OncoTargets and Therapy

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

LINC00152 promotes the growth and invasion of oral squamous cell carcinoma by regulating miR-139-5p

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Minghe Li[1,2]
Jun Ning[3]
Zhihong Li[4]
Jing Wang[5]
Cong Zhao[5]
Lei Wang[6]

[1]Department of Oral and Maxillofacial Surgery, School of Stomatology Hospital of Jilin University, Changchun, China; [2]Key Laboratory of Tooth Development and Bone Remodeling, School of Stomatology Jilin University, Changchun, China; [3]Department of Gynaecology II, The First Hospital of Jilin University, Changchun, China; [4]Department of Thoracic Surgery, The First Hospital of Jilin University, Changchun, China; [5]Department of Endodontics, School of Stomatology Hospital of Jilin University, Changchun, China; [6]Department of Periodontology, School of Stomatology Hospital of Jilin University, Changchun, China

Background: LINC00152 plays a crucial role in tumorigenesis and progression of multiple types of cancer. However, the biological significance of LINC00152 and its potential role in oral squamous cell carcinoma (OSCC) remain to be determined. In the present study, we investigated the role of LINC00152 and the underlying mechanism of its oncogenic activity in OSCC.

Materials and methods: The expression of LINC00152 in OSCC tissues and cell lines was detected using qRT-PCR. Cell proliferation, colony formation, migration, and invasion were measured using a cell counting kit, colony formation assay, wound healing, and transwell invasion assays, respectively. The target gene of LINC00152 was confirmed using a dual-luciferase reporter assay and qRT-PCR. A nude mouse model was established to analyze the function of LINC00152 in vivo.

Results: LINC00152 expression was significantly upregulated in OSCC tissues and cell lines compared with that in normal counterparts. Upregulated LINC00152 served as an independent prognostic predictor in patients with OSCC. Moreover, knockdown of LINC00152 inhibited cell proliferation, colony formation, migration, and invasion, and suppressed the epithelial to mesenchymal transition in vitro, as well as impairing tumor growth in vivo. A mechanistic investigation indicated that LINC00152 could directly bind to miR-139-5p in OSCC. LINC00152 expression was inversely correlated with miR-139 expression in OSCC tissues.

Conclusion: Taken together, these results suggested that LINC00152 may function as oncogene in OSCC and could be a potential therapeutic target in patients with OSCC.

Keywords: lncRNA, LINC00152, miR-139-5p, oral squamous cell carcinoma

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most aggressive neoplasms among head and neck carcinomas.[1] OSCC is an aggressive, invasive epithelial malignancy that is sometimes associated with inflammatory changes and periodontal disease.[2–4] Although there has been significant progress in the diagnosis and clinical treatment of these diseases, the overall 5-year survival rate after diagnosis remains less than 50%, mainly because of cancer metastasis or recurrence.[5] Therefore, increasing our understanding of the molecular mechanisms involved in OSCC growth and metastasis might help to find effective therapeutic targets and improve the overall prognosis of patients with OSCC.

Long non-coding RNAs (lncRNAs) are a group of non-protein-coding RNAs of more than 200 nucleotides in length. Increasing evidence indicates that lncRNAs play crucial roles in diverse cellular processes, such as proliferation, apoptosis, dedifferentiation, cycle arrest, migration, and invasion.[4,5] LncRNAs have been reported to have

Correspondence: Zhihong Li
Department of Thoracic Surgery, The First Hospital of Jilin University, 71# Xinmin Street, Chaoyang District, Changchun 130021, China
Email lizhihong5436@126.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/OTT.S168807

important functions in human cancer biology by regulating malignant biological behaviors in tumor cells, such as proliferation, invasion, and metastasis.[6,7] Their high tissue specificity, high efficiency, and increased stability, have led to the suggestion that lncRNAs could act as therapeutic targets and biomarkers for diagnosis or prognosis in various cancers, including OSCC.[8,9]

LINC00152, a 828 bp lncRNA that maps to chromosome 2p11.2,[10] was recently reported to be involved in tumorigenesis and metastasis in multiple types of cancers.[11–19] A recent study showed that LINC00152 was the most significantly upregulated lncRNA in tongue squamous cell carcinoma (a kind of OSCC), and elevated LINC00152 expression was associated with poor prognosis.[20] However, the biological functions and underlying molecular mechanisms for LINC00152 in OSCC remain unclear. Therefore, the present study aimed to explore whether LINC00152 is involved in OSCC progression. The results showed that LINC00152 expression was increased in OSCC tissues and cell lines. LINC00152 knockdown inhibited OSCC development by increasing the level of miR-139-5p. Thus, we hypothesized that LINC00152 is a competing endogenous RNA (ceRNA) that acts as an miRNA sponge for miR-139-5p in OSCC.

Materials and methods

Subject and tissue collection

Forty patients diagnosed with OSCC were included in this study. Written informed consent was obtained from all the participants, and this study was approved by the Ethics Committee of Jilin University (Changchun, China). The OSCC tissues and corresponding adjacent nontumor tissues were harvested from 40 patients who were pathologically diagnosed with OSCC and underwent surgery between March 2012 and March 2014. The patients were recruited from the School of Stomatology Hospital of Jilin University. During surgery, all samples were immediately frozen in liquid nitrogen until RNA extraction. None of the patients received any preoperative chemotherapy, radiotherapy, or other therapy.

Cell lines and transfection

Four OSCC cell lines: Tca8113, OSCC-15, SCC-9, and SCC-25; and the human normal oral keratinocytes (hNOKs) cell line, were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). All cells were routinely cultured in DMEM/Nutrient Mixture F12 (HyClone, Logan, UT, USA) containing 10% FBS (HyClone), 100 U/mL penicillin (Sigma-Aldrich Co., St Louis, MO, USA), or 100 µg/mL streptomycin (Sigma-Aldrich Co.) at 37°C in a humidified incubator containing 5% CO_2.

A short-hairpin RNA (shRNA) directed against human LINC00152 (sh-LINC00152) and a scrambled shRNA as a negative control (sh-NC), both in plasmids, were synthesized by GenePharma (Shanghai, China). An miR-139-5p mimic and corresponding negative control (miR-NC) were obtained from GenePharma. Before transfection, SCC-9 cells (1×10^5) were cultured until it reached 80% confluence. The vectors and miRNAs were transfected separately into SCC-9 cells using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The silencing efficiencies were evaluated using qRT-PCR at 48 hours post-transfection.

