Long non-coding RNA UCA1 contributes to the progression of oral squamous cell carcinoma by regulating the WNT/β-catenin signaling pathway

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With the development of functional genomics studies, a mass of long non-coding RNAs (LncRNA) were discovered from the human genome. Long non-coding RNAs serve as pivotal regulators of genes that are able to generate LncRNA–binding protein complexes to modulate a great number of genes. Recently, the LncRNA urothelial carcinoma-associated 1 (UCA1) has been revealed to be dysregulated, which plays a critical role in the development of a few cancers. However, the role of the biology and clinical significance of UCA1 in the tumorigenesis of oral squamous cell carcinoma (OSCC) remain unknown. We explored the expression, function, and molecular mechanism of LncRNA UCA1 in OSCC. In the present work, we revealed that UCA1 silencing suppressed proliferation and metastasis and induced apoptosis of OSCC cell lines *in vitro* and *in vivo*, which might be related to the activation level of the WNT/β-catenin signaling pathway. Our research results emphasize the pivotal role of UCA1 in the oncogenesis of OSCC and reveal a novel LncRNA UCA1–β-catenin–WNT signaling pathway regulatory network that could contribute to our understanding in the pathogenesis of OSCC and assist in the discovery of a viable LncRNA-directed diagnostic and therapeutic strategy for this fatal disease.

Oral carcinoma is the third most common carcinoma in developing countries and the sixth most common carcinoma around the world.[1,2] Oral squamous cell carcinoma (OSCC) is one of the most frequent head and neck tumors, accounting for approximately 3% of all newly diagnosed clinical cancer cases. In spite of recent significant progress in the diagnosis and clinical treatment of these tumors, the general 5-year survival rate of OSCC patients has not distinctly improved over the last few decades and remains at < 50%.[3,4] Although a lot of scientific research has been undertaken to comprehend the basic cellular activity in OSCC, the precise molecular mechanisms underlying OSCC oncogenesis are still unclear. Therefore, in order to improve the prognosis of patients with OSCC, it is important to identify effective diagnostic biomarkers and therapeutic targets.

Over the last few years, many studies have reported that a group of long non-coding RNAs (LncRNAs), with no protein-coding capacity and over 200 base pairs in length, play a role in carcinogenesis and suggested that this type of gene might serve as a biomarker in cancer.[5–7] Long non-coding RNAs have various functions; they can act as molecular signals, tethers, decoys, guides, and scaffolds at almost every level of gene modulation, that is, epigenetic, transcriptional, post-transcriptional, and translational.[8,9] Long non-coding RNAs carry out their functions in a wide range of processes and can regulate gene expression by various mechanisms.[10,11] Increasing evidence has indicated that LncRNAs play a potent role in the processes of cell proliferation, differentiation, apoptosis, and cancer metastasis.[12,13] However, it is still unknown whether this distinct function of LncRNAs is involved in the tumorigenesis of OSCC.

Urothelial carcinoma-associated 1 (*UCA1*) gene is located in chromosome 19p13.12, which has three exons and encodes two transcripts. Long non-coding RNA UCA1 has two isoforms. One is 1.4 kb in length,[14] and the other is 2.2 kb in length, which has been verified by other groups as cancer upregulated drug-resistant (CUDR).[15] Several groups have reported that UCA1 is aberrantly expressed in bladder cancer, breast cancer, and colorectal cancer,[14,16,17] suggesting that UCA1 may be used as a biomarker for the diagnosis of these cancers. Its enhancement of the tumorigenic behavior of these cancer cells *in vitro* and *in vivo* strongly revealed that UCA1 has tumorigenic functions in these cancers progression.

In our present study, we show that UCA1 is aberrantly expressed in tongue squamous cell carcinoma (TSCC) and that it may play an oncogenic role in promoting malignancy of OSCC cells, including proliferation and metastasis. Notably, systematic analysis uncovered that UCA1 may regulate β-catenin expression and the downstream target molecules of MMP-9, transcription factor-4 (TCF-4), and cyclin D1, indicating that UCA1 may induce the activation of the WNT/β-catenin pathway. Our results provide the first evidence for a novel LncRNA–mRNA signaling pathway regulatory network that is the UCA1–β-catenin–WNT signaling pathway in OSCC.

Materials and Methods

Tissue samples. From January 2012 to October 2015, TSCC samples were obtained from 124 patients of the Department of Oral and Maxillofacial Surgery, Shenzhen Hospital, Peking University (Shenzhen, China). A detailed description of clinical and tumoral features is listed in Table S1. Adjacent normal mucosal tissues (ANTs) located at least 1.5 cm from the macroscopically unaffected margins of the tumor were defined as normal controls. All the paired TSCC/ANT samples were collected from patients undergoing surgery for TSCCs. The TSCC samples were graded into four groups according to common criteria of SCC staging devised by the American Joint Committee on Cancer: stage 1, $n = 24$); stage 2, $n = 50$; stage 3, $n = 31$; and stage 4, $n = 19$. The rest of the process was the same as previously described.[18] All patients were informed about the aims of specimen collection and gave signed written consent in accordance with the ethical guidelines of Peking University (Protocol No. 37923/2-3-2012).

Real-time quantitative PCR analysis. Total RNA was isolated from tissues by using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reaction mixture (20 μL) containing 1 μg total RNA was reverse transcribed to cDNA using PrimeScript RT-polymerase (Takara, Dalian, China). Primers can be found in Table S2. Exploratory data analysis using scatter plots was applied to visually identify the expression level of target mRNA. Statistical analysis was undertaken using the paired *t*-test or non-parametric test. Values of $P < 0.05$ were considered statistically significant.

Cell culture and transfection. Human OSCC cell lines SCC15 and Cal27 were used in this study. The two cell lines were generously gifted from the College of Stomatology, Wuhan University (Wuhan, China). To construct UCA1 siRNA vector, the sequences targeting UCA1 were designed and synthesized by Qiagen (California, USA) and RiboBio (Guangzhou, China), named UCA1-si. A negative control, named as Ctrl-si, was also designed by Santa Cruz Biotechnology (Dallas, TX, USA) (sc-37007) (see Table S2). Transfection was carried out with Lipofectamine 3000 reagent (Invitrogen) following the manufacturer's protocol.

