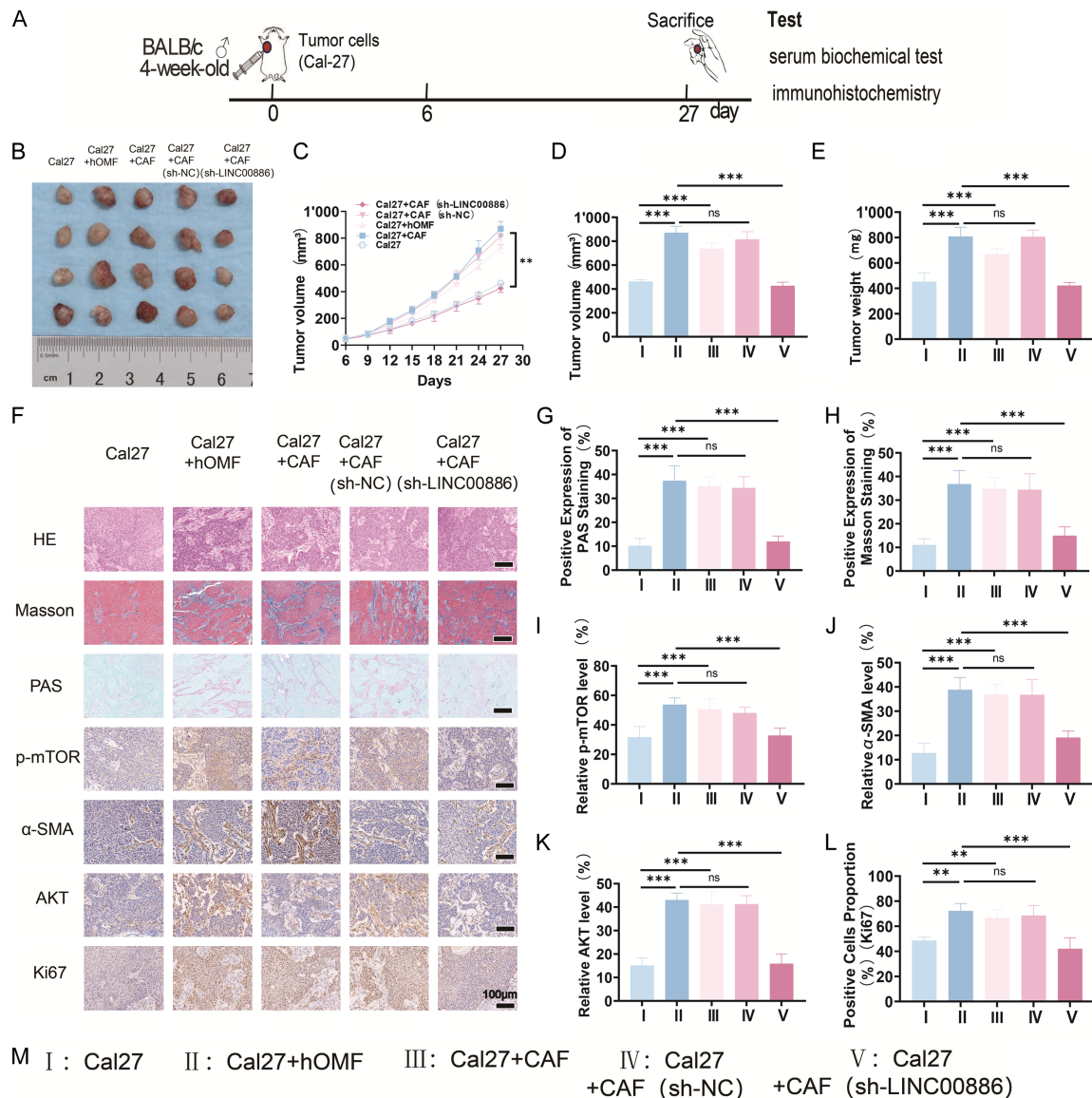


Figure 6. CAF (sh-LINC00886) affects tumor progression in a co-transplantation model. A. Schematic diagram of the animal tumor-xenograft model. B. Representative images of tumors (n = 4). C. Tumor growth curves (n = 4). D. Quantitative analysis of tumor volumes (n = 4). E. Quantitative analysis of tumor weights (n = 4). F. Representative micrographs of HE staining, PAS staining, MASSON staining, and immunohistochemical staining for α-SMA, Ki67, AKT, and p-mTOR in tumor sections (scale bar, 100 μm) (n = 4). G. Proportion of PAS-positive areas in tumor sections (n = 4). H. Proportion of MASSON-positive areas in tumor sections (n = 4). I. Proportion of p-mTOR-positive areas in tumor sections (n = 4). J. Proportion of α-SMA-positive cells in tumor sections (n = 4). K. Proportion of AKT-positive areas in tumor sections (n = 4). L. Proportion of Ki67-positive areas in tumor sections (n = 4). M. Experimental group labeling in the Figure 6. The data were expressed as the mean ± standard deviation. *p* values were calculated using two-sided Student's *t*-test (^{ns}*P* > 0.05, **P* < 0.05, ***P* < 0.01, ****P* < 0.001).

reprogramming. This finding expands the cell-type-specific functions of hsa-miR-15b-5p, suggesting its role may differ between tumor cells and stromal cells. Together, these findings demonstrate the context-specific functional diversity of critical signaling molecules and non-coding RNAs in tumorigenesis, which offer

useful information on the tissue-specific regulation of cancer progression.

