

LncRNA MALAT1 expression inhibition suppresses tongue squamous cell carcinoma proliferation, migration and invasion by inactivating PI3K/Akt pathway and downregulating MMP-9 expression

J. YUAN, X.-J. XU, Y. LIN, Q.-Y. CHEN, W.-J. SUN, L. TANG, Q.-X. LIANG

¹Department of Stomatology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou City, Guangdong Province, China.

Jia Yuan and Xiongjun Xu contributed equally to this work

Abstract. – **OBJECTIVE:** LncRNA MALAT1 has been proved to be involved in the development of various types of human cancers while the involvement of MALAT1 in tongue squamous cell carcinoma has not been reported. In view of this, our study aimed to investigate the functionality of MALAT1 in tongue squamous cell carcinoma.

PATIENTS AND METHODS: The expression of MALAT1 in tumor tissues and adjacent healthy tissues of tongue cancer patients, and the serum from tongue cancer patients as well as healthy controls, were detected by quantitative Real Time-PCR (qRT-PCR). ROC curve analysis was performed to analyze the diagnostic value of plasma MALAT1 for tongue cancer. Survival curves were plotted using the Kaplan-Meier method to evaluate the prognostic value of plasma MALAT1 for tongue cancer. CCK-8 assay, transwell migration and invasion assay were performed to investigate the effects of MALAT1 knockdown on the proliferation, migration and invasion of tongue cancer cells, respectively. The effects of MALAT1 overexpression on the PI3K/Akt pathway and MMP-9 expression were detected by Western blot.

RESULTS: The expression level of MALAT1 was remarkably higher in tumor tissues than that in adjacent healthy tissues. Serum MALAT1 was significantly higher in tongue cancer patients than in healthy controls. MALAT1 knockdown markedly inhibits the proliferation, migration and invasion of tongue cancer cells. MALAT1 knockdown also reduced the phosphorylation level of Akt as well as the expression level of MMP-9. It showed no significant effects on Akt expression, while PI3K activator treatment reduced the inhibitory effects of MALAT1 knockdown on the proliferation, migration and invasion of tongue cancer cells.

CONCLUSIONS: LncRNA MALAT1 expression inhibition can inhibit the proliferation, migration and invasion of tongue cancer cells by inactivating the PI3K/Akt pathway and downregulating

MMP-9. MALAT1 may serve as a target for the treatment of tongue squamous cell carcinoma.

Key Words

LncRNA MALAT1, Tongue squamous cell carcinoma, PI3K/Akt pathway, MMP-9.

Introduction

Tongue squamous cell carcinoma is one of the most common types of aggressive epithelial cancers identified in the oral cavity, which accounts for about 1.5% of all cancer cases¹. Although early-stage tongue squamous cell carcinoma can be cured by proper treatments, tongue squamous cell carcinoma patients are prone to develop secondary or recurrent tumors in the surrounding organs and tissues². Once lymph nodes are invaded by tumor cells, the overall mortality rate will be significantly increased and the 5-year overall survival rate is usually below 50%, which lead tongue squamous cell carcinoma to be one of the most deadly cancers³. Although smoking and alcohol addiction are two major risk factors for tongue squamous cell carcinoma, other factors such as viral infection, Epstein-Barr virus and human papillomavirus infection might induce the occurrences of this disease⁴.

The development of tongue squamous cell carcinoma is a complex process with various internal and external factors involved. Studies⁵ on differentially expressed genes in tumor tissues have identified numerous oncogenes or tumor suppressor genes that play critical roles in tumorigenesis. Besides protein-coding messenger RNAs (mRNAs), human transcriptomes contain a large set of non-coding RNAs (ncRNAs) that have regulatory func-

tions in both normal physiological processes and pathological changes⁶. Long non-coding RNA (lncRNA) is a group of non-coding RNAs with length longer than 200 nucleotides. Numerous studies⁷ have shown that different lncRNAs may play different roles in the pathogenesis of various types of malignancies that either promote or inhibit tumor development. MALAT1 plays oncogenic roles in certain types of malignant tumor⁸, but its involvement in tongue squamous cell carcinoma remains unclear. Our study aimed to investigate the functionality of MALAT1 in tongue squamous cell carcinoma, and to explore the possible mechanisms.