Cell wound healing assay. Cells were plated in 6-well plates and transfected when cultured to confluency. Wounds were created by scratching cell monolayers with a sterile 200-μL plastic pipette tip and pictures were taken at the 0 and 24 h time points to measure the wound-healing distance. All the assays were carried out in triplicate.

Cell migration and invasion assays. Matrigel matrix (30 μg/well; BD Biosciences, San Jose, CA, USA) was added to Transwell chambers in a 24-well plate. A total of 3×10^4 post-transfection SCC15 and Cal27 cells in 100 μL serum-free DMEM were plated onto the upper chambers (24-well insert, pore size 8 μm; Corning, NY, USA), and the lower chambers were filled with 700 μL DMEM with 20% FBS. After 24 h of incubation, the membranes were fixed with 4% methanol, stained by 0.1% crystal violet, and washed three times. Finally, the stained cells were counted in five random fields (original magnification, ×200). The migration assay was carried out in a similar fashion without coating with Matrigel matrix.

Lentiviral construction and cell transfection. To generate clones stably overexpressing LncRNA UCA1, SCC15 and Cal27 cells were first infected at an MOI of 50 with a lentiviral vector (Genechem, Shanghai, China) encoding full-length human LncRNA UCA1 gene sequence or an empty lentiviral vector control. Stable clones were selected for 2 weeks using puromycin and the expression level of LncRNA UCA1 was determined by quantitative RT-PCR (qRT-PCR). Lentiviral vectors containing the human LncRNA UCA1 were constructed and designated "LV-UCA1". A lentiviral vector only expressing the puromycin-resistance gene was used as the negative control and designated "LV-NC".

Immunocytochemistry assay. After culture on a microscope cover glass (Thermo Fisher Scientific, Waltham, MA, USA), cells were transfected with UCA1-si or Ctrl-si. After a 48-h incubation, cells were fixed with 4% formaldehyde and blocked in 10% BSA for 30 min. Slides were then incubated with a rabbit monoclonal anti-human β-catenin (1:100; CST, Shanghai, China) overnight at 4°C, washed with PBS, and incubated with an Alexa Flour 555 conjugated anti-rabbit IgG secondary antibody (1:1000; CST) for 45 min. The cells were washed three times with PBS, subsequently stained with DAPI (Merck, Darmstadt, Germany), then washed three times with PBS. The images were acquired using a Leica 4000 fluorescence microscope (original magnification, ×400) with a JVC digital device camera and collected using DISKUS software (version 4.60; Hilgers, Dortmund, Germany) and analyzed by Image-Pro Plus 6.0 software (Media Cybernetics, Georgia Avenue SilverSpring, MD).

Tumorigenicity assay in nude mice. Animal experiments were undertaken with the approval of the Institutional Committee for Animal Research and in conformity with national guidelines for the care and use of laboratory animals. The UCA1-si and Ctrl-si transfected SCC15 cells (1×10^7 cells in 200 μL) were injected s.c. into the flanks of 4-week-old male athymic nude mice (Experimental Animal Center, Guangdong, China). Tumor growth was examined weekly for at least 6 weeks. Then the mice were killed, necropsies were carried out, and tumors were weighed. Tumor volumes were calculated by the formula: $V = \pi LW^2/6$, where L is the largest diameter, and W is the perpendicular diameter.

Statistical analysis. All experimental data from three independent experiments are presented as mean ± SD. All statistical data were analyzed by SPSS 17.0 software (SPSS, Chicago, IL, USA). Cell Counting Kit (CCK)-8 data were analyzed by ANOVA, and paired samples *t*-test was used to analyze other

data. A two-tailed value of $P < 0.05$ was considered statistically significant (Doc. S1).

Results

Aberrant upregulation of UCA1 in TSCC tissues and association with disease metastasis and TNM stage. In the preliminary work,[18] six cancer-related LncRNAs were examined in a portion of the collected TSCC specimens and corresponding ANTs by qRT-PCR analysis. These LncRNAs were NEAT1, HOTAIR, HULC, MALAT-1, MEG3, and UCA1. However, the differences were not significant in any of these LncRNAs except UCA1. The expression levels of UCA1 were conspicuously higher in TSCC tissues than that in pair-matched ANTs. The difference was more significant when all the 124 paired samples were included (Fig. 1a). Clinicopathological analysis showed that UCA1 was significantly correlated with metastasis and TNM stage (Fig. 1b,c), whereas there was no significant correlation between UCA1 and other clinicopathological features such as gender, age, and tumor size ($P > 0.05$, Table 1). Collectively, these results suggest that the upregulation of UCA1 might be involved in the progression, metastasis, and prognosis of the majority of human TSCC.

Silencing of UCA1 suppresses cell proliferation in OSCC cell lines. Based on these observations, an analysis of UCA1 expression was carried out among five different OSCC cell lines (SCC9, SCC15, SCC25, Cal27, and Tca8113). Compared with human normal oral keratinocyte cell line HOK, obvious upregulation in UCA1 levels was identified in OSCC cell lines, especially in SCC15 and Cal27 cells (Fig. 2a). Thus, SCC15 and Cal27 cell lines were selected as research representatives of OSCC cells in the following studies.

Then, we constructed siRNA targeting UCA1, namely UCA1-si. The knockdown efficiency was approximately 78% in Cal27 and 86% in SCC15 cells after transfection with

Table 1. Correlation between clinicopathological fetures and UCA1 expression levels in 124 tongue squamous cell carcinoma patients

Features	No. of patients	LncRNA UCA1 expression levels		P-value
		Low expression, n (%)†	High expression, n (%)†	
Gender				
Male	83	45 (67.16)	38 (66.67)	0.242
Female	41	22 (32.84)	19 (33.33)	
Age, years				
<55	62	30 (51.72)	32 (48.48)	0.705
≥55	62	28 (48.28)	34 (51.52)	
Tumor size, cm				
<5	84	39 (70.91)	45 (65.22)	0.090
≥5	40	16 (29.09)	24 (34.78)	
TNM stage				
I + II	74	63 (64.95)	11 (40.74)	0.042
III + IV	50	34 (35.05)	16 (59.26)	
LNM				
Yes	55	20 (32.26)	35 (56.45)	0.011
No	69	42 (67.74)	27 (43.55)	

†Compared with normal control tissues. LncRNA, long non-coding RNA; LNM, lymph node metastasis.