To select OSCC cells with stable depletion of LINC00152, sh-LINC00152 or sh-NC vectors were transfected into SCC-9 cells and were selected with neomycin (800 µg/mL) for 4 weeks.

qRT-PCR

Total RNA extraction was performed from tumor tissues and cell lines using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA was reverse transcribed to cDNA using PrimeScript™ RT Reagent Kit (Takara, Dalian, China) according to the manufacturer's instructions. RT-PCR was performed using TransStart Top Green qPCR SuperMix (Transgen, Beijing, China) on an ABI 7900 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). The primers for U6, miR-139-5p, LINC00152, and *GAPDH* used in this study have been described previously.[19,21] The expression levels of *GAPDH* and U6 were used as endogenous controls. Relative quantification of the target genes was performed with the comparative cycle threshold (CT) values using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation and colony formation

The proliferation of cells transfected with the indicated shRNAs or plasmid constructs was determined using the cell counting kit 8 (CCK-8) assay, as described previously.[19] For the colony formation assay, transfected cells were seeded into 6-well plates at a density of 1,000 cells per well and cultured at 37°C in a humidified 5% CO_2 incubator for 10 days to form colonies. After staining with 0.2% crystal violet, the colonies were imaged and counted under a light microscope (Olympus Corporation, Tokyo, Japan).

Cell migration and invasion assays

The cell migration ability was determined using a wound healing assay. Briefly, transfected cells were seeded into 6-well plates and grown to 100% confluence. A linear wound was created using a pipette tip. After washing three times with PBS, the cells were cultured in medium containing 1% FBS for 24 hours. Three random images were taken at the time of wounding. Migration distance (units) was analyzed as a reduction in the wound gap, using the NIH ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Cell invasion was determined using Matrigel invasion assays. Briefly, 2×10^5 transfected cells in serum-free DMEM were seeded into the upper chamber of a BD BioCoat Matrigel Invasion Chamber (BD Biosciences, San Jose, CA, USA) with 8 µm pores, coated with Matrigel matrix (BD Biosciences), and 600 µL medium containing 10% FBS as a chemoattractant was added to the lower chamber. After incubation for 48 hours, the cells that had invaded through the membrane and remained on the lower membrane surface were fixed in 20% methanol and stained with 0.1% crystal violet. The fixed cells in five randomly selected fields were photographed and counted under an X71 inverted microscope (Olympus Corporation).

Western blotting

Cells or tumor tissues were incubated on ice with lysis buffer (Beyotime, Beijing, China) for 30 minutes, and centrifuged at 20,000× *g* at 4°C for 15 minutes. Protein concentrations were determined using a Pierce Bicinchoninic Acid Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein (30 µg) were subjected to 10% SDS-PAGE and then transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk in Tris-buffered saline ([TBS] 10 mM Tris and 150 mM NaCl) for 1 hour. Immediately following blocking, the membranes were incubated with the following primary antibodies overnight at 4°C, all of which were raised in mice and supplied by Santa Cruz Biotechnology Inc. (Dallas, TX, USA): anti-epithelial (E)-cadherin (1:1,000 dilution), anti-neural (N)-cadherin (1:1,000 dilution), anti-Vimentin (1:1,000 dilution), and anti-GAPDH (1:2,000 dilution). After washing three times with TBS, the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (1:5,000 dilution; Santa Cruz Biotechnology Inc.) at room temperature for 2 hours. Immunoreactive protein bands were visualized using an enhanced chemiluminescence-based FluorChem® FC2 imaging system (Alpha Innotech, San Jose, CA, USA).

Luciferase reporter assay

The 3′UTR of LINC00152, containing the potential binding sites of miR-139-5p, was synthesized by RiboBio (Guangzhou, China), inserted into vector psiCHECK2 (Promega Corporation, Fitchburg, WI, USA), and named WT-LINC00152. A mutant 3′UTR of LINC00152 was constructed using a QuikChange XL Site-Directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA, USA), and named MT-LINC00152. For the luciferase reporter assay, SCC-9 cells were co-transfected with the miR-139-5p mimic or miR-NC and the reporter plasmid WT-LINC00152 or MT-LINC00152 using the Lipofectamine 3,000 reagent (Invitrogen) according to the manufacturer's instructions. The luciferase activity assays were performed 48 hours after transfection using a Dual Luciferase Reporter Gene Assay Kit (Beyotime), following the manufacturer's protocol. The relative luciferase activity was normalized to the Renilla luciferase activity.

Tumor xenograft model

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Jilin University. Male athymic BALB/c nude mice (4–5 weeks old) were obtained from the Experimental Animal Center of Jilin University (Changchun, China) for animal studies. All animals were maintained and used under specific pathogen-free conditions in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Jilin University (Changchun, China).

SCC-9 cells (2×10^6) stably overexpressing sh-LINC00152 or the corresponding sh-NC were subcutaneously injected into the flanks of nude mice, respectively. The tumor volume (V) was monitored and calculated according to the formula: $V=0.536\times(L \times W^2)$ by measuring tumor length (L) and width (W) every 7 days until the mice were sacrificed. At 35 days after injection, the mice were sacrificed, and the tumors were carefully excised, weighed, photographed, and stored in liquid nitrogen for further experiments.

Statistical analysis

The data shown in this study were expressed as the mean ± SD from at least three independent experiments. The differences among treatment groups were analyzed using Student's *t*-test or one-way ANOVA followed by Dunnett's multiple test, as appropriate. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Significant differences between groups were assessed as $P<0.05$ and $P<0.01$.

Results

LINC00152 is overexpressed in OSCC and is associated with decreased survival in patients with OSCC

We first detected the expression of LINC00152 in tumor tissues collected from 40 patients with OSCC and adjacent normal tissues (ANTs). The results of qRT-PCR demonstrated that LINC00152 levels were significantly higher in tumor tissues than in ANTs ($P<0.01$; Figure 1A). The clinicopathological parameters that might be related to LINC00152 expression levels in the 40 patients with OSCC were also analyzed. Based on the median values, patients could be divided into two groups: those with a high level of LINC00152 (n=25) and those with a low level of LINC00152 (n=15). Statistical analysis showed that LINC00152 expression levels were significantly associated with the TNM stage and lymph node metastasis (Table 1), but were not significantly associated with other clinicopathological parameters, including age, gender, and differentiation (Table 1). Moreover, the expression levels of LINC00152 were also examined in four OSCC cell lines (Tca8113, OSCC-15, SCC-9, and SCC-25) and in normal oral cells (hNOK) using qRT-PCR. The result showed that the OSCC cells also expressed higher levels of LINC00152 than the hNOK cells (Figure 1B). We also found that high expression of LINC00152 was associated with a reduced survival rate in patients with OSCC (Figure 1C, $P<0.05$). These results