Patients and Methods

Patients

72 patients with tongue squamous cell carcinoma enrolled in the Third Affiliated Hospital of Sun Yat-sen University from July 2008 to July 2011 were selected to serve as research subjects. All patients were diagnosed by pathological and imaging examinations. Patients with other oral cavity disease and mental disorders were excluded from this study. These 72 patients included 38 males and 34 females, and the age ranged from 21 years to 71 years with an average age of 46 ± 12.1 . Primary tumors were classified into different stages according to the following standards: tumor invades adjacent structures, such as extrinsic muscle of the tongue, skin of the face, maxillary sinus, or even pterygoid plates, masticator space, or skull base and/or encases internal carotid artery. There were 13 cases of T1, 16 cases of T2, 22 cases of T3 and 21 cases of T4. All patients were subjected to surgical resection of the primary tumor, and tumor tissues as well as adjacent healthy tissues were collected during surgical operations. Tumor tissues and adjacent healthy tissues were confirmed by pathological examinations. 25 people with normal physical conditions were also enrolled at the same time to serve as controls. The control group included 13 males and 12 females, and the age ranged from 19 to 73 years, with an average age of 48 ± 13.1 . The Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University approved this study. All the participants signed the informed consent.

Cell Lines and Cell Culture

Human tongue squamous cell carcinoma cell lines SCC4 cell, SCC15 and SCC25, as well as normal human tongue cell line Hs 680.Tg, were

purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured under the conditions recommended by ATCC and harvested during the logarithmic growth phase for subsequent experiments.

Construction of LncRNA MALAT1 Knockdown Cell Lines

MALAT1 siRNA silencing cell lines were constructed using commercial MALAT1 siRNA (cat. no. 4390771; Thermo Fisher Scientific, Waltham, MA, USA) and Silencer™ Select Negative Control No. 1 siRNA (cat. no. 4390843; Thermo Fisher Scientific, Waltham, MA, USA). Prior to transfection, cells were cultured in Eagle's Minimum Essential Medium (MEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) overnight to reach 80-90% confluence. Lipofectamine 2000 reagent (cat. no. 11668-019; Invitrogen, Carlsbad, CA, USA) was used to transfect 40 nM siRNA into 6×10^5 cells by incubating with the cells at 37°C in a 5% CO₂ incubator, followed by incubation in Roswell Park Memorial Institute-1640 medium (RPMI-1640; Hyclone, South Logan, UT, USA) at 37°C for 48 h prior to subsequent experimentation.

Preparation of Serum Samples

Fasting blood (10 ml) was extracted from each participant in the morning of the day after admission. The serum was obtained by keeping the blood at room temperature for 3 h, followed by centrifugation at 2000 rpm for 15 min. Serum samples were stored at -80 °C before RNA extraction.

Cell Proliferation Assay

Cell proliferation assay was performed using cell counting kit 8 (CCK-8; Sigma-Aldrich, St. Louis, MO, USA). Cells were harvested during the logarithmic growth phase to prepare a cell suspension. The cell suspension was diluted and 100 µl of diluted cell suspension containing 4×10^4 cells was transferred to each well of a 96-well plate. 10 µl of CCK-8 solution was added into each well 24, 48, 72 and 96 h later. Cells were cultured at 37°C for another 4 h, and OD values at 450 nm were measured with Fisherbrand™ accuSkan™ GO UV/Vis Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Three replicate wells were set for each treatment, and this experiment was performed three times to calculate the mean value.

Transwell Migration and Invasion Assay

Transwell cell migration and invasion assay (BD Biosciences, Franklin Lakes, NJ, USA) were performed. For migration assay, the upper chamber was filled with serum-free RPMI-1640 medium (Hyclone, South Logan, UT, USA) containing 4×10^4 cells, while the lower chamber was filled with RPMI-1640 medium containing 20% fetal calf serum (FCS; Sigma-Aldrich, St. Louis, MO, USA). Cells were incubated at 37°C for 24 h; the membranes were then collected and subjected to 0.5% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) staining at room temperature for 20 min. A total of 10 visual fields were randomly selected and stained cells were counted under an optical microscope (Olympus Corporation, Tokyo, Japan). The invasion assay was performed in the same way of the migration assay except that the upper chamber was pre-coated with Matrigel (cat. no. 356234; EMD Millipore, Billerica, MA, USA).

