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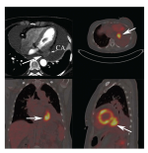

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RESEARCH PAPER

Knockdown of lncRNA LEF1-AS1 inhibited the progression of oral squamous cell carcinoma (OSCC) via Hippo signaling pathway

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ABSTRACT

It is verified that long non-coding RNAs (lncRNAs) play crucial roles in various cancers. LncRNA LEF1-AS1 is a reported oncogene in colorectal cancer and glioblastoma. In this study, we unveiled that LEF1-AS1 markedly increased in oral squamous cell carcinoma (OSCC) tissues and cell lines. Besides, OSCC patients with high levels of LEF1-AS1 were apt to poor prognosis. Functionally, LEF1-AS1 knockdown inhibited cell survival, proliferation and migration, whereas enhanced cell apoptosis and induced G0/G1 cell cycle arrest in vitro. Consistently, LEF1-AS1 silence hindered tumor growth in vivo. Moreover, LEF1-AS1 inhibition stimulated the activation of Hippo signaling pathway through directly interacting with LATS1. Furtherly, we disclosed that LEF1-AS1 silence abolished the interaction of LEF1-AS1 with LATS1 while enhanced the binding of LATS1 to MOB, therefore promoting YAP phosphorylation but impairing YAP1 nuclear translocation. Additionally, we demonstrated that LEF1-AS1 regulated YAP1 translocation via a LATS1-dependent manner. Furthermore, we also uncovered that YAP1 overexpression abolished the suppressive impact of LEF1-AS1 repression on the biological processes of OSCC cells. In a word, we concluded that LEF1-AS1 served an oncogenic part in OSCC through suppressing Hippo signaling pathway by interacting with LATS1, suggesting the therapeutic and prognostic potential of LEF1-AS1 in OSCC.

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KEYWORDS
LEF1-AS1; OSCC progression; LATS1; Hippo pathway

Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers with high mortality all over the world.[1,2] Squamous cell carcinoma rising in the oral cavity accounts for 90% of head and neck cancers.[3] Oral squamous cell carcinoma (OSCC) is a kind of cancer affecting the oral cavity region and accounts for about 90% of oral cancers.[4] Although great improvements have been made in diagnosis and treatment, such as surgery and radiation therapies in the past few decades, OSCC still threats human health today.[5–7] So it is urgent to investigate the molecular mechanisms of OSCC progression and find a novel effective therapeutic target for OSCC.

Long non-coding RNAs (lncRNAs) are defined as a novel class of transcripts that cannot encode proteins.[8,9] LncRNAs are longer than 200 bases, and they are involved in many processes, including tumorigenesis and the development of various human cancers.[10–12] For instance, lncRNA ZFAS1 has been found as an oncogene in bladder cancer.[13] Lymphoid enhancer-binding factor 1 antisense RNA 1 (LEF1-AS1), is a newfound lncRNA that locates in chromosome 4q25, which was firstly found to be upregulated and predict prognosis in colorectal cancer (CRC)[14] and further revealed to promote malignancy in glioblastoma.[15] However, it remains obscure whether LEF1-AS1 implicates in OSCC.

Hippo signaling pathway is an evolutionarily conserved kinase cascade which plays critical roles in regulating cell proliferation and survival by modulating the effect of the tumor suppressor Hippo (Mst1 and Mst2 in mammals) on the carcinogenic transcriptional coactivators Yki (YAP and TAZ in mammals).[16] The mutations of its components have been verified to contribute to various human diseases, even in cancer.[17,18] For example, it has been proved that Verteporfin inhibits YAP-induced bladder cancer cell growth and invasion via Hippo signaling pathway.[19] Besides, Hippo pathway also functions in breast cancer, pancreatic cancer, colon adenocarcinoma and oral cancer.[20–23] Recently, it has already been discovered that Hippo pathway plays a pivotal role in tumorigenesis and progression of OSCC.[22]

In the present study, we probed the functional role and potential mechanism of LEF1-AS1 in OSCC.

Materials and methods

Clinical samples

An amount of 88 pairs of OSCC tumor tissues and adjacent non-tumor tissues were obtained from patients who were diagnosed with OSCC at Wenzhou People's Hospital from 2012 to 2017. All of the patients didn't receive any other treatment before surgery. This study was approved by the Research Medical Ethics Committee of Wenzhou People's Hospital. All participants had signed a statement of informed consent before using the tissues in this study.

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Cell lines and culture

Normal Human Oral Keratinocyte cells (NHOK) and OSCC cell lines (SCC9, FADU, SCC25, SCC1, TU183, HSU3, OEC-M1, SNU1041, SCC4, and SCC15) were purchased from the ATCC (Manassas, VA). All kinds of Cells were cultured in the mixture of DMEM/Ham's F-12 (Invitrogen, Carlsbad, CA), 10% fetal bovine serum (FBS, GIBCO, Thermo Fisher Scientific, Waltham, USA) and 0.4μg/ml hydrocortisone at a humidified atmosphere with 5% CO_2 under 37°C.

Interfering RNA transfection

The shRNA for knockdown of LEF1-AS1 as well as its negative scrambled shRNA were purchased from Genecopoeia (Rockville, USA). So did si-YAP1. The shRNA transfection was performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's indications.

Real-time quantification PCR (RT-qPCR)

Total RNAs from tissues and cells were obtained by TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer's instructions. Reverse transcription was implemented using PrimeScript RT reagent kit (Takara Bio, Inc., Otsu, Japan) under the manufacturer's instructions. RT-qPCR was conducted by SYBR Prime Script RT-PCR kits (Takara Bio, Inc.) on the basis of the manufacturer's instructions. GAPDH was an endogenous control. The LEF1-AS1 level was evaluated by using the 2-ΔΔCt method. The PCR primers were as the following: LEF1-AS1, forward 5′-AAGGACGAGAGAAAAGCAC-3′, reverse 5′-CACA CAAAGGGGAAGACC-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5′-AGCAAGAGCACAAGAG GAAG-3′, reverse 5′-GGTTGAGCACAGGGTACTTT-3′.

