

# lncRNA SOX2-OT regulates laryngeal cancer cell proliferation, migration and invasion and induces apoptosis by suppressing miR-654

GUANG LI<sup>1-3</sup>, CHUNCHEN PAN<sup>3</sup>, JIAQIANG SUN<sup>3</sup>, GUANGLUN WAN<sup>3</sup> and JINGWU SUN<sup>1,3</sup>

<sup>1</sup>School of Medicine, Shandong University, Jinan, Shandong 250012;

<sup>2</sup>Department of Otorhinolaryngology-Head and Neck Surgery, The Affiliated Hospital of Yangzhou University, Yangzhou, Jiangsu 225001; <sup>3</sup>Department of Otorhinolaryngology-Head and Neck Surgery,

The First Affiliated Hospital of University of Science and Technology of China, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui 230001, P.R. China

Received June 4, 2019; Accepted January 13, 2020

DOI: 10.3892/etm.2020.8577

**Abstract.** Laryngeal carcinoma is the most common type of malignant tumor in the head and neck. Long non-coding RNAs (lncRNAs) serve crucial roles in numerous biological processes. The present study aimed to investigate the role of lncRNA SOX2-OT in laryngeal cancer and to reveal the underlying mechanisms. Reverse transcription-quantitative PCR assays were used to measure the expression levels of SOX2-OT in the laryngeal cell lines. Furthermore, cell proliferation, apoptosis, migration and invasion were assessed by CCK-8, flow cytometry, wound healing and Transwell assays, respectively. Western blot assay was performed to detect the protein expressions. In addition, a dual-luciferase reporter assay was performed to confirm the direct interaction between SOX2-OT and microRNA (miR)-654. The data demonstrated that SOX2-OT level were significantly increased in the laryngeal cell lines. Furthermore, SOX2-OT silencing markedly promoted apoptosis and suppressed the proliferation, migration and invasion of TU-177 cells. A dual-luciferase reporter assay revealed that miR-654 was a direct target of SOX2-OT. Moreover, downregulation of miR-654 could attenuate cell apoptosis and accelerate cell proliferation, migration and invasion in TU-177 cells. In summary, the present study reported that knockdown of SOX2-OT could suppress cell proliferation, migration and invasion, and induce apoptosis in laryngeal cancer by targeting miR-654.

## Introduction

With changes in diet and lifestyle, the incidence of laryngeal cancer has increased in recent years. According to the statistical data presented in GLOBOCAN in 2018, the number of new cases of laryngeal cancer reached 177,422, accounting for 1% of all new cancer cases worldwide (1-3). As the most common type of malignant tumor in the head and neck, laryngeal cancer has become a worldwide problem (4). Although there have been considerable developments in treatment options in recent years, there remains a number of problems and the prognosis of patients has not reached what is desired (5,6). Therefore, it is of great significance to investigate the potential molecular mechanisms and provide new therapeutic targets for the clinical treatment of laryngeal cancer.

Long non-coding RNAs (lncRNAs) have attracted much attention due to their extensive biological activities (7). lncRNAs exhibit tissue specificity and functional diversity, which makes them potentially effective diagnostic and prognostic markers (8). Recent studies have revealed that lncRNA SOX2-OT functions as an oncogenic factor and serves an important role in numerous types of cancer, including osteosarcoma, cholangiocarcinoma and lung squamous cell carcinoma (9-11). Notably, using microarrays, Feng *et al* (12) identified that the expression of SOX2-OT in cancer tissues was significantly higher compared with that in adjacent non-neoplastic tissues in advanced laryngeal squamous cell carcinoma (LSCC). Furthermore, Tai *et al* (13) suggested that SOX2-OT promotes the development of LSCC through silencing of phosphatase and tensin homolog, which is induced by the methyltransferase EZH2. These studies suggest that SOX2-OT is closely associated with the development of laryngeal cancer. However, the underlying mechanism by which SOX2-OT functions remains unclear in laryngeal cancer.

MicroRNAs (miRNAs) are composed of endogenous non-coding small RNAs that can regulate mRNA stability and protein translation (14). It has been proved that miRNAs play take part in the development of various cancer processes, such as proliferation, differentiation and metastasis (15). miR-654

---

*Correspondence to:* Dr Jingwu Sun, Department of Otorhinolaryngology-Head and Neck Surgery, The First Affiliated Hospital of University of Science and Technology of China, Division of Life Sciences and Medicine, University of Science and Technology of China, 17 Lujiang Road, Hefei, Anhui 230001, P.R. China  
E-mail: entsunjingwu@hotmail.com

**Key words:** SOX2-OT, laryngeal cancer, TU-177, microRNA-654

was found to be abnormally expressed in many squamous cell carcinoma including laryngeal squamous cell carcinoma (16). Nonetheless, the biological role of miR-654 in laryngeal squamous cell carcinoma is still unclear.

The present study aimed to investigate whether SOX2-OT is involved in the development of laryngeal cancer by regulating microRNA (miR)-654. It was identified that the expression of SOX2-OT is significantly increased in laryngeal cancer cells. In order to evaluate the potential function of SOX2-OT, RNA interference was applied to knockdown the expression level of SOX2-OT, and further experiments were conducted to identify the association between SOX2-OT and miR-654 in TU-177 cells.

## Materials and methods

**Cell culture and treatment.** All cell lines, including the normal human nasopharyngeal epithelial cell line NP69 and laryngeal cancer cell lines TU-177, M4E, AMC-HN-8 and TU686, were purchased from the American Type Culture Collection. Cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) in an incubator containing 95% air and 5% CO<sub>2</sub> at a constant temperature of 37°C.

**Cell transfection.** The short hairpin RNA (shRNA) sequence targeting SOX2-OT (shRNA-SOX2-OT-1/2), the negative control (shRNA-NC), the miR-654 inhibitor, inhibitor NC (miR-NC), miR-654 mimic and mimic NC (miR-654 NC) were designed and synthesized by Shanghai GenePharma Co., Ltd. The shRNA-SOX2-OT-1 sequence was GCACCGCTATACAGAGAAACCTTATCCTCGAGGATAAGGTTTCTCTGTATAGCTTTTTTG, the shRNA-SOX2-OT-2 sequence was GCACCGGAGCAAAGGTGCTGTCATTTCTCGAGAAATGACAGCACCTTTTGCTCCTTTTTG, the shRNA-NC sequence was CGCGTCCCCCACCTTTTCGGCACTCTCCCTTCAAGAGGGGAGAGTGCCGAAAGGTGTTTTTGGAAAT, The miR-654 inhibitor sequence was 5' ACACAUGUUCUGCGGCCACCA 3', the negative control (miR-NC) sequence was 5' CAGUACUUUUGUGUAGUACAA 3', the miR-654 mimic sequence was 5' UGGUGGGCCGCAGAACAUUGUC 3' and the miR-654 NC sequence was 5' UUGUACUACACAAAAGUACUG 3'. TU-177 cells were seeded in six-well plates at a density of  $3 \times 10^5$ /well and incubated for 24 h. Subsequently, TU-177 cells were transfected with 100 pmol shRNA-SOX2-OT-1/2 or shRNA-NC with or without 100 nM miR-654 mimic, miR-654 inhibitor or corresponding controls using Lipofectamine<sup>®</sup> 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). At 48 h after transfection, cells were harvested for further experiments.