The AKT-mTOR pathway is essential in the proliferation, survival, migration, and immune evasion of tumor cells [51]. The activation of the aberrant AKT pathway in oral tongue squamous

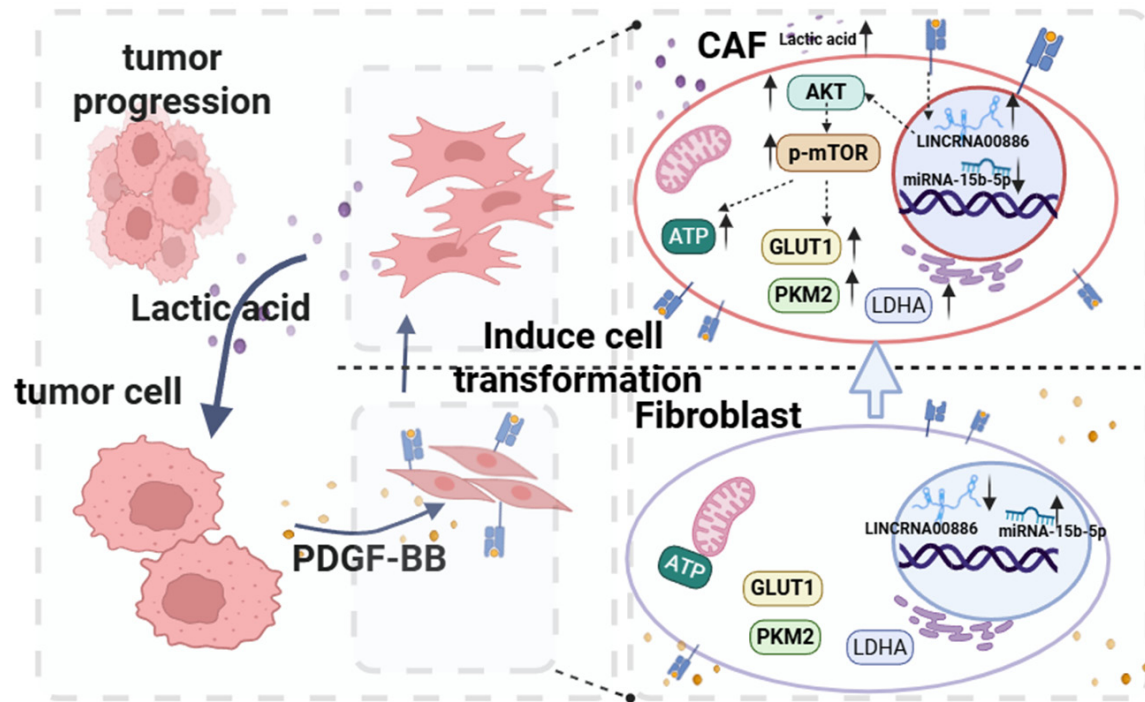


Figure 7. Schematic diagram illustrating the mechanism by which PDGF-BB mediates the LINC00886/hsa-miR-15b-5p/AKT axis to induce the transformation of fibroblasts into CAFs (Graphical abstract).

cell carcinoma (OTSCC) is closely linked to tumor growth, invasion, and metastasis [52]. We further expanded our knowledge of these context-dependent regulatory networks in order to extrapolate these observations and outline the mechanisms underlying tongue squamous cell carcinoma. We discovered that the LINC00886/hsa-miR-15b-5p axis triggers the AKT/mTOR pathway when stimulated with PDGF-BB but it is blocked by LINC00886 knockdown or hsa-miR-15b-5p overexpression. These results are in line with earlier studies that the AKT/mTOR pathway enhances CAF activation, but this is the first study to explain how this pathway is regulated upstream by non-coding RNA (LINC00886/hsa-miR-15b-5p) to activate the CAFs, thus completing the entire regulatory cascade: tumor cytokine (PDGF-BB)-lncRNA/miRNA (LINC00886/hsa-miR-15b-5p) - signaling pathway (AKT/mTOR)-CAF activation. Besides, activation of AKT-mTOR pathways is reported to control the activity of glycolytic enzymes (e.g., HK2 and LDHA) through phosphorylation. In line with this, we also found out that LINC00886 knockdown simultaneously blocked the AKT/mTOR pathway and glycolysis, which implies that AKT/mTOR is the “effector pathway” by which LINC00886 regulates CAF

metabolic reprogramming. This pathway regulates CAF energy metabolism through the incorporation of upstream ceRNA signaling and is a new way to understand “signal-metabolism coupling” in the TME.