MTT assay

Cell survival ability was measured by MTT assay. Cells (1×10^3 per well) were placed in 96-well plates and cultured for 24 h. Then, the cells were incubated for another 0 h, 24 h, 48 h, and 72 h, respectively. After that, the cells were treated with MTT and further cultured at 37°C for another 4 h. Then, DMSO was added after removing the medium. And the mixture was maintained for 10 min. Finally, a microplate reader was used to measure the absorbance at a wavelength of 490 nm.

Colony formation assay

The colony formation ability was evaluated by colony formation assay. In brief, after transfection for 24 h, cells (800/well) were seeded on a 6-well plate and then further cultured for 14 days at 37°C. After incubation, the plate was washed twice by PBS and stained using crystal violet, and the number of colonies was counted artificially.

Cell cycle analysis

After transfection for 48 h, cells distributed in different cell cycle phases were examined by DNA content using flow cytometry analysis. Then, cells were stained with propidium iodide (PI) and analyzed by flow cytometer FACSVerse (BD, NJ, USA) following the DNA content.

Transwell assay

Cell migration ability was tested by Transwell assay. 200μL serum-free medium of cells (8×10^4) was placed in the upper inserts of transwell, meanwhile 700 μL of medium with 10% FBS was added into the lower inserts of transwell. After incubation for 24 h under a condition of 37°C with 5% CO_2, cells that did not transit the membrane were removed and the migrated cells were stained by 0.1% crystal violet.

Western blot

Total proteins extracted from cells were separated using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) gels and then transferred to nitrocellulose (NC) membranes. After incubated with primary antibodies overnight at 4°C, the membranes were blocked with 10% skim milk for 1 h. The primary antibodies used in this study included Hippo pathway kit (cat: 8579, Cell Signal Technology, Beverly, MA, USA), YAP (ab52771; Abcam, Cambridge, UK), MOB1 (3863; Cell Signal Technology) and GAPDH (cat: 5174, Cell Signal Technology). GAPDH acted as the endogenous control here. And then membranes were washed using TBST (Tris-buffered saline with 0.1% Tween 20) and incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Cell Signaling Technology). Membranes were detected by the LI-COR Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

In vivo xenograft experiments

Six BALB/c-nu male mice with 4-week-old were obtained from Shanghai laboratory animal center of Chinese academy of sciences, Shanghai, China. SCC15 cells (6×10^6) were subcutaneously inoculated into the flank region of mice. Then, the tumor volume was detected every three days based on the formula: $V = 0.5 \times D \times d^2$ (V represents volume, D is longitudinal diameter and d means latitudinal diameter). Three weeks later, the mice were euthanized with the tumors excised for analysis. All procedures in the animal experiments were under the approval of the Institutional Animal Care and Use Committees of Wenzhou People's Hospital.

Immunohistochemical (IHC) staining

Paraffin sections made using the excised tumors in animal studies were applied for immunohistochemistry assays to evaluate the expression level of Ki67 protein. The tissue sections used for IHC staining were, respectively, estimated by two pathologists, and the staining was conducted using indirect streptavidin-peroxidase method in line with the manufacturer's instructions. And the antibody used here were rabbit anti-Ki67 (Cat. No. Ab16667, 1:100, Abcam).

RNA immunoprecipitation (RIP) assay

In the light of the manufacturer's protocols, the RIP experiments were performed using the Magna RNA-binding protein immunoprecipitation kit (Millipore, Bedford, MA). In brief, cell lysis obtained by the use of lysis buffer (Gibco) with RNase inhibitor were incubated with anti-LATS1 (Abcam) or anti-IgG (negative control; Abcam). Then, the proteins interacted with LATS1 were identified by western blotting.

Co-immunoprecipitation (CoIP) assay

Cell lysates were obtained by using lysis buffer (20 mM Tris-HCl pH 8.0, 137 mM NaCl, 10% glycerol, 1% NP-40, and 2 mM EDTA) containing protease inhibitor (Roche). After centrifugation, the collected supernatant was incubated with magnetic beads (Sigma, M8823-5 mL) bonded with anti-LATS1 at 4°C overnight. Afterward, the beads were rinsed while the bound proteins were eluted and then evaluated by western blot.

Statistical analysis

All the data were analyzed using GraphPad Prism 5 software. Students' t-test, one-way ANOVA and two-way ANOVA were applied to analyze the differences among treatment groups in this study. All the data were shown in the manner of mean ± SD, and it was thought to be of statistical significance when P values were lower than 0.05.

Results

LEF1-AS1 was upregulated in OSCC tissues and cell lines

To uncover the role of LEF1-AS1 in OSCC, the expression levels of LEF1-AS1 in 88 pairs of OSCC tissues and corresponding non-tumor tissues were firstly detected by RT-qPCR. As a result, LEF1-AS1 expression was found to be notably higher in OSCC tissues than that in adjacent non-cancerous tissues (Figure 1a). Next, we examined the levels of LEF1-AS1 in 10 OSCC cell lines (SCC9, FADU, SCC25, SCC1, TU183, HSU3, OEC-M1, SNU1041, SCC4, and SCC15) and normal human oral keratinocyte (NHOK) cells, and discovered that LEF1-AS1 was also upregulated in OSCC cells in comparison with NHOK cells, further confirming the upregulation of LEF1-AS1 in OSCC (Figure 1b). In addition, the expression of LEF1-AS1 was comparatively higher in SCC4 and SCC15 cells than other eight OSCC cells. Collectively, LEF1-AS1 was highly expressed in OSCC, implying its tumorigenic role in OSCC.

Upregulated LEF1-AS1 was greatly relevant to poor prognosis in OSCC

To make sure the relevance of LEF1-AS1 expression level to the progression and prognosis in OSCC, the relationship between clinicopathological features and LEF1-AS1 expression level was analyzed here. As shown in Table 1, the expression level of LEF1-AS1 was strongly correlated with the stage but irrelevant to others. Furthermore, Kaplan–Meier curve suggested that patients with high LEF1-AS1 expression usually suffered from unsatisfied overall survival (OS) in contrast to those with low level of LEF1-AS1 (Figure 2). In addition, the prognosis of OSCC patients was strongly relevant to LEF1-AS1 level ($P = 0.029$) and stage ($P = 0.025$) by using the univariate analysis (Table 2), whereas it was indicated to be closely associated with LEF1-AS 1 level ($P < 0.001$), stage ($P = 0.008$) and N stage ($P = 0.005$) under the multivariate analysis (Table 3). These results demonstrated that LEF1-AS1 might serve as a biomarker for prognosis of OSCC.