**Reverse transcription-quantitative PCR (RT-qPCR).** TU-177 cells were lysed and total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc.). For the mRNAs, complementary DNA (cDNA) was synthesized using the Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics). For miR-654, cDNA was synthesized using specific stem-loop primers combined with TaqMan MicroRNA Reverse Transcription kit (Takara Bio, Inc.). The reverse transcription reaction was performed at 42°C for 5 min and 95°C

for 10 sec. qPCR was then performed using the SYBR Green kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) in the ABI 7500 Real-time PCR system (Applied Biosystems). The thermocycling conditions were as follows: One cycle of 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. GAPDH and U6 were used as the internal controls for SOX2-OT and miR-654, respectively. Primer sequences were exhibited at Table I.

**Cell Counting Kit-8 (CCK-8).** TU-177 cells were seeded in 96-well plates at a density of  $2 \times 10^4$ /well and incubated for 24 h. Subsequently, the shRNAs were transfected into TU-177 cells. At 24, 48 and 72 h after transfection, cell proliferation was evaluated with a CCK-8 kit (catalog no. C0038; Beyotime Institute of Biotechnology), according to manufacturer's instructions. The absorbance value of each well was measured at 450 nm.

**Western blot assay.** The western blot assay was performed as previously described (17). Briefly, total protein was extracted from TU-177 cells using RIPA lysis buffer (Beyotime Institute of Biotechnology). Following separation by 10% SDS-PAGE, protein samples were transferred onto a PVDF membrane (EMD Millipore). After blockage in 10% non-fat milk for 1 h at 37°C, the membranes were incubated with primary antibodies (p21, 1:500, cat. no. 64016, Cell Signaling Technology; CDK2, 1:1,000, cat. no. 2546, Cell Signaling Technology; cyclin E1, 1:500, cat. no. sc-377100, Santa Cruz Biotechnology; MMP-7, 1:500, cat. no. 3801, Cell Signaling Technology; MMP-9, 1:1,000, cat. no. 3667, Cell Signaling Technology; Bcl-2, 1:1,000, cat. no. 15071, Cell Signaling Technology; Bax, 1:1,000, cat. no. 14796, Cell Signaling Technology; cleaved caspase 3, 1:1,000, cat. no. 9661, Cell Signaling Technology; GAPDH, 1:1,000, cat. no. MAB374, EMD Millipore) overnight at 4°C. Subsequently, the protein bands were probed with rabbit anti-mouse IgG-HRP (1:10,000, cat. no. sc-358914) or mouse anti-rabbit IgG-HRP secondary antibodies (1:10,000, cat. no. sc-2357; both Santa Cruz Biotechnology) for 2 h at room temperature. Finally, the ECL detection reagent (EMD Millipore) was used to visualize the chemiluminescent signals. ImageJ software (version 1.8, NIH) was used to quantify the protein bands.

**Transwell assay.** Cell invasion was assessed using a Transwell assay. During this assay, the inserts of the Transwell chamber (Corning Inc.) were filled with Matrigel (BD Biosciences). At 48 h post-transfection, TU-177 cells ( $5 \times 10^3$  cells/ml) were plated in the upper chambers with serum-free RPMI-1640 medium. In addition, complete DMEM containing 10% FBS was added to the lower chamber as a chemoattractant. Following incubation for 24 h, cells that migrated through the membrane were fixed with 4% formaldehyde at room temperature. Finally, following staining with 0.1% crystal violet, the invaded cells were counted with a light microscope (Nikon Corporation) at x200 magnification.

**Wound healing.** TU-177 cells were seeded in six-well plates at a density of  $3 \times 10^5$ /well and allowed to growth to 90% confluence in DMEM with 10% FBS at 37°C. A 10- $\mu$ l sterile pipette tip was used to generate a wound, and an inverted microscope

Table I. PCR primer sequences.

Primers (5'-3')	
SOX2-OT forward	5'-TTGGAAGGATGGCATACAC-3'
SOX2-OT reverse	5'-CAATGAAGTTGACTGGACTC-3'
GAPDH forward	5'-GGAGCGAGATCCCTCCAAAAT-3'
GAPDH reverse	5'-GGCTGTTGTCATACTTCTCATGG-3'
miR-654 forward	5'-TGGTGGGCCGAGAACATGTGC-3'
miR-654 reverse	5'-GCGAGCACAGAATTAATACGAC-3'
U6 forward	5'-CGCTTCGGCAGCACATATACTA-3'
U6 reverse	5'-CGCTTCACGAATTGCGTGTCA-3'
miR, microRNA.	

was used to monitor the migration of the cells at 0 and 24 h. The widths of the wounds were measured to assess the cell migration.

**Cell apoptosis.** At 48 h post-transfection, TU-177 cells were harvested and an Annexin V-FITC/PI Apoptosis kit (catalog no. KA3805; Abnova) was used to assess apoptosis. In brief, cells were suspended in 100  $\mu$ l 1X binding buffer. Following staining with 5  $\mu$ l Annexin V-FITC for 10 min, cells were stained with 5  $\mu$ l PI for a further 5 min in the dark at room temperature. Apoptotic cells were analyzed using a flow cytometer (FACScan; BD Biosciences).

**Dual-luciferase reporter assay.** To verify the direct interactions between SOX2-OT and miR-654, a dual-luciferase reporter assay was performed. Briefly, the mutant type and wild type of SOX2-OT binding sequences were cloned into a pGL3-promoter (Promega Corporation) to generate the recombinant vectors pGL3-SOX2-OT-WT and pGL3-SOX2-OT-MUT. Following seeding onto 24-well plates for 24 h, TU-177 cells were co-transfected with 50 ng pGL3-SOX2-OT-WT/MUT and 20  $\mu$ M miR-654 mimic/NC using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). At 48 h after transfection, the luciferase activity of each well was determined using the Dual-Luciferase Reporter assay system (Promega Corporation), and the results were normalized to the Renilla luciferase activity.