Real Time-Quantitative PCR

Total RNA was extracted from tumor tissues as well as adjacent healthy tissues from patients, serum from patients and controls, and *in vitro* cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). NanoDrop™ 2000 Spectrophotometers (Thermo Fisher Scientific, Waltham, MA, USA) was used to test RNA samples, and only the ones with an A260/A280 ratio between 1.8 and 2.0 were retained and used to synthesize cDNA through the reverse transcription. SYBR® Green Real-Time PCR Master Mixes (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA were used to prepare PCR reaction system. Following primers were utilized in PCR reactions: 5'-CTTAAGCGCAGCGCCATTTT-3' (forward) and 5'-CCTCCAAACCCCAAGACCAA-3' (reverse) for MALAT1; 5'-GACCTCTATGCCAACACAGT-3' (forward) and 5'-AGTACTTGCGCTCAGGAGGA-3' (reverse) for β -actin. PCR reaction conditions were: 95°C for 1 min, followed by 40 cycles of 95°C for 15 s and 60°C for 35 s. Ct values were processed using the $2^{-\Delta\Delta CT}$ method. The relative expression level of MALAT1 was normalized to endogenous control β -actin.

Western Blot

Total protein was extracted from *in vitro* cultured cells using radioimmunoprecipitation assay solution (RIPA; Thermo Fisher Scientific, Waltham, MA, USA), followed by bicinchoninic acid (BCA) method to quantify the protein samples. 20 μ g from each sample was subjected to 10% SDS-PAGE gel electrophoresis followed by transmembrane

to polyvinylidene difluoride (PVDF) membrane. Membranes were blocked with 5% skimmed milk for 1 h at room temperature, followed by incubation with primary antibodies including rabbit anti-Akt (1:2000, ab8805, Abcam, Cambridge, MA, USA), rabbit anti-p-AKT (phospho T308, 1:1000, ab8933, Abcam, Cambridge, MA, USA) and anti-GAPDH primary antibody (1:1000, ab8245, Abcam, Cambridge, MA, USA) overnight at 4°C. The next day, the membranes were washed and incubated with anti-rabbit IgG-HRP secondary antibody (1: 1000, MBS435036, MyBioSource, San Diego, CA, USA) at room temperature for 4 h. Then, enhanced chemiluminescence (ECL; Sigma-Aldrich, St. Louis, MO, USA) was added and signals were detected using MYECL™ Imager (Thermo Fisher Scientific, Waltham, MA, USA). Image J software was used to normalize relative expression level of each protein to endogenous control GAPDH.

Statistical Analysis

SPSS19.0 (SPSS Inc., Chicago, IL, USA) was used to process all the data. Count data were processed by the chi-square test. Measurement data were expressed as ($\bar{x} \pm s$). The comparisons of measurement data between the two groups were performed by the *t*-test, and analysis of variance and LSD test were used for comparisons among multiple groups. $p < 0.05$ was considered to be statistically significant.

Results

Expression of MALAT1 in Tumor Tissues and Adjacent Healthy Tissues of Patients With Tongue Squamous Cell Carcinoma

The expression of MALAT1 in tumor tissues and adjacent healthy tissues of 72 patients with oral squamous cell carcinoma was detected by qRT-PCR. As shown in Figure 1, the expression of MALAT1 was remarkably upregulated in tumor tissues compared with adjacent healthy tissues in 61 out of 72 patients ($p < 0.05$). The expression of MALAT1 was markedly downregulated in tumor tissues compared with adjacent healthy tissues in only 2 patients ($p < 0.05$). No significant differences in the expression level of MALAT1 were found between tumor tissues and adjacent healthy tissues in 9 patients ($p > 0.05$). These data suggest that the upregulation of MALAT1 is very likely to be involved in the pathogenesis of tongue squamous cell carcinoma.