Knockdown of LEF1-AS1 inhibited cell survival and proliferation in OSCC cell lines

To make clear the precise function of LEF1-AS1 in OSCC, the loss-of-function assays were carried out in SCC4 and SCC15 cells by transfecting with specific shRNAs (sh-LEF1-AS1#1 and sh-LEF1-AS1#2) while those with shCtrl as a negative control. As illustrated in Figure 3a, in contrast to the mock group, LEF1-AS1 expression was effectively silenced in SCC4 and SCC15 cells transfected with either sh-LEF1-AS1#1 or sh-LEF1-AS1#2, whereas that in shCtrl transfected OSCC cells was nearly unaltered. Also, compared with the mock groups,

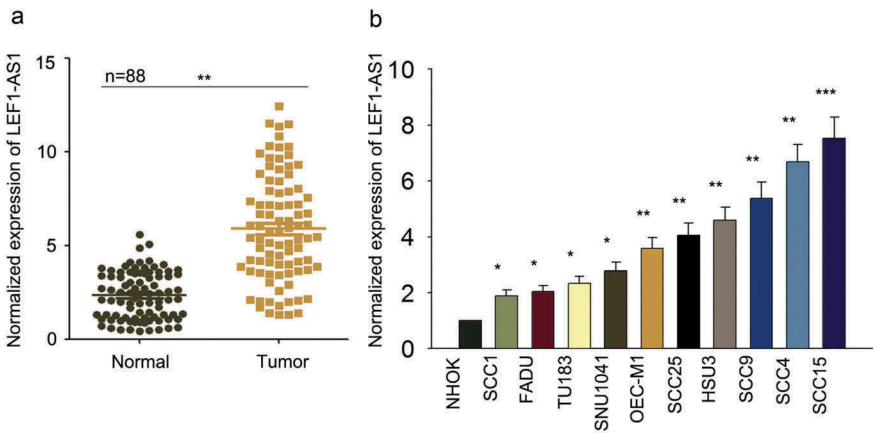

Figure 1. Expression of LEF1-AS1 was upregulated in OSCC tissues and cell lines. The expression of LEF1-AS1 was evaluated by qRT-PCR. (a) LEF1-AS1 expression was remarkably increased in 88 paired OSCC tissues compared to corresponding non-cancerous tissues. (b) LEF1-AS1 was greatly upregulated in OSCC cell lines in contrast to that in NHOK cells, and its expression in SCC4 and SCC15 cells were higher than other OSCC cell lines. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 1. Correlation between LEF1-AS1 expression and clinical features. (n = 88).

Variable	LEF1-AS1 Expression		*P*-value
	low	high	
Age			
>60	11	23	0.547
≤60	27	27	
Gender			
Male	2	10	0.664
Female	36	40	
T Stage			
T1-T3	18	25	0.399
T4	20	25	
N Stage			
N0	28	21	0.068
N+	10	29	
Stage			
I,II,III	21	13	0.006
IV	17	37	
Nodal Invasion			
positive	10	21	0.382
negative	28	29	
Differentiation			
differentiated	15	23	0.183
undifferentiated	23	27	

Low/high by the sample mean. Pearson χ^2 test. $P < 0.05$ was considered statistically significant.

Table 2. Univariate analysis of prognostic parameters in patients with oral squamous cell carcinoma by Cox regression analysis.

Variable	Category	*P*-value
Age		
	>60	0.439
	≤60	
Gender		
	Male	0.320
	Female	
T Stage		
	T1-T3	0.679
	T4	
N Stage		
	N0	0.140
Stage	N+	0.025
	I,II,III	
	IV	
Nodal Invasion		
	positive	0.681
	negeative	
Differentiation		
	differentiated	0.439
	undifferentiated	
LEF1-AS1 level	High	0.029
	Low	

Proportional hazards method analysis showed a positive, independent prognostic importance of LEF1-AS1 expression (P = 0.029). *$P < 0.05$ was considered statistically significant.

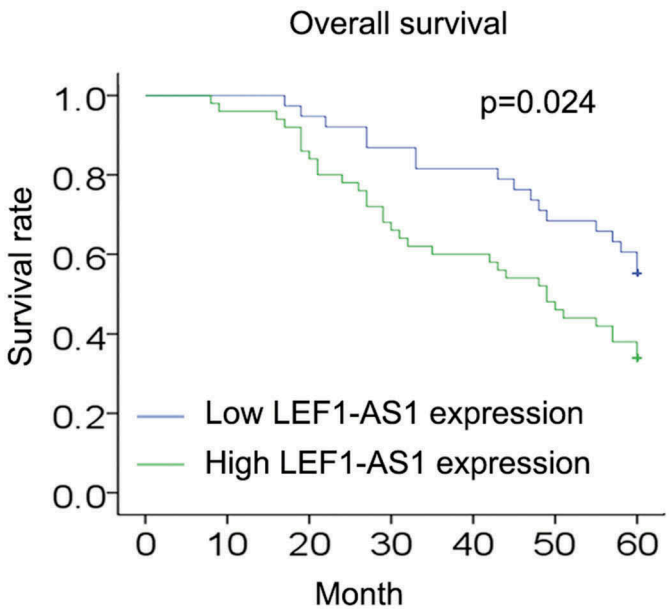

Figure 2. LEF1-AS1 upregulation was associated with poor prognosis in OSCC. Kaplan-Meier analysis and the log-rank test were utilized to analyze the relationship between LEF1-AS1 expression level and overall survival (OS) in patients with OSCC, as well as early-stage patients.

Table 3. Multivariate analysis of prognostic parameters in patients with oral squamous cell carcinoma by Cox regression analysis.