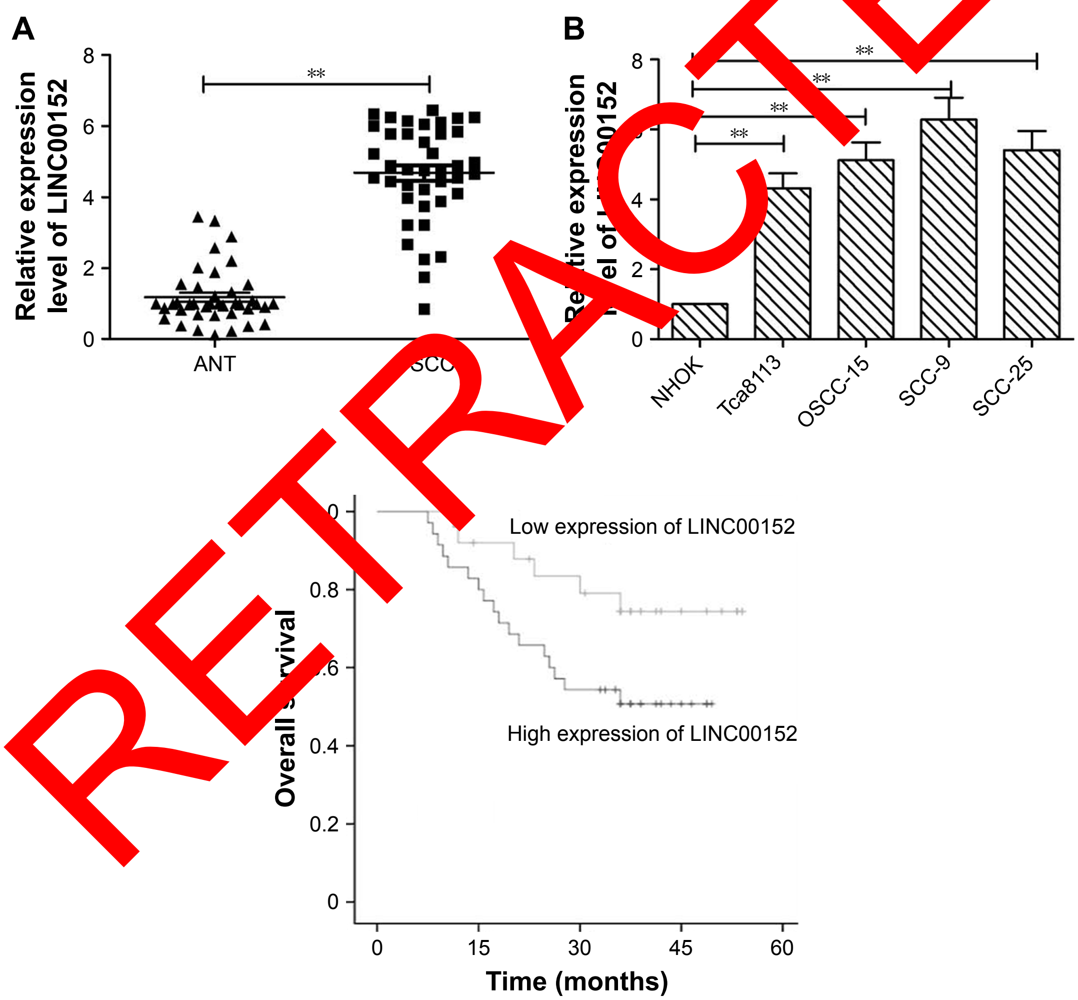

Figure 1 LINC00152 is overexpressed in OSCC samples and is associated with reduced survival of patients with OSCC.

Notes: (**A**) Relative expression of LINC00152 in OSCC tissues (n=40) and in paired adjacent normal tissues (n=40). LINC00152 expression was detected by qRT-PCR and normalized to GAPDH expression. (**B**) Relative expression of LINC00152 in four OSCC cell lines (Tca8113, OSCC-15, SCC-9, and SCC-25) and in normal oral cells (hNOK). (**C**) Kaplan–Meier survival analysis showed that high LINC00152 expression was associated with worse overall survival compared with low expression. $^{**}P<0.01$.

Abbreviations: ANT, adjacent normal tissue; hNOK, human normal oral keratinocytes; OSCC, oral squamous cell carcinoma.

Table 1 Association of LINC00152 expression with clinicopathologic factors of 40 OSCC patients

Variables	Number of cases	LINC00152 expression		*P*-value
		High (n %)	Low (n %)	
Age (years)				*P*>0.05
<60	17	11 (64.7)	6 (36.3)	
≥60	23	14 (60.9)	9 (39.1)	
Gender				*P*>0.05
Male	22	14 (63.6)	8 (34.4)	
Female	18	11 (61.1)	7 (38.9)	
Clinical stage				*P*<0.01
I–II	29	15 (51.8)	14 (48.2)	
III–IV	11	10 (90.9)	1 (9.1)	
Differentiated				*P*>0.05
Well/moderate	25	15 (60.0)	10 (40.0)	
Poor	15	10 (66.7)	5 (33.3)	
Lymph node metastasis				*P*<0.01
No	28	14 (50.0)	14 (50.0)	
Yes	12	11 (91.7)	1 (8.3)	

Abbreviation: OSCC, oral squamous cell carcinoma.

implied that high LINC00152 expression is related to poor prognosis of OSCC.

Knockdown of LINC00152 inhibits cell proliferation and colony formation in OSCC cells

To investigate the role of LINC00152 in OSCC cells, we reduced the levels of LINC00152 by transfecting SCC-9 cells with the sh-LINC00152 plasmid. Transfection with sh-LINC00152 significantly reduced the expression levels of LINC00152 in SCC9 cells (Figure 2A). The CCK-8 assay results showed that knockdown of LINC00152 significantly inhibited the proliferation of SCC-9 cells (Figure 2B). Furthermore, knockdown of LINC00152 significantly inhibited cell colony formation of SCC-9 cells (Figure 2C).

Knockdown of LINC00152 inhibits cell migration and invasion in OSCC cells

The roles of LINC00152 in cell migration and invasion of OSCC were then evaluated. As shown in Figure 3A and B, transfection with sh-LINC00152 significantly decreased the cell migration and invasion abilities of SCC-9 cells.

To investigate the mechanism underlying LINC00152 knockdown-mediated inhibition of migration and invasion in OSCC cells, we also assessed whether LINC00152 had an effect on the epithelial to mesenchymal transition (EMT) process by examining the protein levels of certain EMT-related markers. As shown in Figure 3C, when LINC00152 expression was knocked down, the levels of the mesenchymal markers N-cadherin and Vimentin were downregulated, and the levels of epithelial marker E-cadherin were upregulated in SCC-9 cells.