**Statistical analysis.** In the present study, each experiment was repeated a minimum of three times and data are presented as the mean  $\pm$  standard deviation. Statistical analysis was performed using SPSS version 20.0. Statistical analysis were performed using Student's t-test and one-way analysis of variance, followed by a Dunnett's post hoc test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Upregulation of SOX2-OT in laryngeal cancer cell lines.** To evaluate the expression levels of SOX2-OT in laryngeal cancer cell lines, an RT-qPCR assay was performed. As presented in Fig. 1A, the levels of SOX2-OT were significantly increased in the laryngeal cell lines TU-177, AMC-HN-8 and TU686, especially in TU-177 cells, compared with the normal cell line NP69. However, no significant difference in SOX2-OT mRNA expression between NP69 and M4E was observed. With this in mind, TU-177 cells were selected for the following experiments. In order to further investigate the biological function of SOX2-OT, shRNA-SOX2-OT-1/2 or shRNA-NC were transfected into TU-177 cells. At 48 h after transfection, the interference effect was detected, and the results demonstrated that expression of SOX2-OT was markedly decreased by both shRNA-SOX2-OT-1 and -2 transfection (Fig. 1B). Moreover, shRNA-SOX2-OT-1 was selected for subsequent experiments as it exhibited a better interference effect.

**Knockdown of SOX2-OT suppresses the proliferation of TU-177 cells.** CCK-8 assay was performed to assess the effect of SOX2-OT-knockdown on cell proliferation in TU-177 cells. SOX2-OT-silencing markedly suppressed cell proliferation in TU-177 cells (Fig. 1A). Additionally, cell proliferation following shRNA-SOX2-OT-1 transfection was assessed using western blotting. The results demonstrated that SOX2-OT-silencing significantly upregulated the expression of p21, and downregulated the expression of CDK2, cyclin E1 and PCNA (Fig. 2B and C).

**SOX2-OT-knockdown inhibits migration and invasion in TU-177 cells.** Migration and invasion are considered to be key factors in the development of cancer. Therefore, wound healing and transwell assays were used to measure the effects of SOX2-OT on migration and invasion, respectively, in TU-177 cells. The results indicated that both the migration and invasion of TU-177 cells were inhibited following SOX2-OT-knockdown (Fig. 3A and B). Furthermore, western blot analysis was used to determine the protein expression levels of MMP-7 and MMP-9. As presented in Fig. 3C, SOX2-OT-knockdown significantly reduced the protein levels of MMP-7 and MMP-9.

**SOX2-OT-knockdown induces apoptosis in TU-177 cells.** To investigate whether the silencing of SOX2-OT has an effect on the apoptosis of TU-177 cells, annexin V-FITC/PI staining combined with flow cytometry was performed. According to the results, the percentage of the apoptotic cell fraction was significantly increased in shRNA-SOX2-OT-1-treated TU-177 cells (Fig. 4A). In addition, the results of western blot assay revealed that SOX2-OT-knockdown markedly reduced the expression of Bcl-2 protein, and the protein levels of Bax and cleaved caspase 3 were significantly increased in shRNA-SOX2-OT-1-transfected TU-177 cells (Fig. 4B).

**miR-654 serves as a direct target of SOX2-OT.** To assess the role of miR-654 in TU-177 cells, we constructed miR-654 mimic and found that miR-654 expression in TU-177 cells was significantly increased after transfection with miR-654

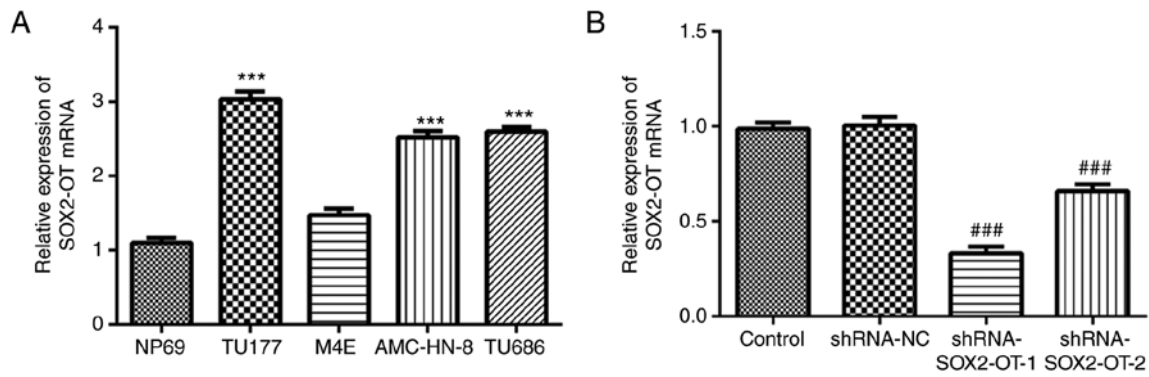


Figure 1. The expression of SOX2-OT in laryngeal cancer cell lines. (A) The relative expression of SOX2-OT in laryngeal cell lines and the normal cell line was measured using RT-qPCR. (B) The expression of SOX2-OT in TU-177 cells transfected with shRNA-NC or shRNA-SOX2-OT-1 was detected using RT-qPCR. GAPDH was used as an internal reference. Data are expressed as mean  $\pm$  standard deviation. \*\*\* $P$ <0.001 vs. NP69 group; ### $P$ <0.001 vs. shRNA-NC. RT-q, reverse transcription-quantitative; sh, short-hairpin; NC, negative control.