Variable	Category	*P*-value
Age		
	>60	0.125
	≤60	
Gender		
	Male	0.061
	Female	
T Stage		
	T1-T3	0.833
	T4	
N Stage		
	N0	0.005*
Stage	N+	0.008*
	I,II,III	
	IV	
Nodal Invasion		
	positive	0.248
	negeative	
Differentiation		
	differentiated	0.177
	undifferentiated	
LEF1-AS1 level	High	<0.001*
	Low	

Proportional hazards method analysis showed a positive, independent prognostic importance of LEF1-AS1 expression ($P < 0.001$). *$P < 0.05$ was considered statistically significant.

evident reductions on cell survival rate was easily revealed in SCC4 and SCC15 cells with either sh-LEF1-AS1#1 or sh-LEF 1-AS1 transfection, whereas no change was observed in shCtrl-transfected OSCC cells (Figure 3b). Given this result, the following researches were only performed in SCC4 and SCC15 cells with the transfection of shCtrl or sh-LEF1-AS1#1 (which was called as sh-LEF1-AS1 subsequently). In addition, the cell proliferative ability tested by colony formation assay was apparently mitigated in either SCC4 or SCC15 cells under LEF1-AS1 silence (Figure 3c). These findings revealed that LEF1-AS1 inhibition repressed cell survival and proliferation in OSCC cells.

Silencing LEF1-AS1 resulted in G0/G1 cell cycle arrest and promoted apoptosis as well as inhibited migration in vitro

In order to investigate the ways in which cell proliferation is affected in OSCC, flow cytometry analysis was carried out to estimate the effects of LEF1-AS1 on cell cycle progression and apoptosis in SCC4 and SCC15 cells. As shown in Figure 4a, the

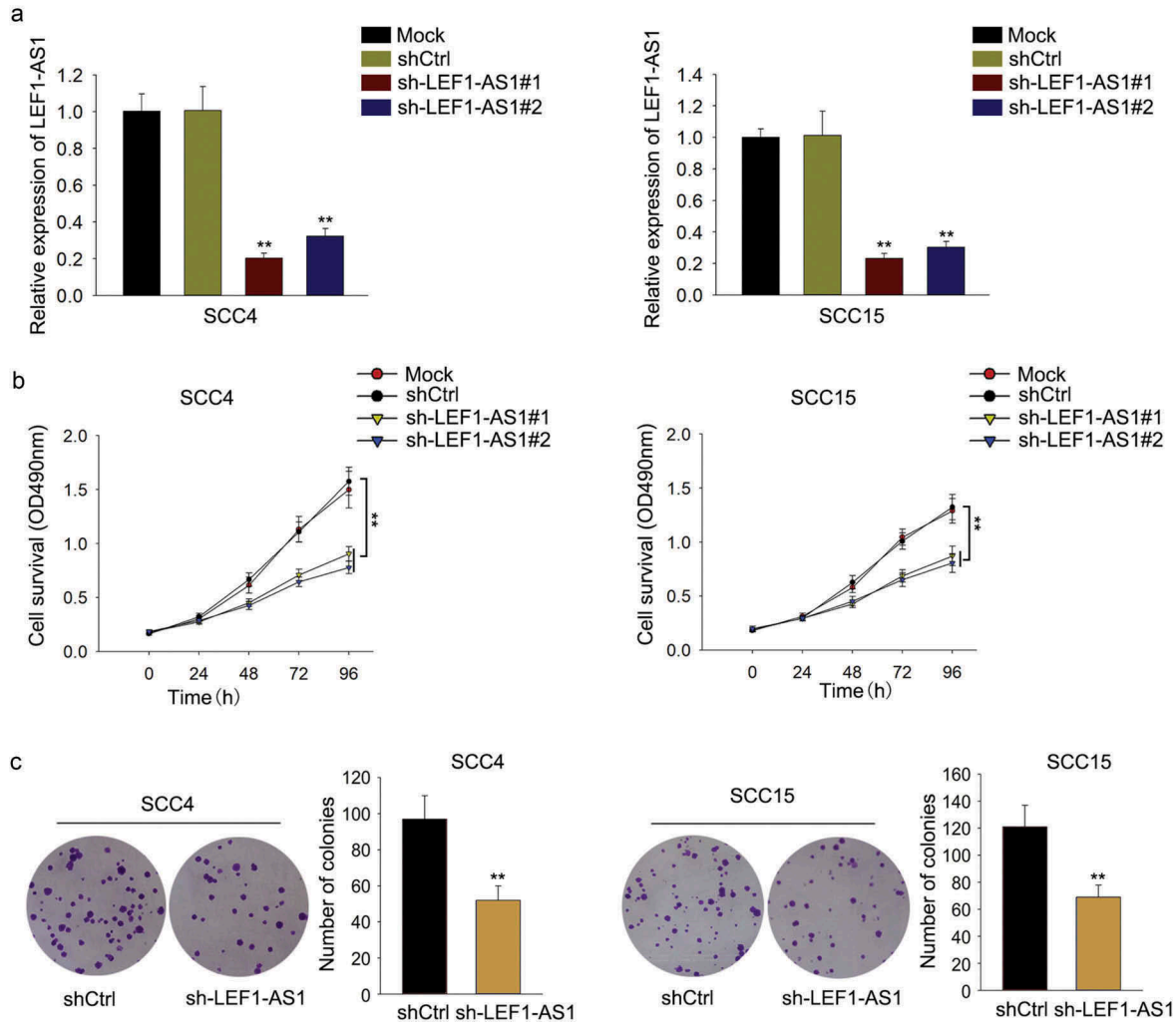

Figure 3. Knockdown of LEF1-AS1 inhibited cell survival and proliferative ability in SCC4 and SCC15 cells. (a) qRT-PCR analysis of the transfection efficiency of two kinds of shRNAs targeting LEF1-AS1 in SCC4 and SCC15 cells. Cells transfected with shCtrl acted as a negative control. (b) MTT assay was carried out to examine cell survival in SCC4 and SCC15 cells in different groups, such as mock group, shCtrl-transfected group and sh-LEF1-AS1#1 or sh-LEF1-AS1#2-transfected group. (c) Colony formation assay was applied to explore the impacts of LEF1-AS1 on cell proliferative ability. $^{**}P < 0.01$.