LINC00152 directly targets miR-139-5p in OSCC

To investigate the interaction between LINC00152 and miRNAs, we predicted the miRNAs that might interact with LINC00152 using two online prediction tools (Starbase v2.0 and miRanda). As shown in Figure 4A, miR-139-5p has a sequence complementary to that of LINC00152. To identify a direct, endogenous link between miR-139-5p and LINC00152, we constructed luciferase reporters that contained the wild-type (WT) or mutated (MUT) miR-139-5p binding sites (Figure 4A). The luciferase reporter assay showed that overexpression of miR-139-5p significantly reduced WT-LINC00152-regulated luciferase activity but did not affect MUT-LINC00152-regulated luciferase activity (Figure 4B). To further determine whether LINC00152 is regulated by miR-139-5p, we detected the LINC00152 expression levels in SCC-9 cells transfected with miR-139-5p mimics or miR-NC mimics. The results showed that miR-139-5p overexpression did not affect LINC00152 expression in SCC-9 cells (Figure 4C). However, knockdown of LINC00152 significantly increased the levels of miR-139-5p (Figure 4D). Furthermore, we assessed the association between the LINC00152 mRNA level and miR-139-5p expression in 40 OSCC tissues. We found that miR-139-5p expression was downregulated in OSCC tissues (Figure 4E), and its expression was significantly negatively correlated with LINC00152 in OSCC tissues, as analyzed by Pearson correlation analysis (Figure 4F, $P<0.01$). Taken together, these data suggested that miR-139-5p might function as a downstream effector of LINC00152 in human OSCC.

Knockdown of LINC00152 suppresses tumor growth in vivo

To evaluate the biological functions of LINC00152 in vivo, SCC-9 cells stably overexpressing sh-LINC00152, or the corresponding sh-NC, were subcutaneously injected into the flanks of nude mice. Tumor growth was measured every week. We found that downregulation of LINC00152 significantly suppressed tumor growth in vivo (Figure 5A). At 35 days after injection, the mice were sacrificed, and tumor tissues were excised and weighed. The results showed that tumor weight and size were smaller in the sh-LINC00152

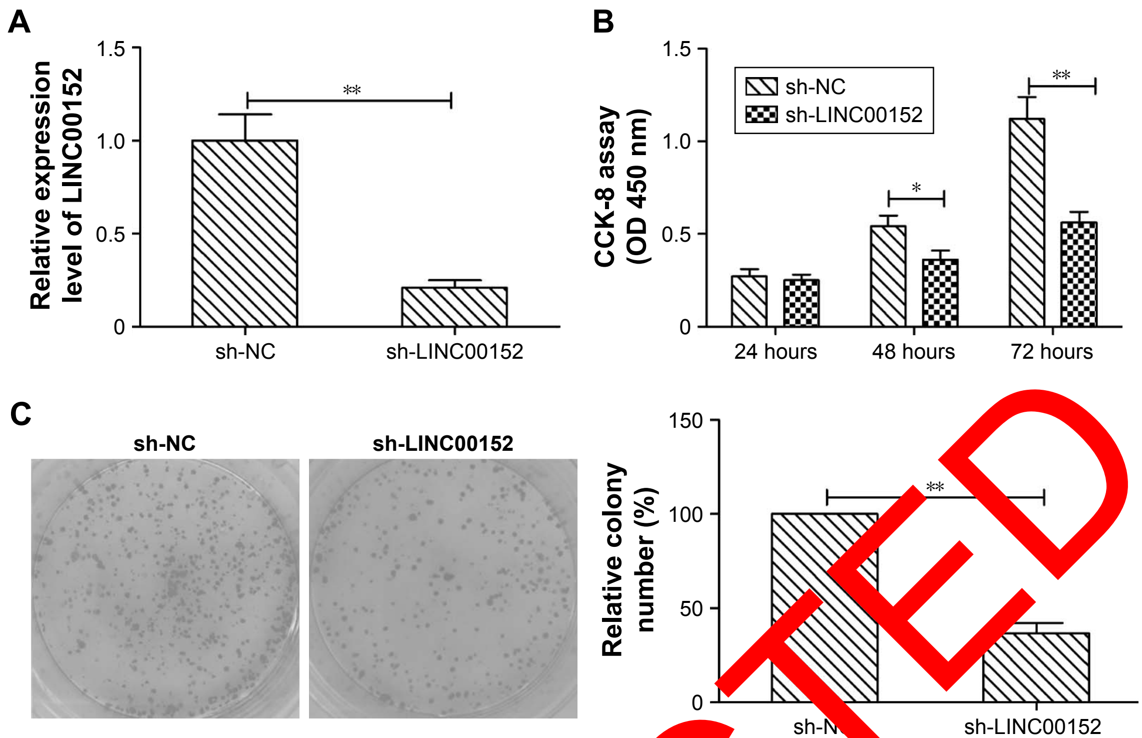

Figure 2 Knockdown of LINC00152 inhibits cell proliferation and colony formation in OSCC cells.

Notes: (**A**) The relative expression of LINC00152 was detected in SCC-9 cells transfected with sh-LINC00152 or sh-NC using qRT-PCR. Cell proliferation (**B**) and colony formation (**C**) were determined in SCC-9 cells transfected with sh-LINC00152 or sh-NC. *$P<0.05$, **$P<0.01$.

Abbreviations: OSCC, oral squamous cell carcinoma; sh-LINC00152, short-hairpin RNA targeting LINC00152; sh-NC, negative control shRNA.