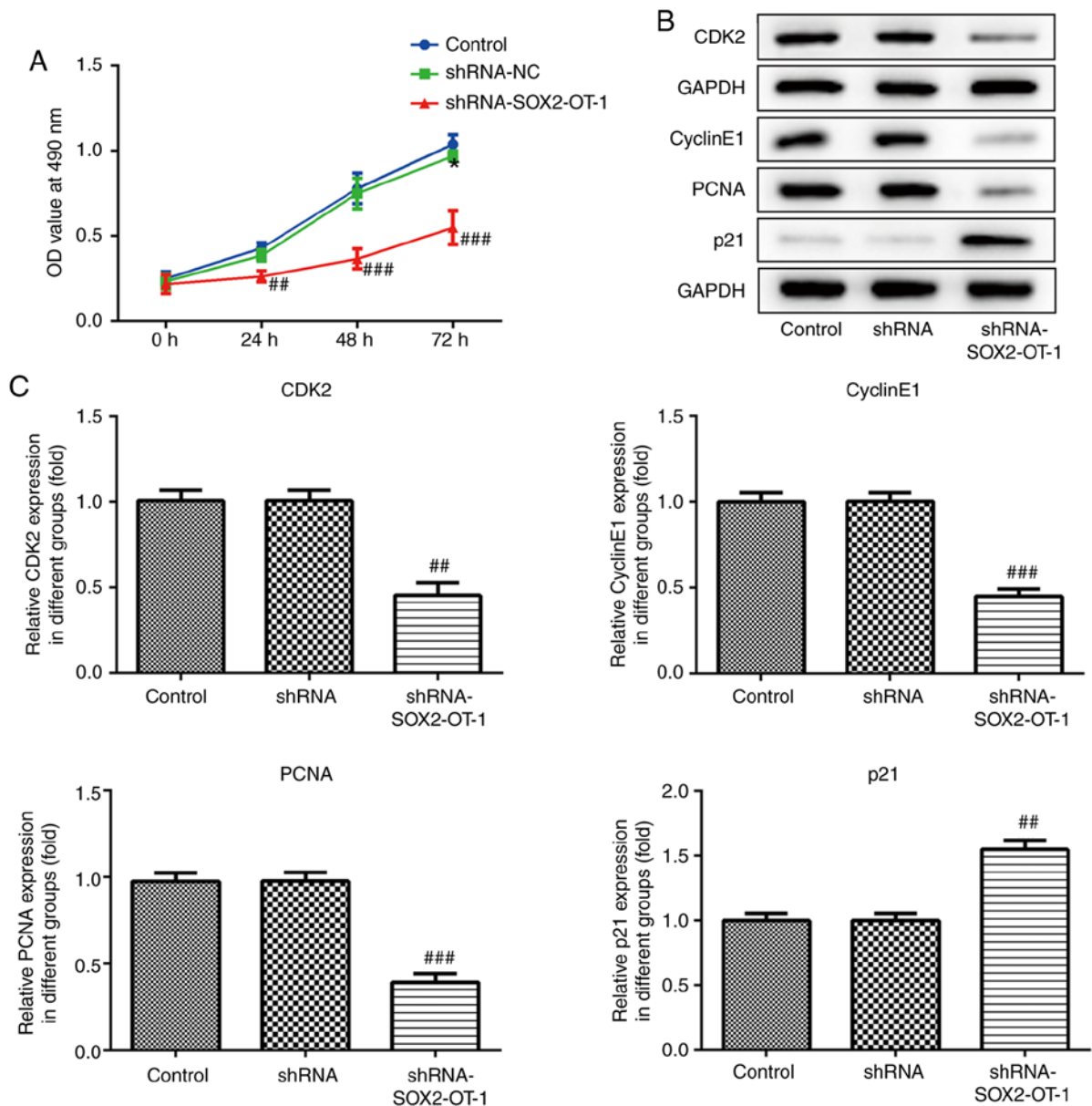


Figure 2. The effect of SOX2-OT knockdown on cell proliferation. (A) Cell counting kit-8 assay was used in TU-177 cells after transfection with shRNA-NC or shRNA-SOX2-OT-1 to determine cell proliferation. (B) Western blot analysis was performed to evaluate the expression of cyclin-dependent kinase 2, cyclinE1, proliferating cell nuclear antigen and p21. GAPDH was used as an internal reference. (C) Densitometric analysis of western blot analysis. Data are expressed as mean  $\pm$  standard deviation. ## $P$ <0.01; ### $P$ <0.001 vs. shRNA-NC. sh, short-hairpin; NC, negative control; OD, optical density.



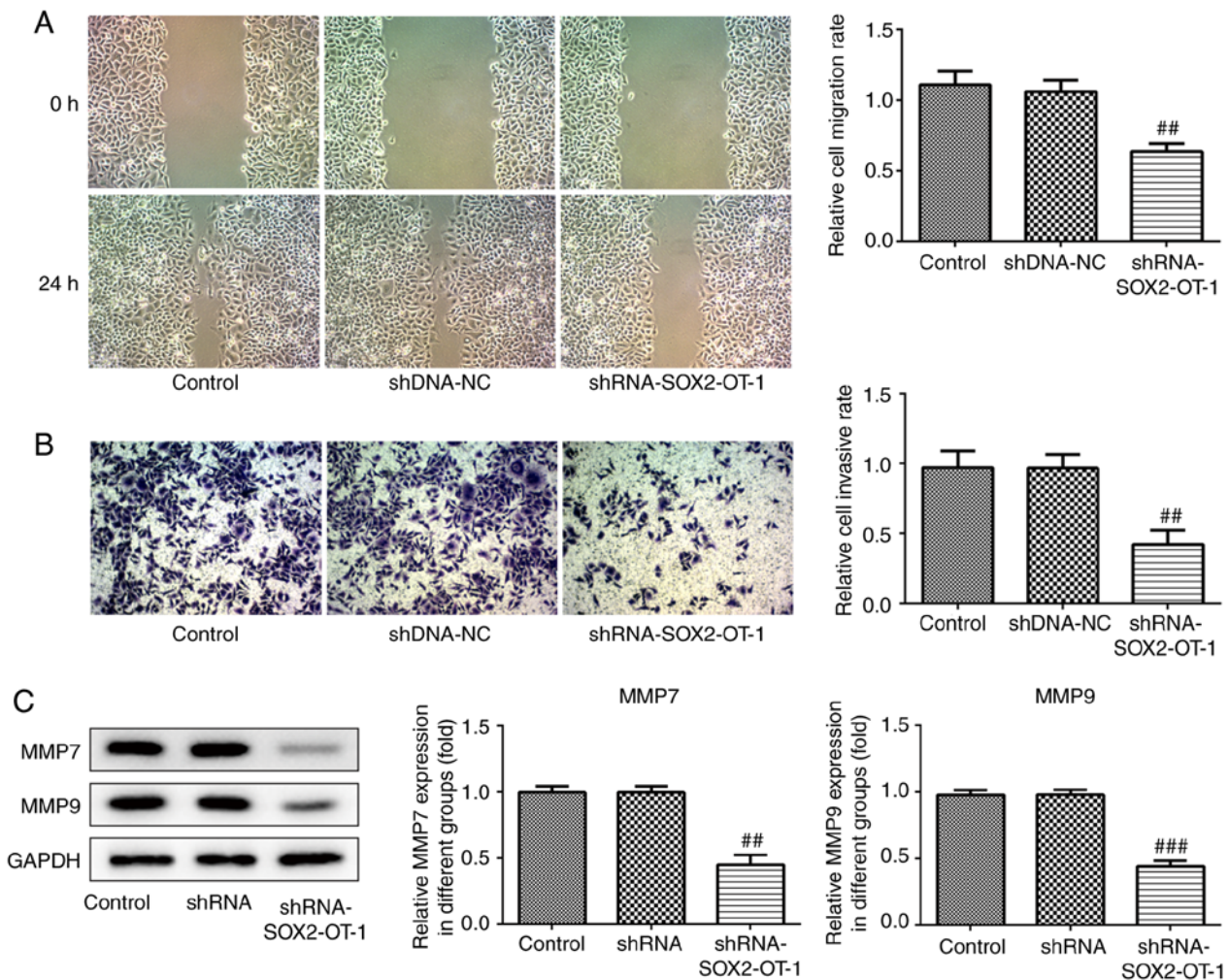


Figure 3. The effect of SOX2-OT knockdown on cell migration and invasion. (A) Cell migration was evaluated by wound healing assay in TU-177 cells transfected with shRNA-NC or shRNA-SOX2-OT-1. (B) Transwell assay was carried out to detect cell invasion in TU-177 cells transfected with shRNA-NC or shRNA-SOX2-OT-1. (C) Western blot analysis was performed to evaluate the expression of MMP-7 and MMP-9. GAPDH was used as an internal reference. Data are expressed as mean  $\pm$  standard deviation. <sup>##</sup>P<0.01; <sup>###</sup>P<0.001 vs. shRNA-NC. sh, short-hairpin; NC, negative control; MMP, Matrix metalloproteinase.