proportion of either SCC4 or SCC15 cells arrested in G0/G1 phase was markedly increased while that in the S and G2/M phase obviously decreased in the sh-LEF1-AS1-transfected group. Additionally, cell apoptosis was remarkably strengthened in LEF1-AS-silenced SCC4 and SCC15 cells (Figure 4b). Next, to examine whether LEF1-AS1 affected the OSCC cell migration, the Transwell assay was conducted in these two OSCC cells. As displayed in Figure 4c, the migratory capacities of SCC4 and SCC15 cells under LEF1-AS1 knockdown were distinctly lessened compared to the control groups. These data indicated that LEF1-AS1 silence contributed to cell cycle arrest and apoptosis but inhibited cell migration in OSCC cells.

LEF1-AS1 inhibition suppressed tumor growth in vivo

Next, we conducted the in vivo experiments to verify the above results that LEF1-AS1 knockdown inhibited cell proliferation in vitro. As displayed in Figure 5a-b, the tumors from sh-LEF1-AS1 transfected cells seemed to be smaller and lighter than those from the controls. Consistently, a markedly slower growth rate of tumors was observed in LEF1-AS1 silenced group while a relatively quicker one in mice injected with the shCtrl transfected SCC15 cells (Figure 5c). Subsequently, the level of Ki67, a protein that indicates cell proliferation, was examined in these two kinds of tumors. The IHC results elucidated that LEF1-AS1 inhibition obviously declined Ki67 expression contrast with the shCtrl transfected controls (Figure 5d). In sum, knockdown of LEF1-AS1 induced a suppressive effect on tumor growth in vivo.

LEF1-AS1 affected Hippo signaling pathway in OSCC through interacting with LATS1

Hippo pathway was a well-known anti-cancer pathway which has been found to function in the majority of human cancers, including OSCC.[18,24] Thus, we next investigated whether LEF1-AS1 also influenced this pathway in OSCC. As shown in Figure 6a-b, the phosphorylation of MST1/2, SAV1, LAST1/2, MOB, and YAP were distinctly improved in SCC4 and SCC15 cells after silencing LEF1-AS1, implying that LEF1-AS1 had an impact on OSCC

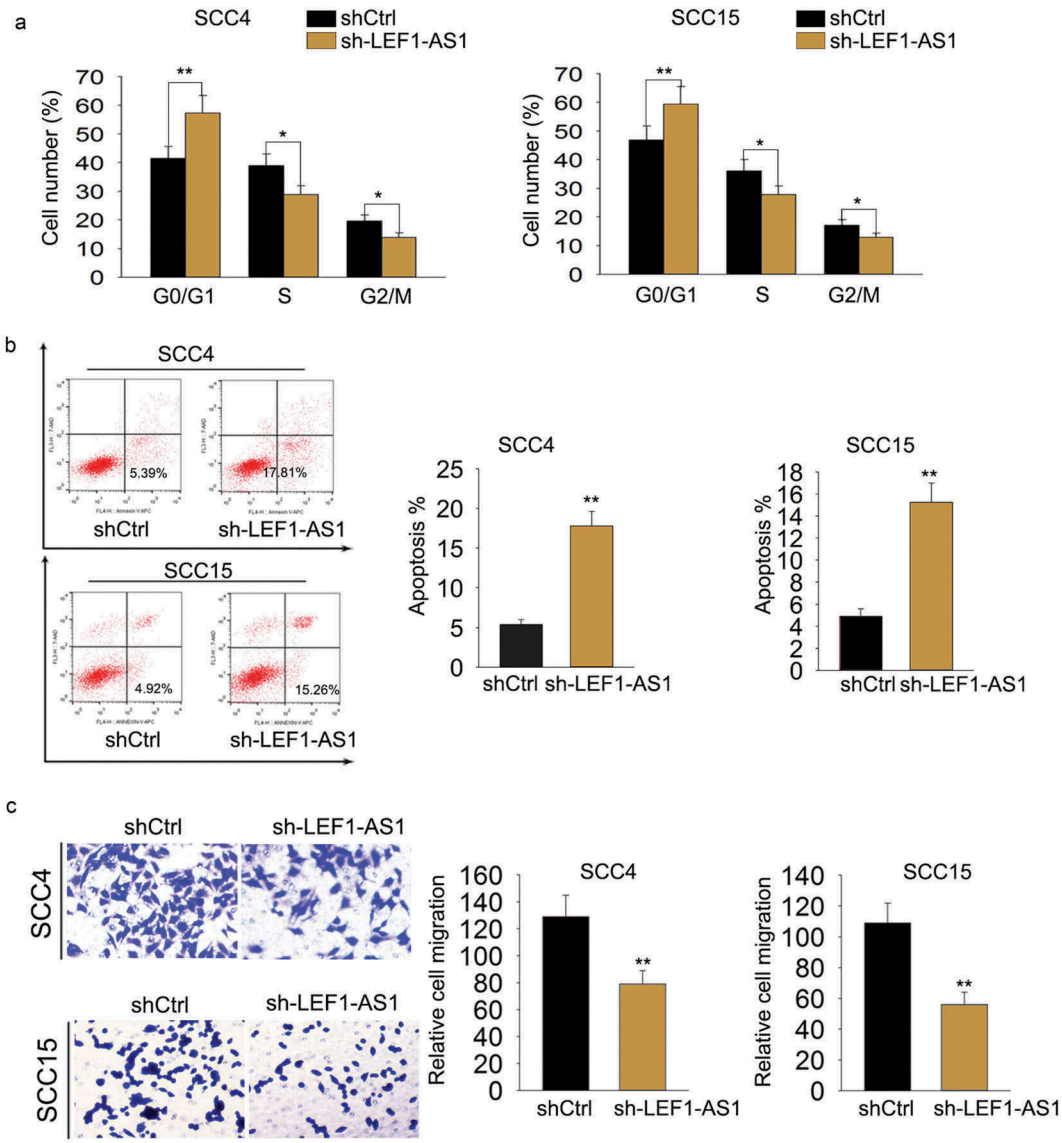

Figure 4. Knockdown of LEF1-AS1 induced cell apoptosis and cell cycle arrest and inhibited cell migration in OSCC cells. (a-b) Flow cytometry analysis was applied to analyze the cell cycle distribution and cell apoptosis rate in SCC4 and SCC15 cells after transfection. (c) Cell migration abilities were detected by Transwell migration assay in SCC4 and SCC15 cells under LEF1-AS1 silence. *$P < 0.05$, **$P < 0.01$.