Moreover, CAF metabolic reprogramming is not a solitary process [53]. Our analysis showed that PDGF-BB-stimulated CAFs (hereafter referred to as CAF (PDGF-BB) cells) had a significant promotion in the proliferation, migration, and invasion of Cal-27 cells, but LINC00886-knockdown CAFs exhibited a significant decrease in protumorigenic potential. This implies that CAF metabolites controlled by LINC00886 (e.g., lactate and ATP) can possibly feedback on tumor cells through metabolic symbiosis. In particular, CAFs stimulate glycolysis to generate lactate that is utilized as an energy source by tongue squamous cell carcinoma cells, and at the same time satisfy their high proliferation needs by aerobic glycolysis. The validation of this model of stroma-tumor metabolic symbiosis in tongue squamous cell carcinoma further emphasizes the pathological role of the PDGF-BB/LINC00886/AKT axis.

CAFs in the TME do not only aid tumor cell proliferation and metastasis, but also tumor progression through tumor metabolism modulation and the initiation of immune evasion [54, 55]. To confirm these in vitro results in a physiological system, we used a co-transplantation model, which once again supported the tumor-promoting activity of LINC00886. In line with in vitro data, the xenograft model exhibited faster tumor growth and more fibrosis, which conferred in vivo evidence of the regulatory effect of LINC00886 on CAF malignant behavior. Moreover, immunohistochemical staining showed that CAF-tumor cell interaction increased the tumor cell proliferative capacity (as demonstrated by markers like Ki-67), which further confirms the important role of LINC00886 in the establishment of a pro-tumorigenic TME. This is the first study to identify the molecular pathway through which OTSCC-secreted PDGF-BB controls the CAF activation and metabolic reprogramming via the LINC00886/hsa-miR-15b-5p/AKT pathway. It also fills the research gap in the non-coding RNA (ncRNA)-mediated CAF regulation in the OTSCC TME and provides valuable potential biomarkers and therapeutic targets in clinical translation.

Although the research highlights the critical role of LINC00886 in the development of OTSCC, there are a number of weaknesses that should be considered. First, it concentrated mainly on the CAF conversion induced by PDGF-BB, subsequent studies need to examine other tumor-associated factors (e.g., TGF- β and IL-6) and their regulation of LINC00886 expression. Moreover, validation in clinical samples is a major future direction although a mouse xenograft model was used. Multicenter clinical trials will also be needed to further support the clinical importance of LINC00886 in tongue squamous cell carcinoma. Nevertheless, future research potentially broadening the multifactorial regulatory network, enhancing the knowledge of underlying molecular processes, confirming results in clinical specimens, and investigating immune regulatory functions of this axis are likely to add more scientific value to the pathway, and eventually offer new approaches to diagnosing OTSCC precisely and treating it.

Conclusions

In summary, OTSCC-secreted PDGF-BB induces normal oral fibroblasts to transform into pro-

tumorigenic CAFs and drives glycolytic metabolic reprogramming of stromal cells. Transcriptomic screening identified LINC00886 as a critical downstream mediator of PDGF-BB signaling during CAF activation. Mechanistically, LINC00886 acts as a ceRNA to sponge hsa-miR-15b-5p, thereby relieving miRNA-mediated suppression of the AKT/mTOR pathway to maintain CAF malignant phenotypes and paracrine oncogenic effects on tumor cells. In vivo co-transplant xenograft models further verified that LINC00886 depletion in CAFs attenuates tumor growth, stromal fibrosis, and AKT/mTOR activation.

This study establishes a novel PDGF-BB/LINC00886/hsa-miR-15b-5p/AKT/mTOR regulatory axis that mediates tumor-stroma cross-talk in tongue squamous cell carcinoma. Our findings reveal a lncRNA-dependent mechanism underlying PDGF-BB-triggered CAF activation and provide candidate biomarkers and therapeutic targets for stroma-targeted treatment of OTSCC.

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Disclosure of conflict of interest

None.

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CAF-driven pro-tumorigenic effects

Table S1. Construct the knockdown sequence corresponding to the stable cell line of LINC00886 knockdown

Virus type	Gene name	Vector type	Sequence (5'-3')
shRNA lentivirus	LINC00886-Homo-306	LV3 (H1/GFP&Puro)	GAGGGAAGAGATTGGATAAAT
shRNA lentivirus	LINC00886-Homo-1960	LV3 (H1/GFP&Puro)	CTGCTATAGTGTGACTTAATA
shRNA lentivirus	LINC00886-Homo-849	LV3 (H1/GFP&Puro)	GCAACATTGGACACTTTAAA
shRNA lentivirus	shRNA lentivirus NC	LV3 (H1/GFP&Puro)	-

Table S2. Sequence corresponding to hsa-miR-15b-5p inhibitor

hsa-miR-15b-5p inhibitor	UGUAAACCAUGAUGUGCUGCUA
hsa-miR-15b-5p NC	-