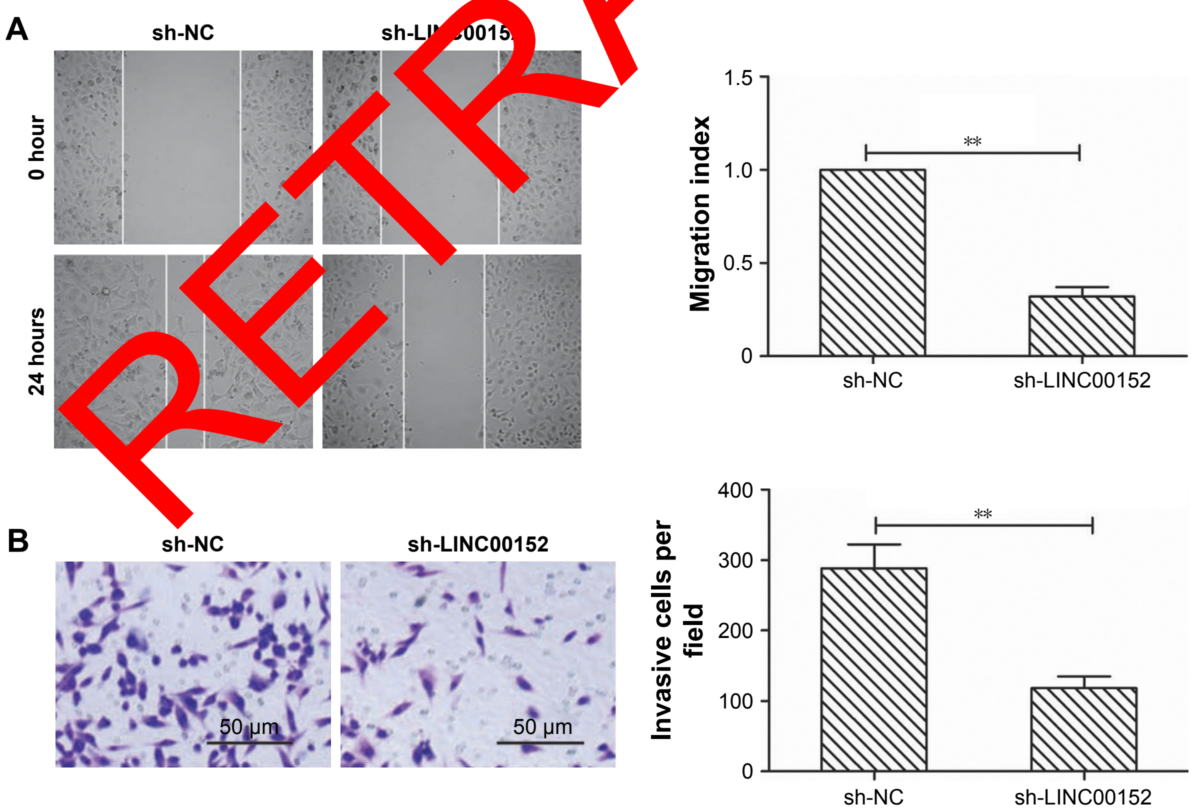

Figure 3 (*Continued*)

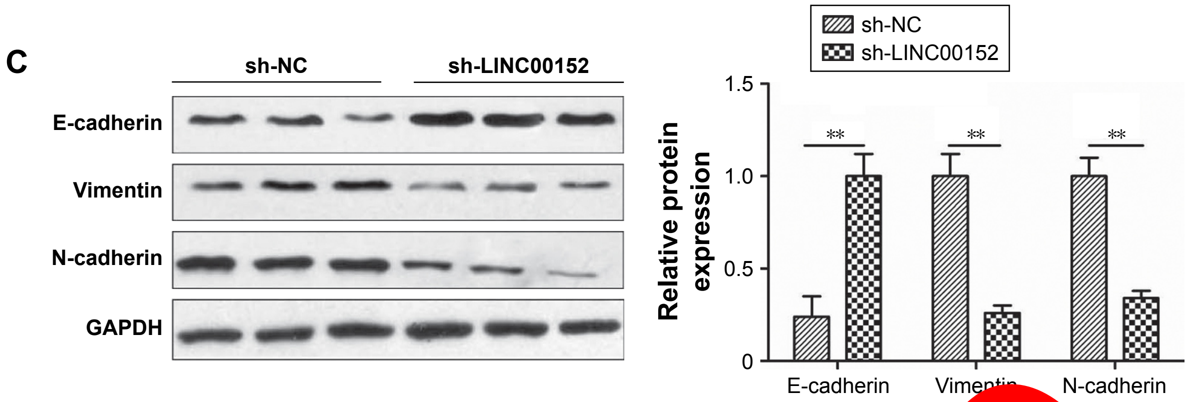

Figure 3 Knockdown of LINC00152 inhibits cell migration and invasion in OSCC cells.
Notes: (**A**) Cell migration was determined in SCC-9 cells transfected with sh-LINC00152 or sh-NC using a wound healing assay. (**B**) Cell invasion was determined in SCC-9 cells transfected with sh-LINC00152 or sh-NC using a transwell invasion assay. (**C**) Western blotting analysis of the levels of epithelial to mesenchymal transition-related proteins (N-cadherin, Vimentin, and E-cadherin) in SCC-9 cells transfected with sh-LINC00152 or sh-NC. $^{**}P<0.01$.
Abbreviations: OSCC, oral squamous cell carcinoma; sh-LINC00152, short-hairpin RNA targeting LINC00152; sh-NC, negative control shRNA.

group than in the sh-NC group (Figure 5B and C). In addition, we also found that the miR-139-5p expression level was increased in the sh-LINC00152 group compared with that in the miR-NC group (Figure 5D).

Discussion

LncRNAs have emerged as critical regulators of gene expression, and play crucial roles in tumorigenesis and progression of various cancers.[6,7] Several lncRNAs were reportedly involved in OSCC progression.[8,9] For example, Zhang et al found that LINC00668 promotes OSCC tumorigenesis via the miR-297/VEGFA axis.[22] Li et al reported that AC132217.4 significantly promotes cell migration and EMT by upregulating IGF2 expression.[23] Kong et al showed that LncRNA-FOXCUT could inhibit cell proliferation and cell migration in vitro, and was accompanied by a reduction in the