progression via inactivating Hippo pathway. It has been well reported that lncRNAs often exert their pathogenic functions by interacting with one or more proteins.[25] Here, we predicted that LEF1-AS1 were most likely to interact with LATS1 by using RPISeq (http://pridb.gdcb.iastate.edu/RPISeq/index.html). And the direct interactivity of LEF1-AS1 with LATS1 was further confirmed by using RIP assays conducted in both SCC4 and SCC15 cells (Figure 6c), whereas such interaction was markedly hampered upon LEF1-AS1 suppression (Figure 6d). Moreover, we discovered that the concentration of MOB in LATS1-immunized precipitates was pronouncedly encouraged in two OSCC cells in face of LEF1-AS1 knockdown (Figure 6e). Interestingly, depletion of LEF1-AS1 strikingly elevated the cytoplasmic YAP1 expression but reduced the nuclear YAP1 level both in SCC4 and SCC15 cells (Figure 6f). Altogether, these findings uncovered that LEF1-AS1 facilitated YAP1 translocation by inactivating Hippo signaling via directly interacting with LATS1.

LEF1-AS1/LATS1/YAP1 axis played a pivotal regulatory role in OSCC progression

Furtherly, we attempted to validate whether LEF1-AS1 regulated OSCC progression through targeting LATS1-participated Hippo signaling. Firstly, we found that the level of phosphorylated YAP1 was noticeably restrained with the decrease of LATS1 protein in LEF1-AS1-silenced SCC15 cells with si-LATS1 co-transfection (Figure 7a). Besides, co-inhibition of LATS1 led to an overt confinement in LATS1-MOB interaction in LEF1-AS1-depleted SCC15 cells (Figure 7b). More importantly, the level of YAP1 protein in the cytoplasm was notably reduced while that in the nucleus was significantly enhanced in LEF1-AS1-inhibited SCC15 cells in response to LATS1 co-suppression (Figure 7c). In other words, LEF1-AS1 effected YAP1 cellular distribution through a LATS1-mediated way, further verifying that LEF1-AS1 had a suppressive effect on the activation of Hippo signaling.

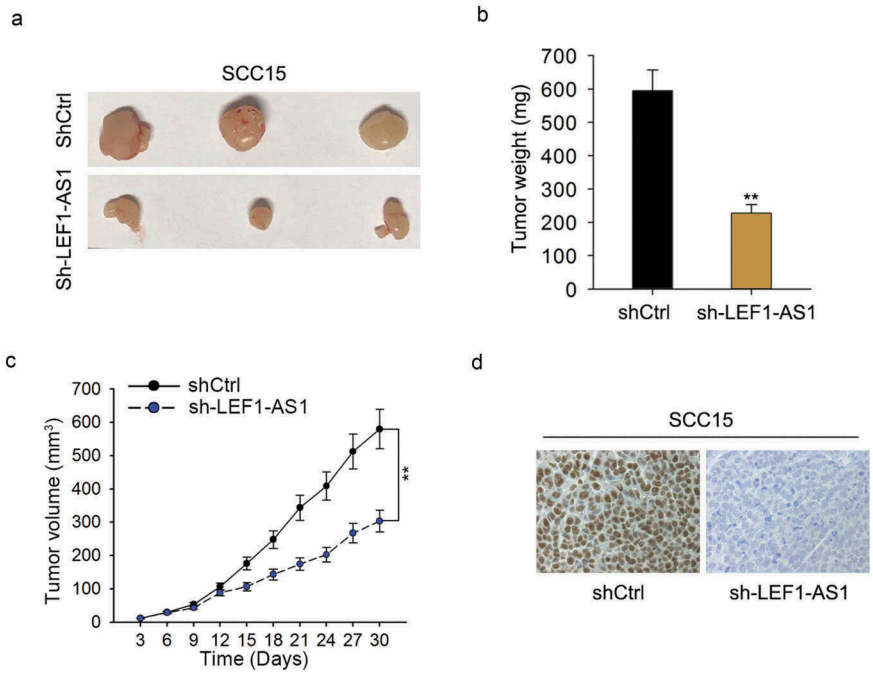

Figure 5. LEF1-AS1 silence inhibited tumor growth in vivo. (a) Images of tumors originated from mice injected with shCtrl or sh-LEF1-AS1-transfected SCC15 cells. (b) The mean weight of tumors above. (c) Tumor volumes formed in two groups of mice were detected every three days, and LEF1-AS1 silenced group showed a slower growth rate. (d) IHC staining of Ki67 expression in tumors with or without LEF1-AS1 knockdown. $^{**}P < 0.01$.