UCA1-si (Fig. 2b). To further assess the potential effects of siRNA-mediated UCA1 silencing on cell proliferation, CCK-8 assay was carried out 24, 48, 72, 96, and 120 h after siRNA transfection (Fig. 2c–e). Compared with the Ctrl-si transfected cells, a significant decrease of cell viability was detected in SCC15, Cal27, and Tca8113 cells at 48–120 h after treatment with UCA1-si. Thus, the results of the CCK-8 assay indicated that UCA1-si could inhibit *in vitro* proliferation of OSCC cells. That suggested UCA1 may play an oncogenic role in OSCC cells.
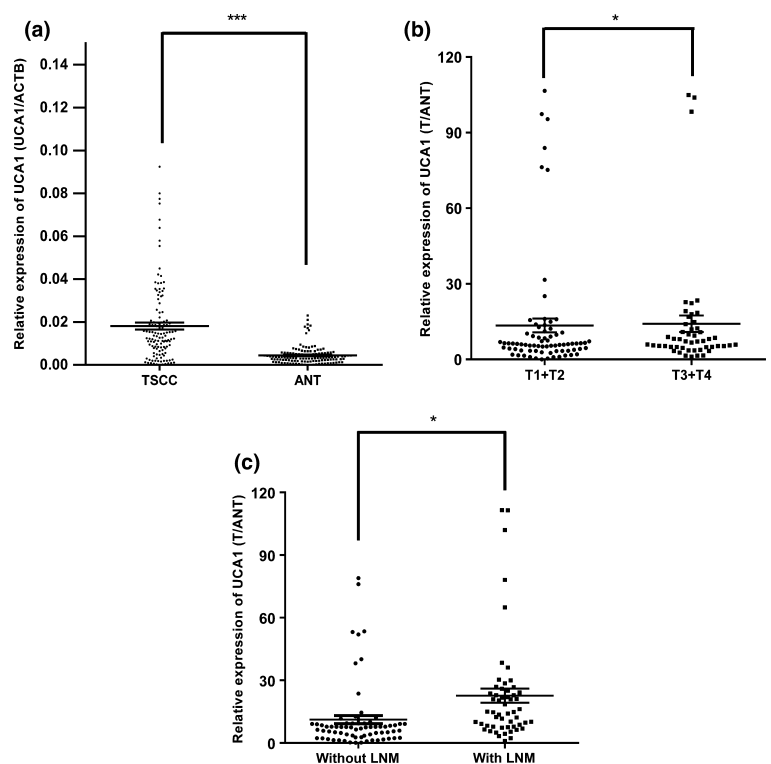

Fig. 1. Relative expression of UCA1 in tongue squamous cell carcinoma (TSCC) tissues and its relationship with TNM stage or lymph node metastasis of TSCC patients. (a) Relative expression of UCA1 was examined by quantitative RT-PCR and normalized to β-actin (ACTB) expression in 124 pairs of TSCC tissues (T) compared with corresponding adjacent normal tissues (ANT). (b) Relative expression of UCA1 as a ratio of TSCC to paired ANT in TSCC patients at different T stages. (c) Relative expression of UCA1 as a ratio of TSCC to paired ANT in TSCC patients with or without lymph node metastasis (LNM). Data are presented as mean ± SD. *$P < 0.05$, ***$P < 0.001$.
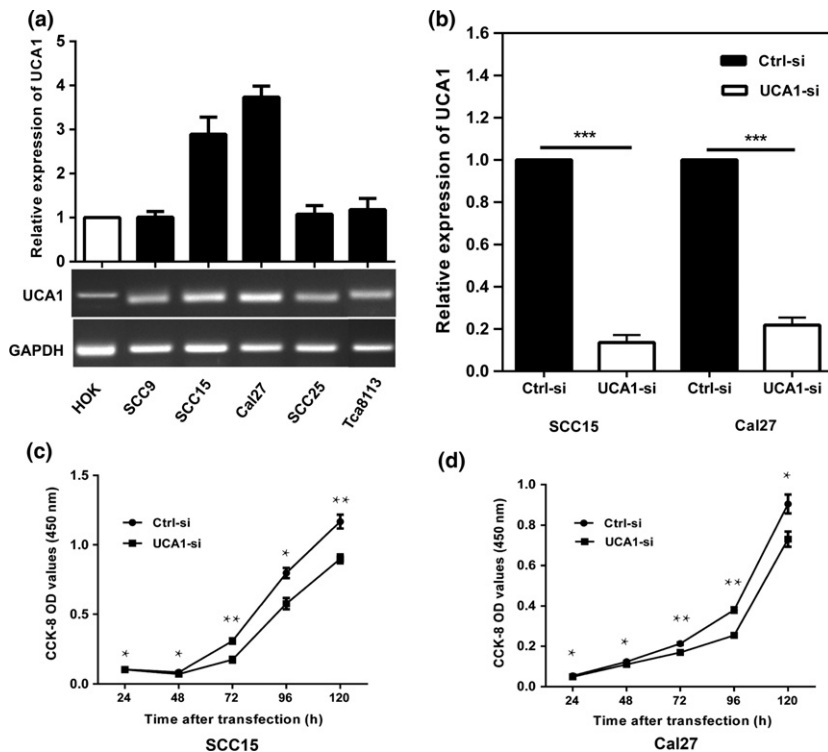
Fig. 2. Silencing of UCA1 inhibits viability of oral squamous cell carcinoma cell lines. (a) UCA1 expression was analyzed in different oral squamous cell carcinoma cell lines by quantitative RT-PCR. GAPDH was treated as internal control. (b) SCC15 and Cal27 cells were transfected with UCA1 siRNA (UCA1-si), and the UCA1 expression level was assayed by quantitative RT-PCR. (c,d) Cell growth viability was assayed in negative control (Ctrl-si) and UCA1-si transfected SCC15 and Cal27 cells by CCK-8 assay at 24, 48, 72, 96, and 120 h time points. Data are presented as mean ± SD. *P < 0.05, **P < 0.01.