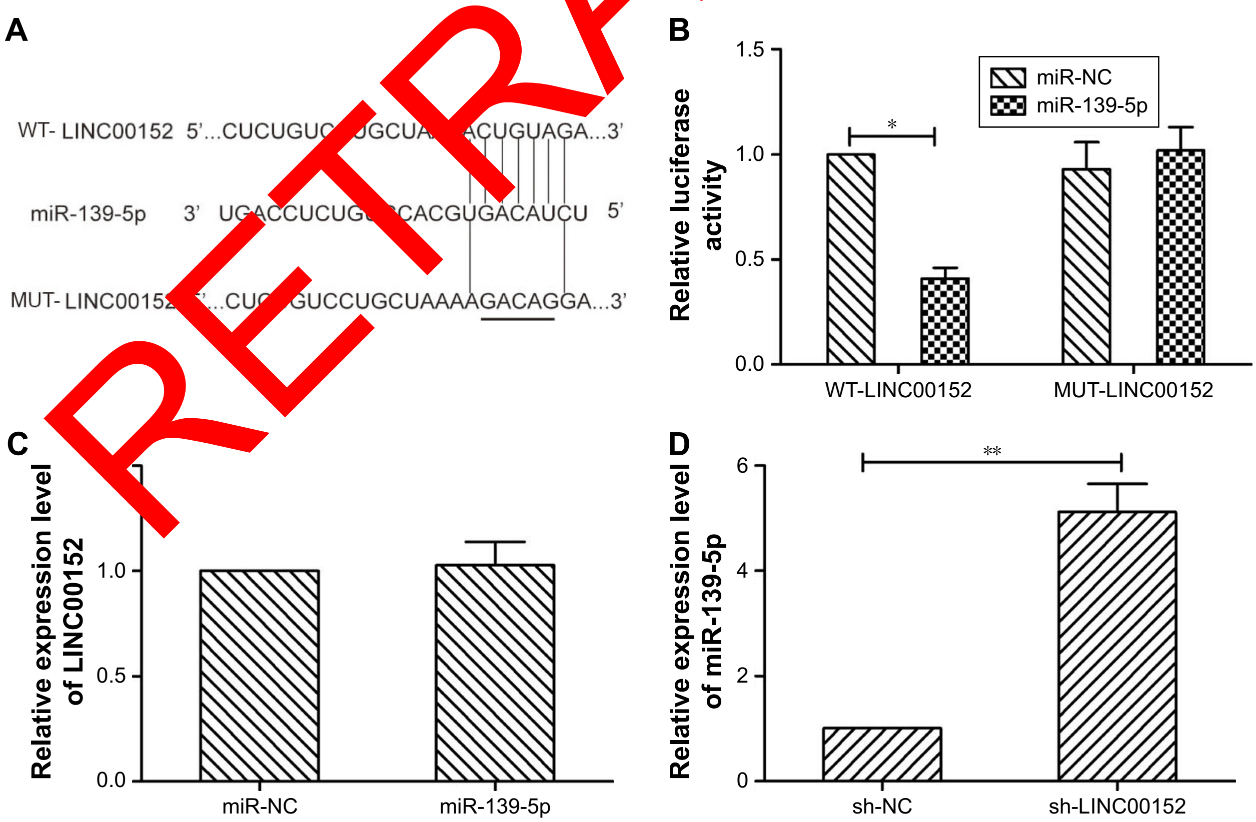

Figure 4 (*Continued*)

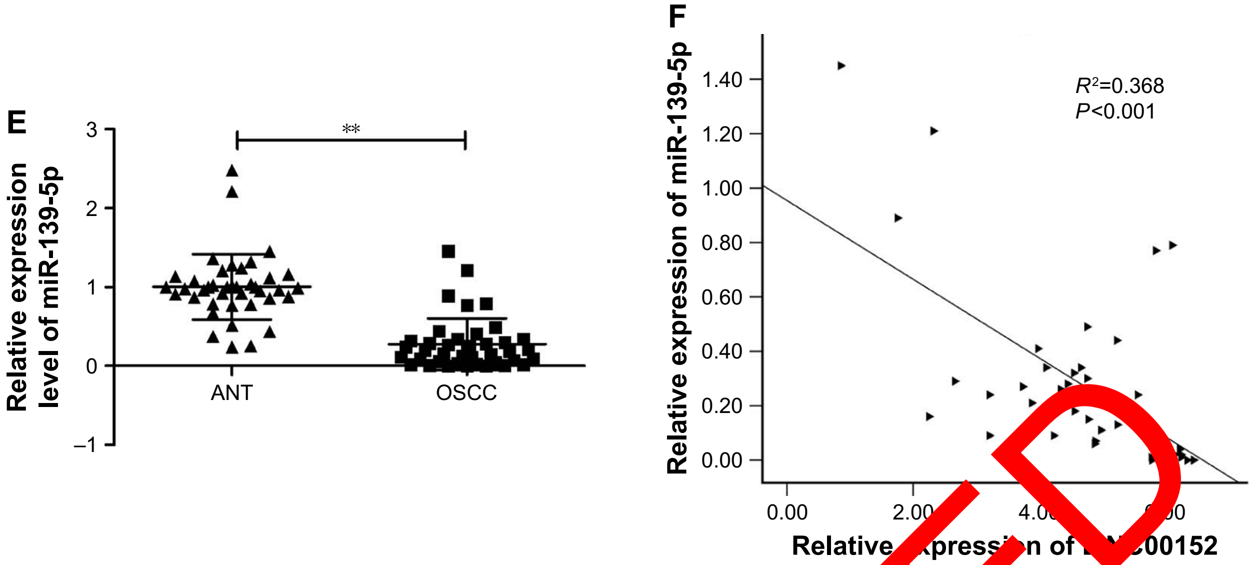

Figure 4 LINC00152 directly targets miR-139-5p in OSCC.
Notes: (**A**) Sequence alignment of miR-139-5p with the putative binding sites within the wild-type (WT) or mutant (MUT) region of LINC00152. (**B**) Luciferase activity was determined in SCC-9 cells co-transfected with WT-LINC00152 or MUT-LINC00152 reporter plasmids and miR-139-5p mimic or miR-NC (negative control). (**C**) LINC00152 expression detected using qRT-PCR in SCC-9 cells transfected with miR-139-5p mimic or miR-NC. (**D**) miR-139-5p expression levels detected using qRT-PCR in SCC-9 cells transfected with sh-LINC00152 or sh-NC. (**E**) miR-139-5p expression levels detected in OSCC tissues and adjacent normal tissues using qRT-PCR. (**F**) The correlation between LINC00152 mRNA and miR-139-5p expression in 40 OSCC tissues, as analyzed by Pearson correlation analysis. *P<0.05, **P<0.01.
Abbreviations: ANT, adjacent normal tissue; OSCC, oral squamous cell carcinoma; sh-LINC00152, short-hairpin RNA targeting LINC00152; sh-NC, negative control shRNA.