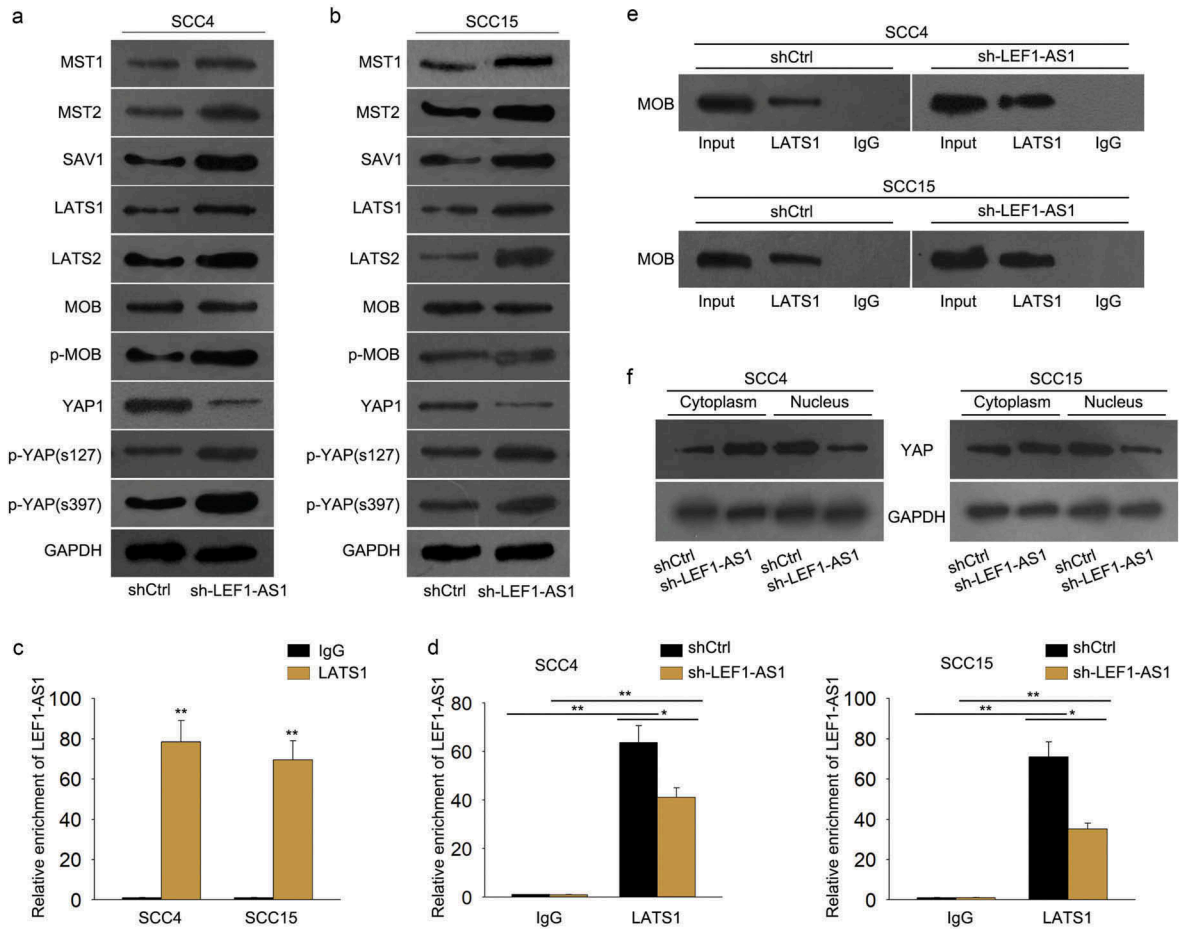

Figure 6. LEF1-AS1 affected Hippo signaling pathway in OSCC through directly interacting with LATS1. (a-b) The protein level changes of Hippo pathway components in SCC4 and SCC15 were determined by western blot assay. (c) RIP results of LEF1-AS1 enrichment in LATS1 or IgG-caused immune-precipitates in SCC4 and SCC15 cells was assessed using qRT-PCR. (d) The influence of LEF1-AS1 depletion on LEF1-AS1-LATS1 interaction in SCC4 and SCC15 cells was evaluated by RIP assay. (e) CoIP assay was performed to determine the impact of LEF1-AS1 inhibition on LATS1-MOB interaction in OSCC cells. (f) The cellular distribution of YAP1 in OSCC cells in face of sh-LEF1-AS1 transfection was estimated using western blot analysis. $^{*}P < 0.05$, $^{**}P < 0.01$.