Silencing of UCA1 suppresses cell migration and invasion in OSCC cell lines. To detect the effect of UCA1 silencing on cell migration, the Transwell migration assay and wound healing assay were carried out in two cell lines. With 24 h of cultivation, the number of migrated cells transfected with UCA1-si in both cell lines was significantly less than those transfected with Ctrl-si (Fig. 3a,b). The Transwell invasion assay results also showed that the cell invasive ability was suppressed in SCC15 and Cal27 cell lines when cells were transfected with UCA1-si compared with the Ctrl-si (Fig. 3c).

Silencing of UCA1 induces cell apoptosis in OSCC cell lines. To assess the influence of UCA1-si on the apoptosis of OSCC cells, we used Hoechst 33258 staining and flow cytometry to examine the apoptosis rate of two cell lines. The OSCC cells were transfected with UCA1-si or Ctrl-si. As Figure 4(a) shows, compared with Ctrl-si transfected cells, the rates of cell apoptosis were notably enhanced in UCA1-si transfected cells. Prominent apoptotic cells were also discovered by Hoechst 33258 staining. Compared to Ctrl-si transfected cells, the percentage of prominent apoptotic cells was distinctly increased in UCA1-si transfected cells (Fig. 4b,c).

Correlation between UCA1 expression and activation level of the WNT/β-catenin signaling pathway. Previous research groups have indicated that UCA1 promotes the progression of cancer and drug resistance through some potential target genes, for instance, *WNT6*, cytochrome P450, and *CYP1A1*.[16,19] Recent studies[20,21] have reported that UCA1 may upregulate the expression level of WNT6, and leads to the subsequent response of the WNT signaling pathway. Thus, we confirmed that UCA1 may serve a potent role in modulating the activation level of the WNT/β-catenin signaling pathway. In order to identify the correlation between UCA1 expression level and the activation level of the WNT/β-catenin signaling pathway, TOP/FOP flash reporters (Howard Hughes Medical Institute, University of Washington, USA) were used to assess the impacts of UCA1 on the WNT/β-catenin signaling pathway in SCC15 and Cal27 cells.

The change of cell luciferase activity is shown in Figure 5(a). While UCA1 expression was silenced, the activation of the WNT/β-catenin signaling pathway was suppressed. We then detected the protein expression levels of TCF-4 using Western blot analysis (Fig. 5a). As is known, the WNT/β-catenin signaling pathway plays a vital role in the modulation of cell proliferation. Using Western blot assay, we detected β-catenin expression and a few of the downstream genes of the WNT/β-catenin signaling pathway, for instance, *CCND1* and *MMP9* (Fig. 5b).[22–24] The results of the immunofluorescence assay showed that the position of β-catenin protein moves from the nucleus to cytoplasm in cells, and decreased in cytoplasm when the level of UCA1 was silenced (Fig. 5c). All results revealed a novel relationship between UCA1 expression and the activation level of the WNT/β-catenin signaling pathway. To further explore other potential mechanisms that might be involved in the UCA1-associated malignant progression of OSCC, we examined the protein expression of MAPK/Erk1/2, phosphorylated (p-)MAPK/Erk1/2, AKT1/2, and p-AKT1/2 in OSCC cell lines. Interestingly, the protein expression of MAPK/Erk1/2, p-MAPK/Erk1/2, AKT1/2, and p-AKT1/2 also have certain degree of difference in UCA1-si treated SCC15 and Cal27 cells (Fig. 5d), which is worthy of further study. Additionally, we analyzed the associations between the LncRNA UCA1 overexpression level and the WNT/β-catenin signaling pathway. Intriguingly, the UCA1 expression level was positively correlated with β-catenin, TCF-4, and cyclin D1 *in vitro* (Fig. 6). Thus, the upregulation of UCA1 could enhance the activation level of the WNT/β-catenin signaling pathway. These results further support the hypothesis that UCA1 has an effect on the biological behavior of OSCC cells.