mimic (Fig. 5A). As presented in Fig. 5B and C, treatment with miR-654 mimic markedly decreased the luciferase activity in pGL3-SOX2-OT-WT-transfected cells. However, no significant effect was identified for the cells transfected with pGL3-SOX2-OT-MUT. Furthermore, RT-qPCR results demonstrated that SOX2-OT-silencing promoted the expression level of miR-654 in TU-177 cells when compared with the control, suggesting that miR-654 is a direct target of SOX2-OT (Fig. 5D).

*miR-654 is responsible for the effects of SOX2-OT-silencing in TU-177 cells.* To further investigate the role of miR-654 in SOX2-OT-silenced laryngeal cancer cells, miR-654 inhibitor was transfected to TU-177 cells and the effect was detected (Fig. 6A). As presented in Fig. 6B, the expression level of miR-654 was significantly reduced in miR-654 inhibitor-transfected TU-177 cells. Moreover, the results of CCK-8 assay demonstrated that downregulation of miR-654 significantly promoted cell proliferation at 24, 48 and 72 h post-transfection (Fig. 6C). Additionally, downregulation of miR-654 markedly inhibited the apoptosis induced by SOX2-OT-knockdown in TU-177 cells (Fig. 6D). In addition, miR-654 silencing significantly reduced the expression of Bax and cleaved

caspase 3 while increased Bcl-2 level compared with the shRNA-SOX2-OT-1-transfected TU-177 cells (Fig. 6E).

## Discussion

lncRNAs are involved in numerous physiological processes, including cell growth, proliferation, apoptosis, migration and differentiation (17-19). The normal expression of lncRNA is necessary to maintain cell homeostasis and function, and its aberrant expression is closely associated with a variety of diseases, including cancer (20,21). The present study reported that the expression level of SOX2-OT was significantly increased in the human laryngeal cancer cell lines TU-177, M4E, AMC-HN-8 and TU686 compared with the normal nasopharyngeal epithelial cell line NP69. Consistent with the present results, Feng *et al* (12) and Tai *et al* (13) revealed that the level of SOX2-OT is markedly upregulated in laryngeal cancer tissues compared with the adjacent tissues. These results suggest that SOX2-OT may play an important role in the occurrence and development of laryngeal carcinoma. In order to further investigate the potential role of SOX2-OT in laryngeal cancer and to reveal the underlying molecular mechanisms,

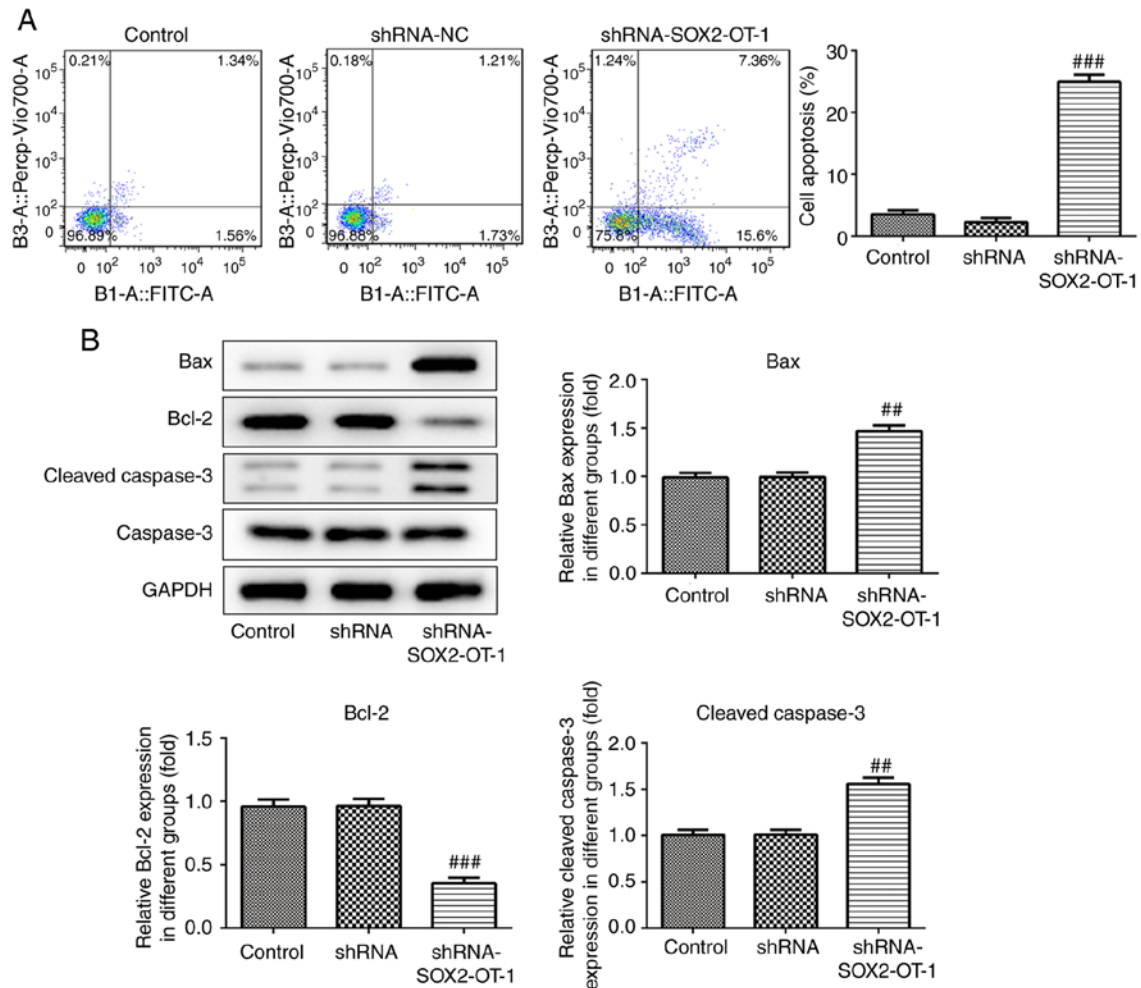


Figure 4. The effect of SOX2-OT knockdown on cell apoptosis. (A) Flow cytometry was performed in TU-177 cells after being transfected with shRNA-NC or shRNA-SOX2-OT-1 to explore cell apoptosis. (B) Western blot analysis was used to detect the protein expression level of Bcl-2, Bax and cleaved caspase3 in TU-177 cells transfected with shRNA-NC or shRNA-SOX2-OT-1. GAPDH was used as an internal reference. Data are expressed as mean  $\pm$  standard deviation. \*\* $P$ <0.01; \*\*\* $P$ <0.001 vs. shRNA-NC. sh, short-hairpin; NC, negative control.