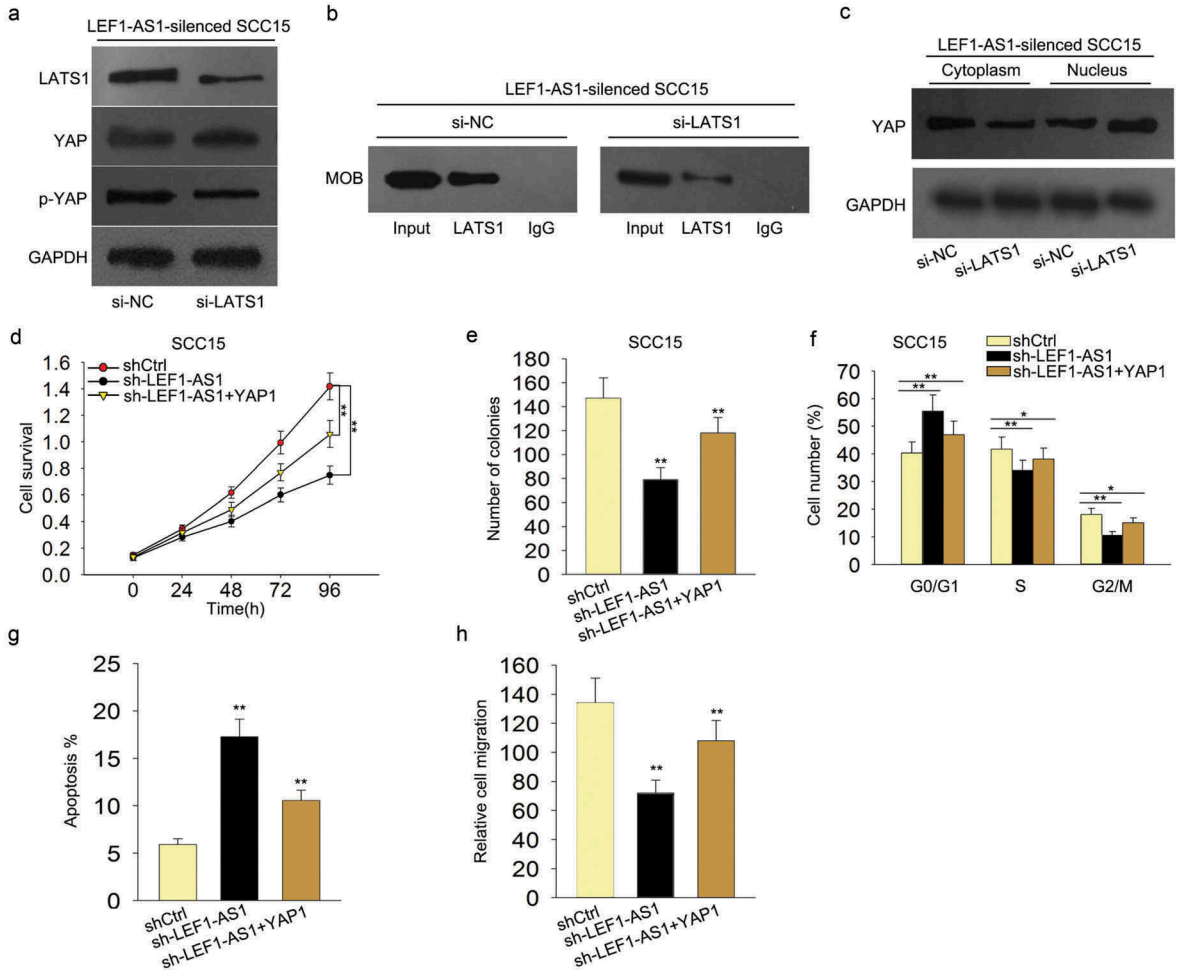

Figure 7. LEF1-AS1 exerted tumorigenic function in OSCC through LATS1/YAP1 signaling. (a) Western blot results of the protein levels of LATS1, YAP1 and p-YAP1 in LEF1-AS1-silenced SCC15 cells upon si-LATS1 co-transfection. (b) The effect of LATS1 knockdown on the binding ability of LATS1 to MOB in LEF1-AS1-depleted SCC15 cells was detected using CoIP assay. (c) Western blot results of the YAP1 protein level in the cytoplasm and the nucleus of SCC15 cells with the co-transfection of sh-LEF1-AS1 together with si-NC or si-LATS1. (d-e) Cell proliferation capacity was evaluated by MTT and colony formation assay in sh-LEF1-AS1-transfected SCC15 cells after overexpressing YAP1. (f-g) Flow cytometry analysis was utilized to detect the changes of cell cycle progression and cell apoptosis induced by YAP1 upregulation in LEF1-AS1 silenced SCC15 cells. (h) Transwell assay was performed to explore the alterations in cell migration ability in SCC15 cells under different transfections. $^{*}P < 0.05$, $^{**}P < 0.01$.

Thereafter, rescue assays were conducted in SCC15 cells with LEF1-AS1 repression via overexpressing YAP1 to further make sure the implication of Hippo pathway in LEF1-AS1-regulated OSCC progression. As uncovered in Figure 7d-e, overexpression of YAP1 sharply increased the cell survival rate and colony formation ability impeded in SCC15 cells with LEF1-AS1 knockdown. By contrast, both parts of cells arrested at G0/G1 phase and the apoptotic rate were notably attenuated under YAP1 encouragement in LEF1-AS1-repressed SCC15 cells (Figure 7f-g). Moreover, YAP1 upregulation facilitated the controlled cell migration in SCC15 cells under LEF1-AS1 knockdown (Figure 7h). All in all, LEF1-AS1 exacerbated OSCC progression through regulating Hippo signaling pathway.

Discussion

Increasing evidences have proved that lncRNAs participate in the pathogenesis of malignant tumors, including tumorigenesis, tumor growth and metastasis.[12,26–31] LncRNAs function through different mechanisms on a lot of significant cancer types by interacting with other cellular macromolecules, for example, altered epigenetic regulation of status of protein-coding genes.[32,33] As for this reason, dysregulation of lncRNAs affects the expression of genes and prompt tumor growth as well. In the past decade, people have found that the expression levels of lncRNAs are associated with the progression and prognosis of various cancer,[26,34–37] including HNSCC and OSCC.[38–42] However, limited lncRNAs have been uncovered in OSCC. Thus, it is necessary to discover OSCC-associated lncRNAs and then investigate their clinical significance and biological function in OSCC.

LEF1-AS1 is a novel lncRNA which has been reported to be upregulated and exert carcinogenesis in CRC and glioblastoma,[14,15] but its role in many other carcinomas including OSCC remains covered. Herein, we investigated whether LEF1-AS1 also functions as an oncogene in OSCC and unsurprisingly observed its upregulation in OSCC tissues and cell lines. Additionally, the upregulated LEF1-AS1 predicted poor prognosis in OSCC patients. Furthermore, we demonstrated that knockdown of LEF1-AS1 significantly reduced cell viability, proliferation, and migration in vitro and suppressed tumor growth in vivo, indicating that LEF1-AS1 acted as an oncogene in OSCC accordantly.

Many signaling pathways have been reported to be dysregulated and function in the progression of HNSCC and OSCC, such as Wnt/beta-catenin,[43] MAPK,[44] PI3K/AKT,[45] mTOR,[46] TGF-β,[47] and Hippo pathway. Hippo pathway involves in the development of multiple cancers including oral cancer.[20–23] In this study, we found that LEF1-AS1 inactivated Hippo signaling pathway through directly interacting with LATS1 so as to hinder the binding of LATS1 to MOB, therefore impairing YAP1 phosphorylation and enhancing nuclear YAP1 level in OSCC cells. Moreover, forced YAP1 expression counteracted the repressive effects of LEF1-AS1 silence on biological behaviors of OSCC cells. In other words, LEF1-AS1 impacted on OSCC through inactivating Hippo signaling pathway.

In conclusion, it has been found for the first time that LEF1-AS1 functioned as an oncogene in OSCC and its overexpression predicted poor overall survival in OSCC patients. Moreover, the carcinogenetic mechanism of LEF1-AS1 in OSCC was relied on the inactivation of Hippo signaling pathway by interacting with LATS1. Our findings provided LEF1-AS1 as a novel potential candidate for the treatment of OSCC. However, intensive researches are also urgently needed to verify the findings of this paper in the future.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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