Knockdown of UCA1 suppresses tumor growth *in vivo*. To assess the effects of UCA1-si on the *in vivo* growth of OSCC cells, we applied a xenograft model in which the SCC15 cells treated with UCA1-si or Ctrl-si were s.c. injected into the flanks of nude mice. There was no nude mice loss in the process of the
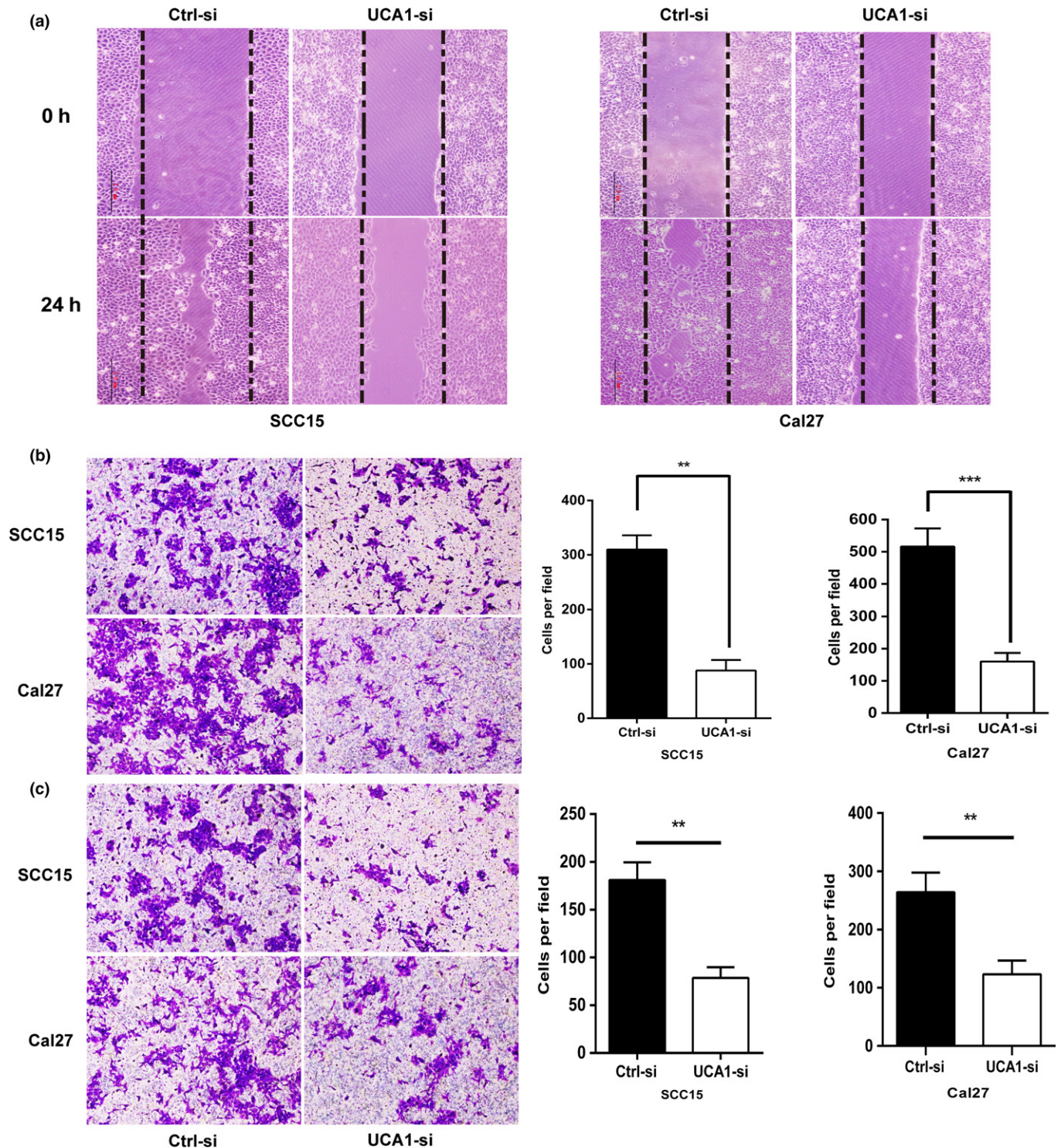
Fig. 3. Silencing of UCA1 inhibits migration and invasion of oral squamous cell carcinoma cell lines. (a) Status of cell migration was detected by wound healing assay. Representative images of migration (b) and invasion (c) of SCC15 and Cal27 cells transfected with UCA1 siRNA (UCA1-si) and negative control (Ctrl-si) are shown in the left panels (magnification, ×200). The number of migrated and invaded cells is measured in the right panel. Data are presented as mean ± SD. **$P < 0.01$, ***$P < 0.001$.

treatment and no other complications were found because of infection. The tumor formation rate in the Ctrl-si transfected group was 80% (8/10), whereas only 60% (6/10) of nude mice in the UCA1-si transfected group gave rise to tumors. During the whole tumor growth period, tumors from the UCA1-si transfected SCC15 cells grew slower than that of Ctrl-si transfected

cells (Fig. 7a). After 6 weeks, the tumor size at the time the animals in the UCA1-si group were killed was 265.6 ± 52.31 mm^3 in SCC15 cells, which was notably smaller than that in the Ctrl-si group with the tumor size of 532.0 ± 30.06 mm^3 (Fig. 7b). The average weight of tumors developed from UCA1-si transfected SCC15 cells (291.5 ± 40.04 mg, $n = 6$) was obviously
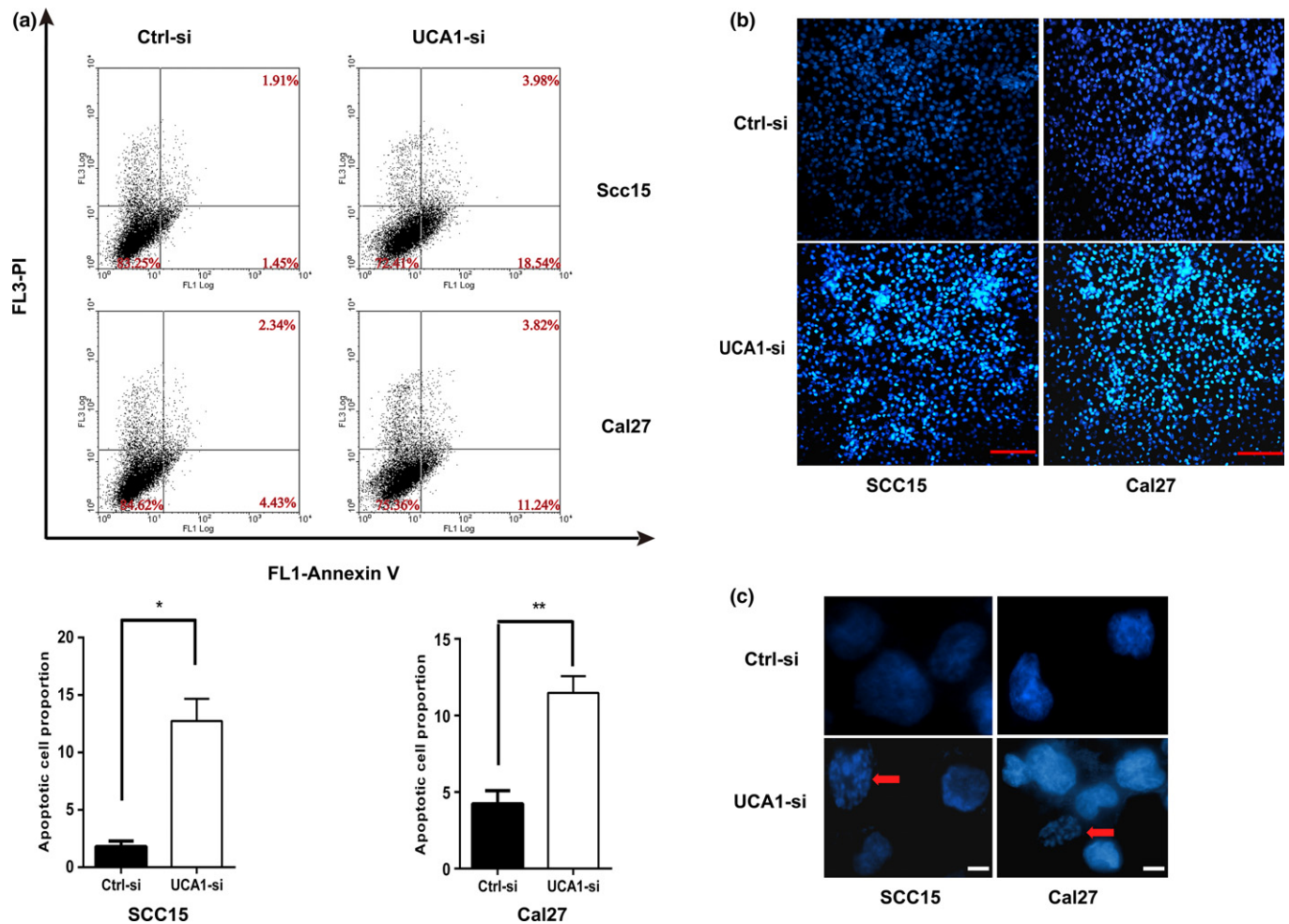
Fig. 4. Silencing of UCA1 induces apoptosis of oral squamous cell carcinoma cells. (a) Apoptotic cell proportion is measured by flow cytometric analysis of annexin V/propidium iodide (PI) double staining after transfection with UCA1 siRNA (UCA1-si) and negative control (Ctrl-si) for 48 h. Upper right and lower right in the cell distribution panels represent late and early apoptotic cell proportions, respectively. (b,c) Hoechst 33258 staining assay was used to detect apoptotic cells. Representative images of changes were shown. Left panel magnification, ×200; right panel magnification, ×1000. The number of apoptotic cells transfected with UCA1-si was more than that transfected with Ctrl-si. Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.