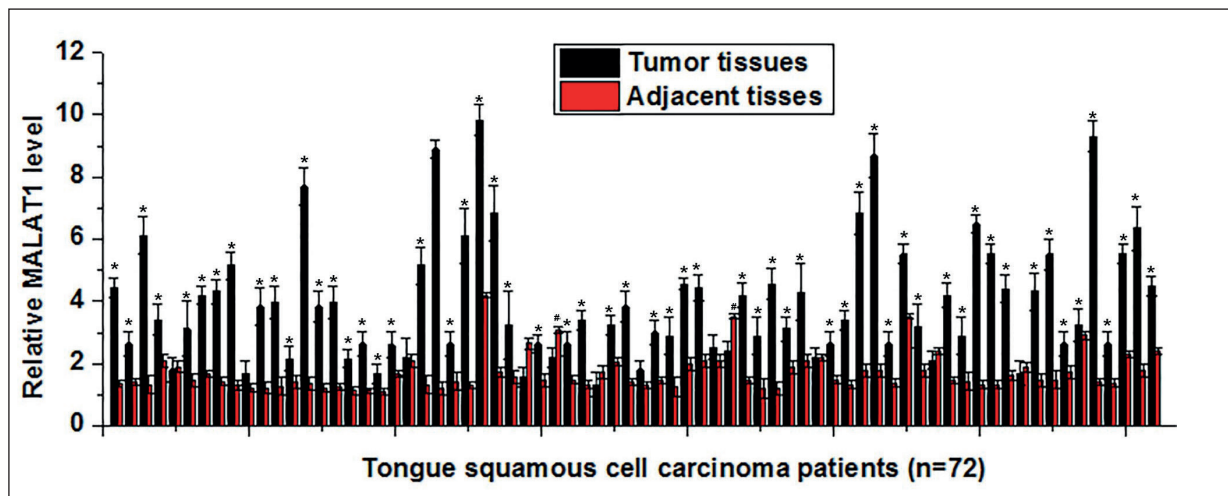


Figure 1. Expression of MALAT1 in tumor tissues and adjacent healthy tissues of patients with tongue squamous cell carcinoma. Notes: *compared with adjacent healthy tissue, $p < 0.05$; #compared with tumor tissue, $p < 0.05$.

Expression of MALAT1 in Serum of Healthy Controls and Patients With Different Stages of Tongue Squamous Cell Carcinoma

As shown in Figure 2, the expression level of MALAT1 in serum was significantly higher in patients with different stages of tongue squamous cell carcinoma than in healthy controls. In addition, the expression level of MALAT1 was further increased with the increased stage of primary tumor. These data suggest that the upregulation of MALAT1 expression is very likely to be involved in the development of tongue squamous cell carcinoma.

Diagnostic and Prognostic Value of Serum MALAT1 for Tongue Squamous Cell Carcinoma

ROC curve analysis was performed to evaluate the diagnostic values of serum MALAT1 for tongue squamous cell carcinoma. The area under the curve (AUC) of serum MALAT1 in the diagnosis of oral squamous cell carcinoma was 0.9514 with 95 intervals of 0.9111 to 0.9917 ($p < 0.0001$, Figure 3a). Patients were divided into the high expression group and low expression group according to the median serum level of MALAT1. The Kaplan-Meier method was used to plot survival curves of both groups and survival curves were compared by log-rank t -test. As shown in Figure 3b, the overall survival rate of patients with high expression level of MALAT1 was significantly lower than that of patients with low expression level of MALAT1. These data suggest that serum MALAT1 may serve

as a diagnostic and prognostic biomarker for tongue squamous cell carcinoma.

Correlation Between Serum Level of MALAT1 and Basic Information of Tongue Squamous Cell Carcinoma Patients

Chi-square test was used to analyze the correlations between the serum levels of MALAT1 and basic clinical data of patients with tongue squamous cell carcinoma. As shown in Table I, serum MALAT1 showed no significant correlations with gender, age, as well as patients' living habits, including diet structure, smoking and alcohol consumption. However, significant correlations were found between the serum levels of MALAT1 and distant tumor metastasis.

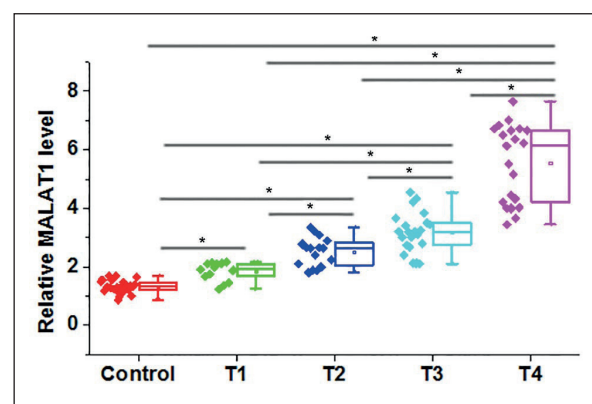


Figure 2. Expression of MALAT1 in serum of healthy controls and patients with different stages of tongue squamous cell carcinoma. Notes: * $p < 0.05$.