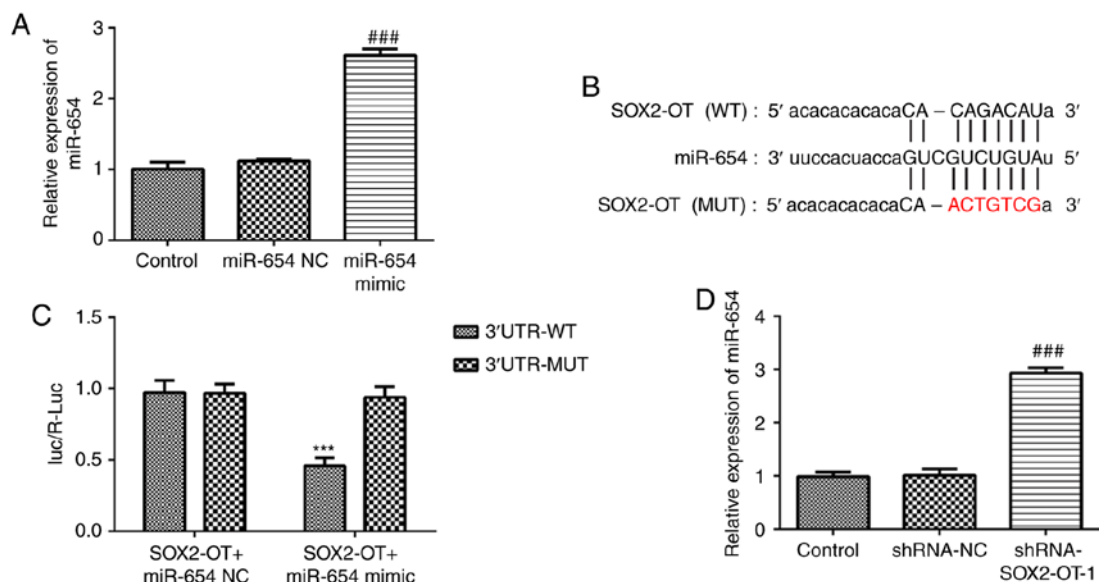


Figure 5. SOX2-OT targets miR-654 in TU-177 cells. (A) The expression of miR-654 in TU-177 cells transfected with miR-654 mimic or negative control. (B) The predictive miR-654 binding sequences in SOX2-OT. (C) Luciferase reporter assay was applied to determine the luciferase activity of TU-177 cells co-transfected with miRNAs and luciferase reporter vectors containing wild-type or mutant SOX2-OT 3'untranslated region. (D) Reverse transcription-quantitative PCR was performed to evaluate the expression of miR-654 in TU-177 cells transfected with shRNA-NC or shRNA-SOX2-OT-1. Data are expressed as mean  $\pm$  standard deviation. \*\*\* $P$ <0.001; \*\*\*\* $P$ <0.001 vs. shRNA-NC. sh, short-hairpin; NC, negative control; miR, microRNA; WT, wild-type; MUT, mutant.