smaller than those of Ctrl-si mice (558.1 ± 50.40 mg, $n = 8$) (Fig. 7c). These results suggest that UCA1 silencing may inhibit the proliferative capacity of OSCC cells *in vivo*.

Discussion

Emerging evidence strongly indicates the importance of LncRNAs in the essential regulation of protein coding genes. They play an important role in normal growth and development and tumorigenesis at both the transcriptional and post-transcriptional levels.[8,9] Several groups have reported that a large variety of LncRNAs are frequently dysregulated in tumors, presenting spatially and temporally regulated expression patterns. These differentially expressed LncRNAs are closely related to tumorigenesis, metastasis, prognosis, or diagnosis, acting as oncogenes or tumor suppressor genes.[8,25,26] Moreover, *UCA1* serves as an oncogene through activating the KLF4-KRT6/13 signaling pathway, promoting the proliferation of prostate cancer cells.[27] Thus, more effort should be devoted to fully elucidate the biological role and potential molecular mechanisms of UCA1 in cancer.

In our present study, we revealed the expression of a novel LncRNA UCA1 in OSCC specimens and their corresponding non-tumorous tissues. The results indicated that UCA1 was upregulated in TSCC tissues and was closely correlated with metastasis and TNM stage. However, our previous report,[18] showed that there was no significant statistical difference in UCA1 expression between TSCC groups at different advanced TNM stages (37 T3–T4 in 94 cases), the probable reasons were the limited number of cases and the randomness of patients. Unfortunately, with too many patients lost to follow-up, a Kaplan–Meier survival analysis was not possible. We then verified the function of UCA1 in OSCC cells by loss-of-function approaches. The downregulation of UCA1 could restrain cell proliferation, migration, and invasion and induce early apoptosis in OSCC cell lines *in vitro*, as well as suppress tumor growth *in vivo*. This is the first report to comprehensively elucidate the functional significance of UCA1 expression in human OSCC, and our data indicate that UCA1 may serve as an oncogene that promotes OSCC malignant progression.