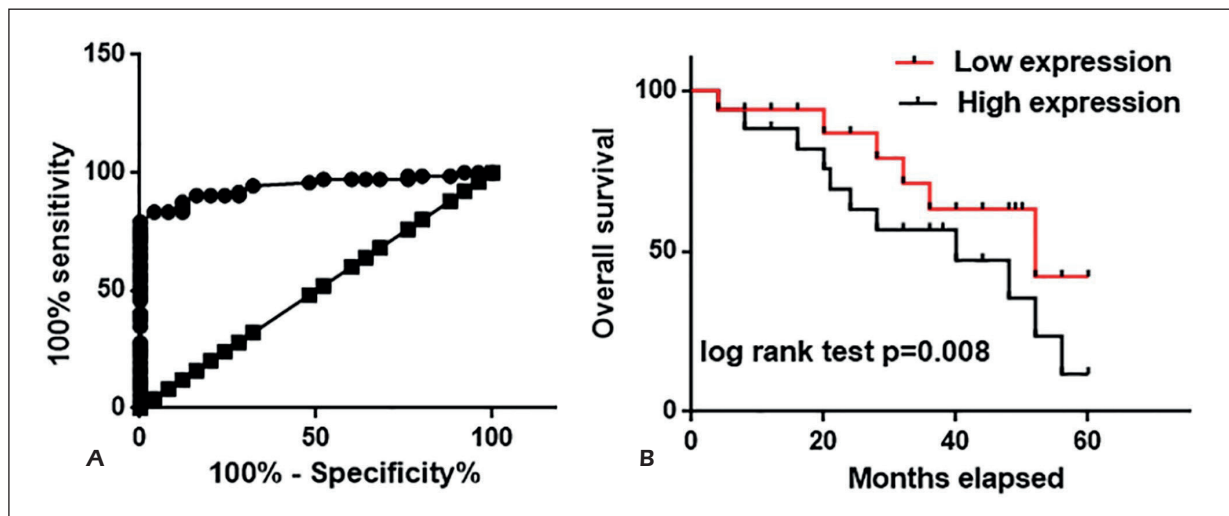


Figure 3. Diagnostic and prognostic value of serum MALAT1 for tongue squamous cell carcinoma. **A**, Diagnostic values of serum MALAT1 for tongue squamous cell carcinoma analyzed by ROC curve analysis. **B**, Comparison of survival curves of patients with high and low serum level of MALAT1.

MALAT1 Knockdown Inhibited the Phosphorylation of Akt and Expression of MMP-9

The PI3K/Akt pathway plays pivotal roles in the development of various types of malignancies. It was reported that MALAT1 can interact with the PI3K/Akt pathway to achieve its biological functions. As shown in Figure 4, MALAT1 knockdown showed no significant effects on the expression of Akt. However, the phosphorylation level of Akt was significantly reduced after MALAT1 knockdown in all three tongue squamous cell carcinoma cell lines ($p < 0.05$). In addition, MALAT1 knockdown significantly reduced the expression level of MMP-9 in all three tongue squamous cell carcinoma cell lines ($p < 0.05$).

These data suggest that MALAT1 can regulate the PI3K/Akt pathway and MMP-9 expression to participate in the pathogenesis of tongue squamous cell carcinoma.

MALAT1 Knockdown Inhibited the Proliferation, Migration and Invasion of Tongue Squamous Cell Carcinoma

The expression level of MALAT1 was significantly higher in all those three tongue squamous cell carcinoma cell lines than that in the normal tongue tissue cell line Hs 680.Tg (data not shown). MALAT1 siRNA was transfected into three tongue squamous cell carcinoma cell lines. Compared with control cells, the expression level of MEG3 was sig-

Table I. Correlation between serum level of MALAT1 and clinical data of tongue squamous cell carcinoma patients.

Items	Groups	Cases	High-expression	Low-expression	p-value
Gender	Male	38	18	20	0.64
	Female	34	18	16	
Age	>45 (years)	40	22	18	0.34
	<45 (years)	32	14	18	
Vegetarian	Yes	16	7	9	0.57
	No	56	29	27	
Smoking	Yes	42	24	18	0.15
	No	30	12	18	
Drinking	Yes	39	22	17	0.24
	No	33	14	19	
Distant Metastasis	Yes	33	28	5	<0.00001
	No	39	8	31	