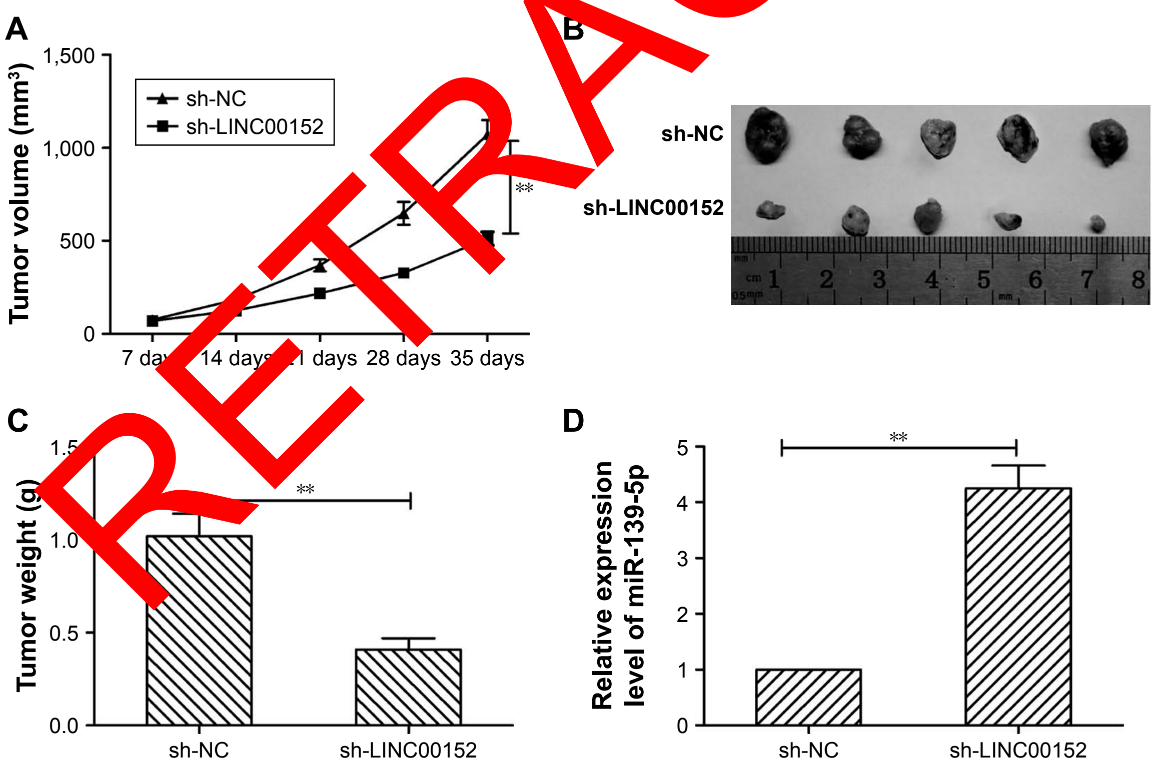

Figure 5 Knockdown of LINC00152 suppresses tumor growth in vivo.
Notes: (**A**) Tumor growth curve of the sh-LINC00152 and sh-NC groups. (**B**) Images of tumor tissues in the sh-LINC00152 and sh-NC groups. (**C**) The weight of the tumor tissues in the sh-LINC00152 and sh-NC groups. (**D**) miR-139-5p expression levels were detected in tumor tissues from sh-LINC00152 and sh-NC groups. **P<0.01.
Abbreviations: sh-LINC00152, short-hairpin RNA targeting LINC00152; sh-NC, negative control shRNA.

expression levels of MMP2, MMP7, MMP9, and VEGFA.[24] In the present study, we discovered that LINC00152 was expressed at a higher level in OSCC tissues than in the corresponding ANTs. Concurrently, LINC00152 levels were associated with TNM stage, lymph node metastasis, and reduced survival in patients with OSCC. We also demonstrated that knockdown of LINC00152 in OSCC cells significantly decreased their proliferation, colony formation, migration, and invasion abilities in vitro, as well as suppressing tumor growth in vivo. To the best our knowledge, this is the first study to show a crucial role for LINC00152 in OSCC tumorigenesis, suggesting that LINC00152 might be a potential therapeutic target for OSCC.

LINC00152, located on 2p11.2, has been reported to have oncogenic roles in gastric cancer,[19] lung cancer,[11,12] glioma,[13] hepatocellular carcinoma,[14] colorectal cancer,[15] gallbladder cancer,[16] and clear cell renal cell carcinoma.[18] However, the effects of LINC00152 on OSCC, and its underlying molecular mechanisms, remain unclear. In the present study, we investigated the functions of LINC00152 in OSCC in vitro and in vivo, and found that its expression was significantly increased in OSCC tissues and cell lines, which was consistent with the results of a previously study.[20] Moreover, transfection of sh-LINC00152 into SCC-9 cells significantly suppressed cell proliferation, colony formation, migration, and invasion in vitro; and inhibited tumor growth in vivo. Our results suggested that LINC00152 might act as an oncogene in OSCC.

EMT is the process that endows epithelial cells with mesenchymal properties, and is implicated in tumor migration and invasion.[25,26] Furthermore, metastatic cancer cells are closely related to EMT, which is characterized by downregulation of intercellular adhesion-related proteins (E-cadherin and occludins), upregulation of mesenchymal markers (Vimentin and N-cadherin), downregulation of epithelial markers (eg, cytokeratins), and the acquisition of a fibroblast-like (spindle) morphology with cytoskeletal reorganization.[27,28] Accumulating evidence shows that EMT plays an important role in OSCC invasion and metastasis.[29,30] Some lncRNAs are involved in mediating the EMT process in OSCC.[23,31] In this study, we found that LINC00152 knockdown suppressed the EMT process, as shown by the decreased levels of mesenchymal markers (N-cadherin and Vimentin) and the increased level of the epithelial marker, E-cadherin. Moreover, LINC00152 knockdown significantly inhibited OSCC migration and invasion. These results implied that LINC00152 knockdown inhibited OSCC cell migration and invasion, to some extent, by inhibiting the EMT process.

LncRNAs can function as ceRNA sponges for miRNAs to regulate the expression levels of the miRNA's target genes.[32] To investigate whether LNC00152 acts as an miRNA sponge, two online predictive software tools (Starbase v2.0 and miRanda) were used to determine possible interactions between miRNAs and LINC00152. Among the target miRNAs, miR-139-5p was of particular interest, because miR-139-5p was reported to be downregulated in OSCC tissues, and ectopic expression of miR-139-5p in OSCC cells significantly inhibited OSCC cell proliferation, migration, and invasion.[21] In the present study, we observed that miR-139-5p was expressed at a low level in OSCC tissues, and that LINC00152 levels were negatively correlated with miR-139-5p levels in OSCC samples. Moreover, a luciferase reporter assay suggested that miR-139-5p could bind to LINC00152 directly via the putative miRNA response element. Knockdown of LINC00152 significantly increased the level of miR-139-5p in OSCC cells. These results suggested that LINC00152 functions in OSCC, at least in part, by regulating miR-139-5p levels.

Conclusion

In summary, the present study provided evidence that LINC00152 is upregulated in OSCC tissues, and its expression is associated with TNM stage, lymph node metastasis, and poor survival of patients with OSCC. Knockdown of LINC00152 expression exerted tumor-suppressive effects by reducing cell proliferation, colony formation, migration, and invasion in vitro; and suppressing tumor growth in vivo. In addition, LINC00152 could target miR-139-5p to regulate its level in OSCC cells. These data suggested that LINC00152 might be an attractive therapeutic target to treat OSCC.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol*. 2009;45(4–5):309–316.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66(2):115–132.
3. Zini A, Czerninski R, Sgan-Cohen HD. Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. *J Oral Pathol Med*. 2010;39(4):299–305.
4. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol*. 2012;9(6):703–719.
5. Cheetham SW, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. *Br J Cancer*. 2013;108(12):2419–2425.
6. Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. *Mol Cancer*. 2011;10:38.