Moreover, mounting evidence indicates that aberrant activation of the WNT/β-catenin signaling pathway may be involved in OSCC development and progression.[28,29] Our
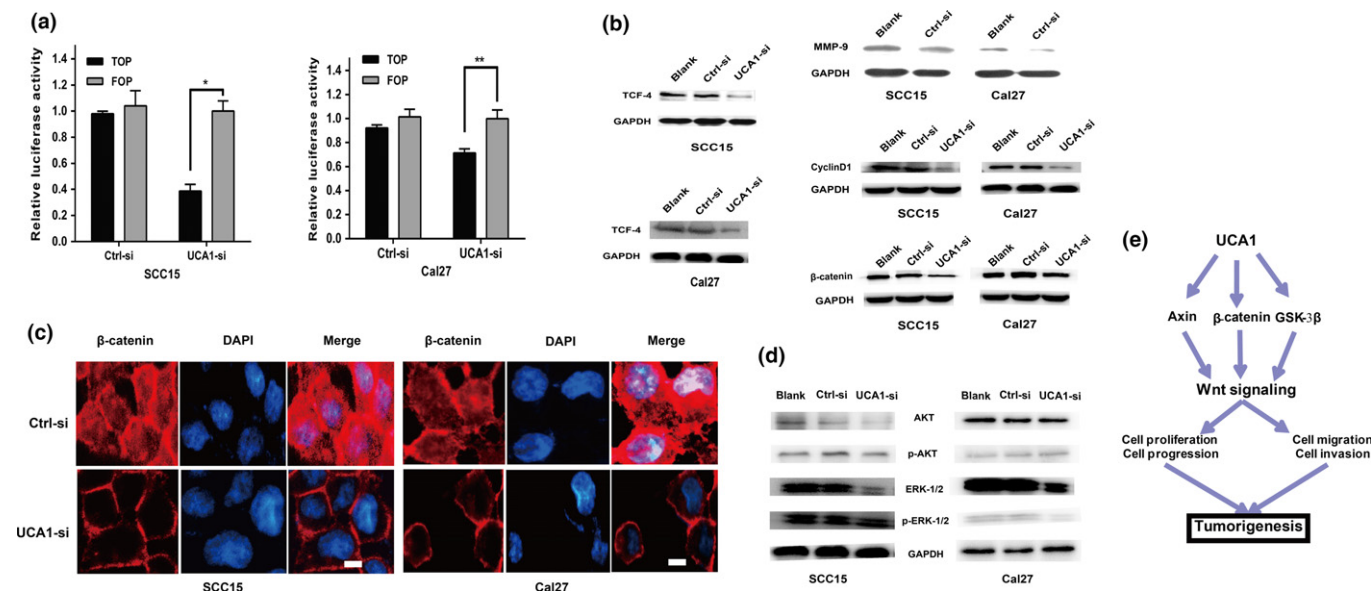
Fig. 5. UCA1 promotes oral squamous cell carcinoma malignant progression through WNT/β-catenin signaling pathway. (a) Luciferase reporter assay using TOPflash vectors was carried out to detect β-catenin transcription factor/lymphoid enhancer binding factor (TCF/LEF) promoter activity; FOPflash has mutated TCF binding sites, acting as a negative control. SCC15 and Cal27 cells were cotransfected with different expression vectors as indicated. UCA1 siRNA (UCA1-si) treatment inhibited β-catenin TCF/LEF promoter activity. (b) Western blot analysis of proteins (β-catenin, TCF-4) in the WNT/β-catenin signaling pathway and of downstream WNT/β-catenin signaling pathway targets, such as cyclin D1 and MMP-9. (c) Immunofluorescence assay for β-catenin implies that the location of β-catenin in cells moves from nuclear to cytoplasmic when the expression of UCA1 is silenced, and that β-catenin expression decreased in the nucleus compared to Ctrl-si. (d) Representative results of Western blot analysis for the protein expression levels of ERK1/2, p-ERK1/2, AKT, and p-AKT from UCA1-si or Ctrl-si treated SCC15 and Cal27 cells. (e) Diagram depicting the regulation mechanism of UCA1 in the tumorigenesis of oral squamous cell carcinoma. GSK-3β, glycogen synthase kinase 3β. Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.
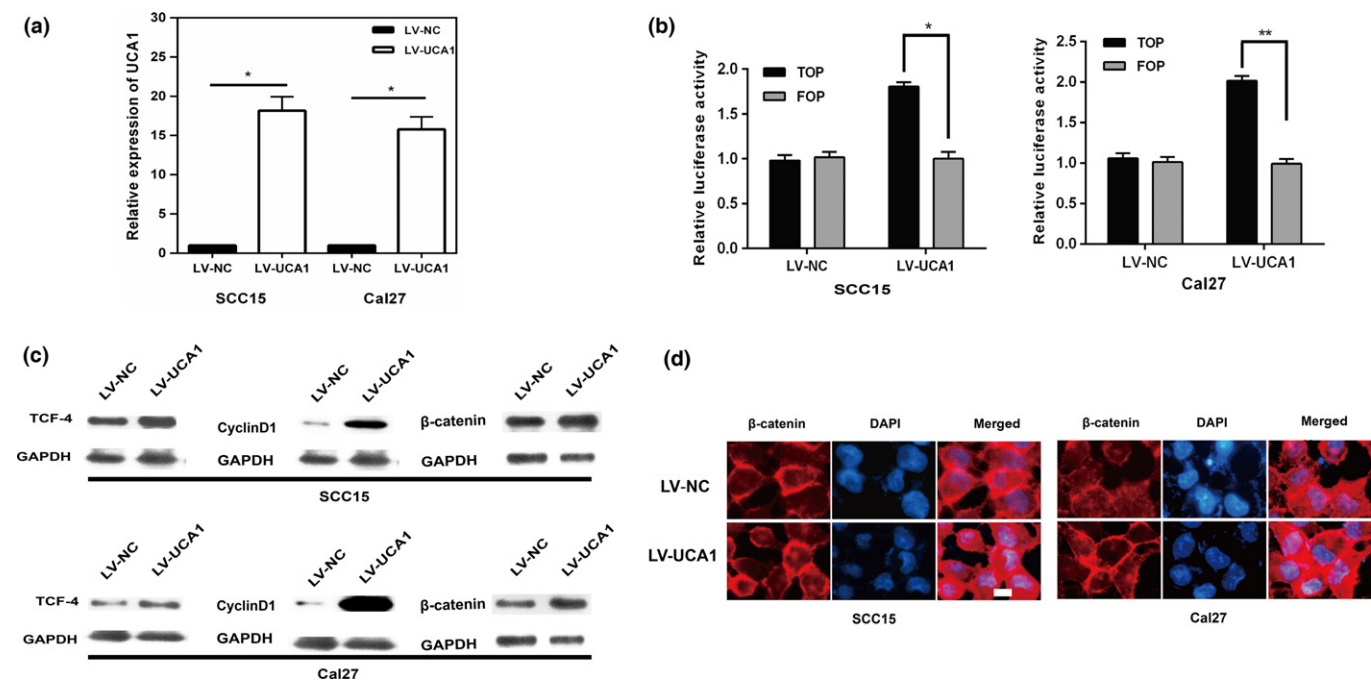