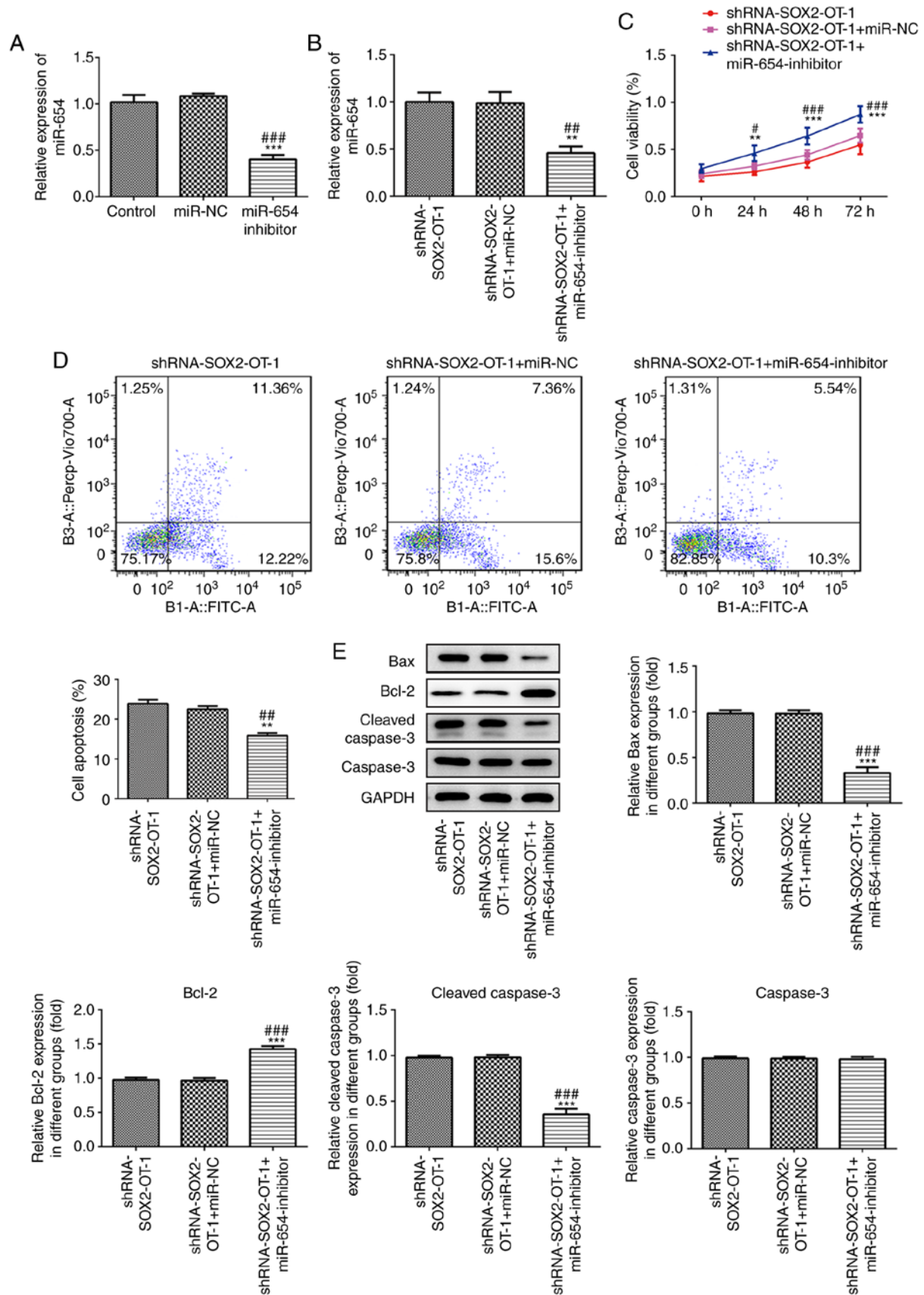


Figure 6. The effects of miR-654 on SOX2-OT silence in TU-177 cells. (A) The expression of miR-654 in TU-177 cells transfected with miR-654 inhibitor or negative control. (B) The relative expression level of miR-654 was assessed using reverse transcription-quantitative PCR in TU-177 cells co-transfected with miR-654 inhibitor or miR-NC and shRNA-SOX2-OT-1. (C) Cell counting kit-8 assay was used in TU-177 cells co-transfected with miR-654 inhibitor or miR-NC and shRNA-SOX2-OT-1 to determine cell viability. (D) Flow cytometry was performed in TU-177 cells co-transfected with miR-654 inhibitor or miR-NC and shRNA-SOX2-OT-1 to explore cell apoptosis. (E) Western blot analysis was performed to measure the protein level of Bcl-2, Bax and cleaved caspase-3 in TU-177 cells co-transfected with shRNA-SOX2-OT-1 and miR-654 inhibitor. Data are expressed as mean  $\pm$  standard deviation. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. shRNA-SOX2-OT-1; # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  vs. shRNA-SOX2-OT-1+miR-NC. sh, short-hairpin; NC, negative control.



shRNA-SOX2-OT-1 was transfected into TU-177 cells in the present study. It was identified that SOX2-OT-knockdown significantly suppressed cell proliferation, migration and invasion, and induced apoptosis of TU-177 cells. Similarly, Tai *et al* (13) suggested that overexpression of SOX2-OT facilitates the tumorigenicity of laryngeal squamous cell carcinoma Hep-2 cells), which indicates that SOX2-OT is involved in the development of laryngeal cancer as an oncogenic factor. Moreover, the present results demonstrated that miR-654 serves as a direct target of SOX2-OT, and downregulation of miR-654 reversed the effects of SOX2-OT-knockdown in TU-177 cells, suggesting that SOX2-OT may facilitate laryngeal cancer development through regulating miR-654.

A study involving oral squamous cell carcinoma revealed that miR-654 expression is correlated with poor prognosis of patients with OSCC, and miR-654-5p promotes the development of OSCC via the GRAP/Ras/Erk signaling pathway (22). However, another study in glioma reported that an upregulation of miR-654 markedly inhibits the proliferation and invasion of the glioma cells U87 and U251 via the IGF2BP3 signaling pathway (23). These results suggest that miR-654 exhibits differences in expression and function in different types of cancer. The present results suggested that miR-654 serves as a tumor-suppressive factor; downregulation of miR-654 significantly promoted cell proliferation and inhibited apoptosis of TU-177 cells. However, further studies are needed to explore the precise mechanism by which miR-654 functions.

Mechanistically, the present study detected the expression of key regulators of cell proliferation, migration, invasion and apoptosis. It has been reported that p21 CDK2, cyclin E and PCNA are strongly implicated in cell proliferation (24,25). The current results demonstrated that SOX2-OT-silencing significantly upregulated the expression of p21 and downregulated the expression of CDK2, cyclin E and PCNA. Furthermore, Bcl-2 family members play crucial roles in cell apoptosis by mediating the chronological activation of caspases (26,27). SOX2-OT-knockdown markedly reduced the expression of Bcl-2 protein, and increased the protein levels of Bax and cleaved caspase 3. In addition, it has been reported that increased expression of matrix metalloproteinases, particularly MMP-7 and MMP-9, is associated with occurrence and development of laryngeal carcinoma malignization (28-30). The present data suggested that the expression levels of MMP-7 and MMP-9 were markedly reduced by SOX2-OT-silencing. These results suggest that the biological function of SOX2-OT is involved in the regulation of multiple factors.

However, this study has several limitations. On one hand, we detected the expression of SOX2-OT in several laryngeal cancer cell lines but we investigated the potential roles of SOX2-OT in only TU-177 cell lines in subsequent experiments. On the other hand, all the experiments in this study were performed at the cellular level thus we only proved the effects of SOX2-OT in laryngeal cancer cells instead of animals or humans. Additionally, 10% FBS was supplemented to TU-177 cells in wound healing assay for more confluence rate. In fact, >5% FBS might significantly increase proliferative activity and it's hard to distinguish the results caused by proliferation or migration. To distinguish proliferation or migration is needed in the future research and the roles of SOX2-OT should be observed in more several OSCC cell lines and in animal experiments.

In conclusion, the present study identified SOX2-OT as a crucial regulator in the development of laryngeal cancer by targeting, at least partially, miR-654. The expression of SOX2-OT was significantly upregulated in laryngeal cancer cell lines, and silencing of SOX2-OT markedly suppressed cell proliferation, migration and invasion, and induced apoptosis in TU-177 cells. Therefore, SOX2-OT may serve as a potential therapeutic target in the treatment of laryngeal cancer.

## Acknowledgements

Not applicable.

## Funding

This work was supported by Anhui Natural Science Foundation (Grant no. 1808085QH248); the National Natural Sciences Foundation of China (Grant no. 81800911); Fundamental Research Funds for the Central Universities (Grant no. WK9110000053).

## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Authors' contributions

GL and JWS designed the study, wrote and revised the manuscript. GL, CP and JQS performed the experiments. GW analyzed the data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68: 394-424, 2018.
2. Steuer CE, El-Deiry M, Parks JR, Higgins KA and Saba NF: An update on larynx cancer. *CA Cancer J Clin* 67: 31-50, 2017.
3. Ferlito A, Devaney KO, Hunt JL and Hellquist H: Some considerations on the WHO histological classification of laryngeal neoplasms. *Adv Ther* 36: 1511-1517, 2019.
4. Wu CW, Dionigi G, Barczynski M, Chiang FY, Dralle H, Schneider R, Al-Quaryshi Z, Angelos P, Brauckhoff K, Brooks JA, *et al*: International neuromonitoring study group guidelines 2018: Part II: Optimal recurrent laryngeal nerve management for invasive thyroid cancer-incorporation of surgical, laryngeal, and neural electrophysiologic data. *Laryngoscope* 128 (Suppl 3): S18-S27, 2018.