RETRACTED

7. Sun W, Yang Y, Xu C, Guo J. Regulatory mechanisms of long non-coding RNAs on gene expression in cancers. *Cancer Genet*. 2017;216–217:105–110.
8. Momen-Heravi F, Bala S. Emerging role of non-coding RNA in oral cancer. *Cell Signal*. 2018;42:134–143.
9. Yu T, Li C, Wang Z, et al. Non-coding RNAs deregulation in oral squamous cell carcinoma: advances and challenges. *Clin Transl Oncol*. 2016;18(5):427–436.
10. Yu Y, Yang J, Li Q, Xu B, Lian Y, Miao L. LINC00152: A pivotal oncogenic long non-coding RNA in human cancers. *Cell Prolif*. 2017;50(4):e12349.
11. Zhang PP, Wang YQ, Weng WW, et al. Linc00152 promotes Cancer Cell Proliferation and Invasion and Predicts Poor Prognosis in Lung adenocarcinoma. *J Cancer*. 2017;8(11):2042–2050.
12. Zhang Y, Xiang C, Wang Y, et al. lncRNA LINC00152 knockdown had effects to suppress biological activity of lung cancer via EGFR/PI3K/AKT pathway. *Biomed Pharmacother*. 2017;94:644–651.
13. Yu M, Xue Y, Zheng J, et al. Linc00152 promotes malignant progression of glioma stem cells by regulating miR-103a-3p/FEZF1/CDC25A pathway. *Mol Cancer*. 2017;16(1):110.
14. Deng X, Zhao XF, Liang XQ, Chen R, Pan YF, Liang J. Linc00152 promotes cancer progression in hepatitis B virus-associated hepatocellular carcinoma. *Biomed Pharmacother*. 2017;90:100–108.
15. Zhang YH, Fu J, Zhang ZJ, Ge CC, Yi Y. LncRNA-LINC00152 down-regulated by miR-376c-3p restricts viability and promotes apoptosis of colorectal cancer cells. *Am J Transl Res*. 2016;8(12):5286–5297.
16. Cai Q, Wang Z, Wang S, et al. Long non-coding RNA LINC00152 promotes gallbladder cancer metastasis and epithelial-mesenchymal transition by regulating HIF-1α via miR-138. *Open Biol*. 2017;7(1):160247.
17. Yang T, Zeng H, Chen W, et al. Helicobacter pylori infection, H19 and LINC00152 expression in serum and risk of gastric cancer in a Chinese population. *Cancer Epidemiol*. 2016;44:147–153.
18. Wu Y, Tan C, Weng WW, et al. Long non-coding RNA Linc001[illegible] is a positive prognostic factor for and demonstrates malignant bio[illegible] logical behavior in clear cell renal cell carcinoma. *Am J C[illegible]cer Res*. 2016;6(2):285–299.
19. Zhao J, Liu Y, Zhang W, et al. Long non-coding [illegible]A Linc0[illegible]52 is involved in cell cycle arrest, apoptosis, epithelial t[illegible]esench[illegible] sition, cell migration and invasion in gastric [illegible]ncer. [illegible]cle. 2015;14(19):3112–3123.
20. Yu J, Liu Y, Guo C, et al. Upregulated long non-coding RNA LINC00152 expression is associated with progression and poor prognosis of tongue squamous cell carcinoma. *J Cancer*. 2017;8(4):523–530.
21. Wang K, Jin J, Ma T, Zhai H. MiR-139-5p inhibits the tumorigenesis and progression of oral squamous carcinoma cells by targeting HOXA9. *J Cell Mol Med*. 2017;21(12):3730–3740.
22. Zhang CZ. Long intergenic non-coding RNA 668 regulates VEGFA signaling through inhibition of miR-297 in oral squamous cell carcinoma. *Biochem Biophys Res Commun*. 2017;489(4):404–412.
23. Li X, Ma C, Zhang L, et al. LncRNAAC132217.4, a KLF8-regulated long non-coding RNA, facilitates oral squamous cell carcinoma metastasis by upregulating IGF2 expression. *Cancer Lett*. 2017;407:45–56.
24. Kong XP, Yao J, Luo W, et al. The expression and functional role of a FOXC1 related mRNA-lncRNA pair in oral s[illegible]ous cell carcinoma. *Mol Cell Biochem*. 2014;394(1–2):177–1[illegible].
25. Singh M, Yelle N, Venugopal C, Sin[illegible] SK. EMT: M[illegible]hanisms and therapeutic implications. *Pharmacol T[illegible]*. 2017;182:80[illegible]4.
26. Amawi H, Ashby CR, Samuel [illegible] Peram[illegible] R, Tiwari [illegible]K. Polyphenolic Nutrients in Cancer C[illegible]emopreventi[illegible] and M[illegible]astasis: Role of the Epithelial-to-Mese[illegible]hymal (E[illegible]T) Path[illegible] *Nutrients*. 2017;9(8):911.
27. Dominguez C, Davi[illegible]M, Pa[illegible]. Epitheli[illegible]mesenchymal transition and inflammati[illegible]t the site of [illegible] primar[illegible]umor. *Semin Cancer Biol*. 2017;47:177[illegible]
28. Jiang ZS, [illegible]n YZ, [illegible]g SM, Ruan [illegible]S. Epithelial-mesenchymal transition: potential regula[illegible] of ABC transporters in tumor progression. *J C[illegible]cer*. [illegible]017;8(12):231[illegible]327.
29. [illegible]el S, Shah K, Mirza S, Daga A, Rawal R. Epigenetic regulators [illegible]verning canc[illegible]stem cells and epithelial-mesenchymal transition [illegible] oral squamou[illegible]ell carcinoma. *Curr Stem Cell Res Ther*. 2015;10[illegible]:140–152.
30. Yanji[illegible]un J. The role of epithelial-mesenchymal transition in [illegible]ral squamous cell carcinoma and oral submucous fibrosis. *Clin Chim [illegible]* [illegible]07;383(1–2):51–56.
31. Zhu G, Wang S, Chen J, et al. Long noncoding RNA HAS2-AS1 mediates hypoxia-induced invasiveness of oral squamous cell carcinoma. *Mol Carcinog*. 2017;56(10):2210–2222.
32. Zheng Y, Liu L, Shukla GC. A comprehensive review of web-based non-coding RNA resources for cancer research. *Cancer Lett*. 2017;407:1–8.