Fig. 6. Overexpression of long non-coding RNA (LncRNA) UCA1 overexpression positively correlates with WNT/β-catenin signaling pathway *in vitro*. (a) Expression of LncRNA UCA1 in SCC15 and Cal27 cells transfected with a lentivirus encoding LncRNA UCA1 (LV-UCA1) or a puromycin control (negative control, LV-NC) was quantified by RT-PCR. (b) Luciferase reporter assay using TOPflash vectors was undertaken to detect β-catenin transcription factor/lymphoid enhancer binding factor (TCF/LEF) promoter activity; FOPflash has mutated TCF binding sites, acting as a negative control. SCC15 and Cal27 cells were cotransfected with different expression vectors as indicated. The upregulation of UCA1 could enhance the β-catenin TCF/LEF promoter activity. (c) Western blot analysis of proteins in the WNT/β-catenin signaling pathway and of downstream WNT/β-catenin signaling pathway targets, such as cyclin D1. (d) Immunofluorescence assay for β-catenin implies that the location of β-catenin in cells moves from cytoplasmic to nuclear when the expression of UCA1 was overexpressed and that β-catenin expression increased in the nucleus compared to LV-NC. Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.
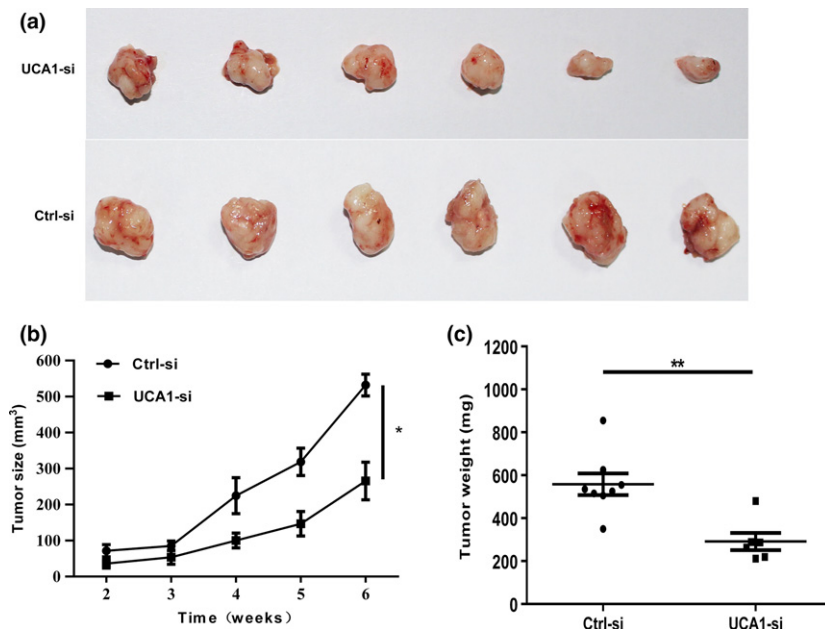
Fig. 7. Silencing of UCA1 suppresses tumor growth *in vivo*. (a) Photographs of tumor xenografts 6 weeks after inoculation. (b) Tumor growth curves measured after injection of SCC15 cells transfected with UCA1 siRNA (UCA1-si) or negative control (Ctrl-si). Tumor volume was calculated every 7 days from 2 to 6 weeks. The tumor size at the time mice were killed in the UCA1-si group was significantly smaller than that in the negative control (Ctrl-si) group. (c) Tumor weight in the UCA1-si group at the end of the experiment was significantly lower than that in the Ctrl-si group. Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.

data suggested that knockdown of UCA1 inhibited β-catenin expression and reversed the activation level of the WNT/β-catenin signaling pathway. In addition, in terms of protein expression level, our findings indicate that UCA1 can also modulate several downstream target genes of the WNT signaling pathway, for instance, *MMP9*, *TCF4*, and *CCND1*. β-Catenin was not only acting as a part of the cadherin-based adhesion protein complex, but also as an important endocellular intermediary for the WNT/β-catenin signaling pathway. Thus, that is why we think that the effects of UCA1 on tumor cell proliferation and metastasis might be involved in β-catenin modulation. We applied a representative luciferase reporter assay, TOP/FOP Flash, to detect the activity of TCF-dependent transcription. As a result, a relationship between UCA1 expression and the activation level of the WNT/β-catenin signaling pathway was revealed. Many other researchers have also found that the WNT/β-catenin signaling pathway is involved in stages (including early and late stages) of apoptosis in other carcinoma cells,[30,31] which is identical to our findings. Based on our results, we therefore considered UCA1 may be associated with the WNT/β-catenin signaling pathway. However, other additional mechanisms exist in the regulation of β-catenin expression to a large extent, such as regulation by miRNA sponges, to affect the expression of β-catenin, or with the co-regulation of other signaling pathways. Thus, our findings provide a new clue for understanding the pathogenesis of OSCC and provide a novel approach for the diagnosis and treatment of OSCC.

Another unresolved question would be the potential reasons for UCA1 overexpression in TSCC or OSCC. We checked the TCGA dataset and referred to many published reports, and found some clues. In the bioinformatics analysis by Xue *et al.*[32,33] UCA1 gene promoter was analyzed using CpG software to predict the CpG island. Matrix Catch and TFSEARCH were used to predict the potential transcription factor binding sites in UCA1 gene core promoter. The ChIP assay was used to identify the transcription factors binding to UCA1 gene core promoter. They then found that UCA1 gene promoter contained no CpG island and was therefore a typical tissue-specific gene. There were four transcription factors (CREB, c-Myb, C/EBPα, and c-ETS-2) associated with human

cancers in UCA1 gene core promoter, but only one of them (c-Myb) interacted with UCA1 gene core promoter. This evidence suggested that both c-ETS-2 and C/EBPα interacted with UCA1 core promoter, leading to the upregulation of UCA1 in bladder cancer. However, the interaction between c-ETS-2 and C/EBPα toward the activation of UCA1 in cancers has not been investigated thus far. Thus, we consider that the activation of transcription factor c-Myb might be the potential reason for UCA1 overexpression in TSCC or OSCC, although further experiments are still needed for verification.

In summary, our present work indicates that UCA1 acts as an oncogene by promoting malignant progression of human OSCC, notably, mechanistic analysis reveals a novel UCA1–β-catenin–WNT signaling pathway regulatory network in OSCC. Nevertheless, several studies have shown that there are LncRNA transcriptional autoregulatory loops that may be feedback to control the expression of LncRNA.[34,35] WNT signaling may activate its downstreaming transcription factors in the nucleus. whether some miRNAs bind with UCA1 to downregulate miRNA expression and activities, subsequently regulating the disinhibition of miRNA targets at the level of post-transcriptional modulation.[34,36,37] In addition, only a few of the functional LncRNAs have been well elucidated in OSCC to date. Therefore, more efforts are needed to better identify the function and critical mechanisms of OSCC-specific LncRNAs in the progression of OSCC, which may effectively enhance our understanding of the occurrence and development of OSCC and ultimately facilitate the development of LncRNA-mediated diagnosis and therapy for this fatal tumor.

Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Doc. S1. Detailed methods.

Table S1. Detailed information of clinical and tumoral characteristics of patients and the information of metastasis.

Table S2. Primers and siRNA sequences used in this study.