5. Calkovsky V, Wallenfels P, Calkovska A and Hajtman A: Laryngeal cancer: 12-year experience of a single center. *Adv Exp Med Biol* 911: 9-16, 2016.
6. Laccourreye O, Bonfils P, Malinvaud D, Ménard M and Giraud P: Survival and laryngeal preservation tradeoff in advanced laryngeal cancer: From the otorhinolaryngology patient to the managing physician. *Head Neck* 39: 1984-1989, 2017.
7. Yang G, Lu X and Yuan L: lncRNA: A link between RNA and cancer. *Biochim Biophys Acta* 1839: 1097-1109, 2014.
8. Sang H, Liu H, Xiong P and Zhu M: Long non-coding RNA functions in lung cancer. *Tumour Biol* 36: 4027-4037, 2015.
9. Teng Y, Kang H and Chu Y: Identification of an exosomal long noncoding RNA SOX2-OT in plasma as a promising biomarker for lung squamous cell carcinoma. *Genet Test Mol Biomarkers* 23: 235-240, 2019.
10. Wang Z, Tan M, Chen G, Li Z and Lu X: lncRNA SOX2-OT is a novel prognostic biomarker for osteosarcoma patients and regulates osteosarcoma cells proliferation and motility through modulating SOX2. *IUBMB Life* 69: 867-876, 2017.
11. Wei CX, Wong H, Xu F, Liu Z, Ran L and Jiang RD: IRF4-induced upregulation of lncRNA SOX2-OT promotes cell proliferation and metastasis in cholangiocarcinoma by regulating SOX2 and PI3K/AKT signaling. *Eur Rev Med Pharmacol Sci* 22: 8169-8178, 2018.
12. Feng L, Wang R, Lian M, Ma H, He N, Liu H, Wang H and Fang J: Integrated analysis of long noncoding RNA and mRNA expression profile in advanced laryngeal squamous cell carcinoma. *PLoS One* 11: e0169232, 2016.
13. Tai Y, Ji Y, Liu F, Zang Y, Xu D, Ma S, Qin L and Ma J: Long noncoding RNA SOX2-OT facilitates laryngeal squamous cell carcinoma development by epigenetically inhibiting PTEN via methyltransferase EZH2. *IUBMB Life* 71: 1230-1239, 2019.
14. Croce CM: Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* 10: 704-714, 2009.
15. Zhang N, Lu C and Chen L: miR-217 regulates tumor growth and apoptosis by targeting the MAPK signaling pathway in colorectal cancer. *Oncol Lett* 12: 4589-4597, 2016.
16. Cybula M, Wieteska L, Józefowicz-Korczyńska M, Karbownik MS, Grzelczyk WL and Szemraj J: New miRNA expression abnormalities in laryngeal squamous cell carcinoma. *Cancer Biomark* 16: 559-568, 2016.
17. Wang FY, Jia J, Song HH, Jia CM, Chen CB and Ma J: Icariin protects vascular endothelial cells from oxidative stress through inhibiting endoplasmic reticulum stress. *J Integr Med* 17: 205-212, 2019.
18. Shields EJ, Petracovici AF and Bonasio R: lncRedibly versatile: Biochemical and biological functions of long noncoding RNAs. *Biochem J* 476: 1083-1104, 2019.
19. Xie ZY, Wang P, Wu YF and Shen HY: Long non-coding RNA: The functional regulator of mesenchymal stem cells. *World J Stem Cells* 11: 167-179, 2019.
20. Xu J, Bai J, Zhang X, Lv Y, Gong Y, Liu L, Zhao H, Yu F, Ping Y, Zhang G, *et al*: A comprehensive overview of lncRNA annotation resources. *Brief Bioinform* 18: 236-249, 2017.
21. Sparber P, Filatova A, Khantemirova M and Skoblov M: The role of long non-coding RNAs in the pathogenesis of hereditary diseases. *BMC Med Genomics* 12 (Suppl 2): S42, 2019.
22. Peng WX, Koirala P and Mo YY: lncRNA-mediated regulation of cell signaling in cancer. *Oncogene* 36: 5661-5667, 2017.
23. Lu M, Wang C, Chen W, Mao C and Wang J: miR-654-5p targets GRAP to promote proliferation, metastasis, and chemoresistance of oral squamous cell carcinoma through Ras/MAPK signaling. *DNA Cell Biol* 37: 381-388, 2018.
24. Jin P, Huang Y, Zhu P, Zou Y, Shao T and Wang O: CircRNA circHIPK3 serves as a prognostic marker to promote glioma progression by regulating miR-654/IGF2BP3 signaling. *Biochem Biophys Res Commun* 503: 1570-1574, 2018.
25. Dhulipala VC, Welshons WV and Reddy CS: Cell cycle proteins in normal and chemically induced abnormal secondary palate development: A review. *Hum Exp Toxicol* 25: 675-682, 2006.
26. Zatonski T, Ciesielska U, Nowinska K, Ratajczak-Wielgomas K, Kobierzycki C, Pula B, Podhorska-Okolow M, Krecicki T and Dziegiel P: Expression of cell cycle-related proteins p16, p27, p53 and Ki-67 in HPV-positive and -negative samples of papillomas of the upper respiratory tract. *Anticancer Res* 36: 3917-3924, 2016.
27. Lee EF, Harris TJ, Tran S, Evangelista M, Arulananda S, John T, Ramnani C, Hobbs C, Zhu H, Gunasingh G, *et al*: BCL-XL and MCL-1 are the key BCL-2 family proteins in melanoma cell survival. *Cell Death Dis* 10: 342, 2019.
28. Chrysovergis A, S Papanikolaou V, Tsiambas E, Kikidis D, Maragoudakis P, Ragos V and Kyrodimos E: Caspase complex in laryngeal squamous cell carcinoma. *J BUON* 24: 1-4, 2019.
29. Lotfi A, Mohammadi G, Saniee L, Mousaviagdas M, Chavoshi H and Tavassoli A: Serum level of matrix metalloproteinase-2 and -9 in patients with laryngeal squamous cell carcinoma and clinical significance. *Asian Pac J Cancer Prev* 16: 6749-6751, 2015.
30. Uloza V, Liutkevičius V, Pangonytė D, Saferis V and Lesauskaitė V: Expression of matrix metalloproteinases (MMP-2 and MMP-9) in recurrent respiratory papillomas and laryngeal carcinoma: Clinical and morphological parallels. *Eur Arch Otorhinolaryngol* 268: 871-878, 2011.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.