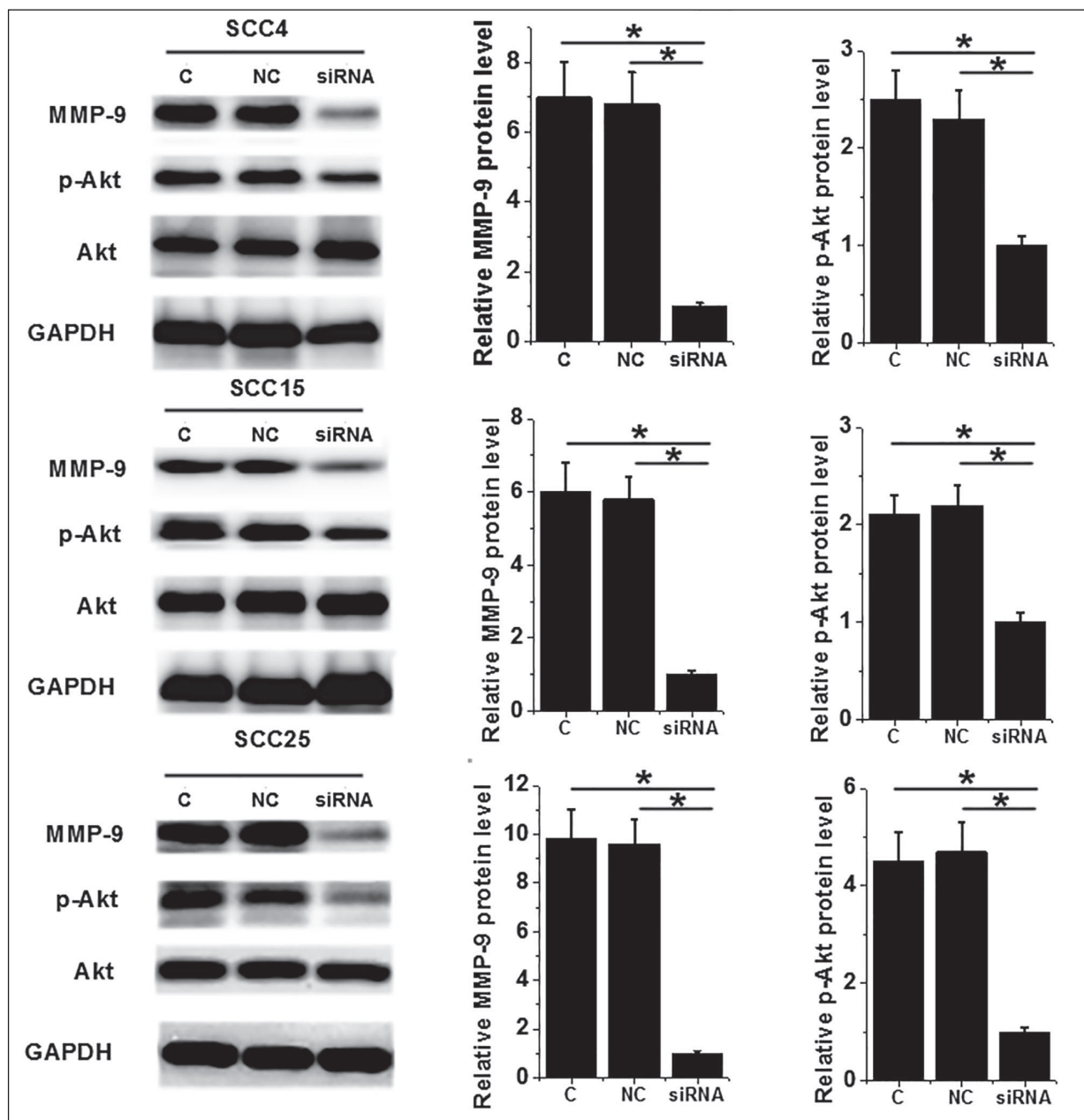


Figure 4. MALAT1 knockdown inhibited the phosphorylation of Akt and expression of MMP-9. Notes: * $p < 0.05$.

nificantly reduced in cells with siRNA transfection (data not shown), indicating the successfully established MALAT1 knockdown cell lines. As shown in Figure 5a, compared with the control cells, the proliferation rate of tongue squamous cell carcinoma cells was significantly decreased after siRNA transfection ($p < 0.05$). Similarly, the migration (Figure 5b) and invasion (Figure 5c) rate of tongue squamous cell carcinoma cells was also significantly decreased after siRNA transfection ($p < 0.05$). Akt activator SC79 (10 $\mu\text{g/mL}$, Sigma-Aldrich, St. Lou-

is, MO, USA) was used to treat cells with MALAT1 siRNA silencing. SC79 significantly increased the phosphorylation level of Akt (data not shown). As shown in Figure 5, PI3K activator treatment significantly reduced the inhibitory effects of MALAT1 knockdown on the proliferation, migration and invasion of tongue cancer cells. These data suggest that LncRNA MALAT1 expression inhibition can inhibit the proliferation, migration and invasion of tongue cancer cells through the inactivation of the PI3K/Akt pathway.

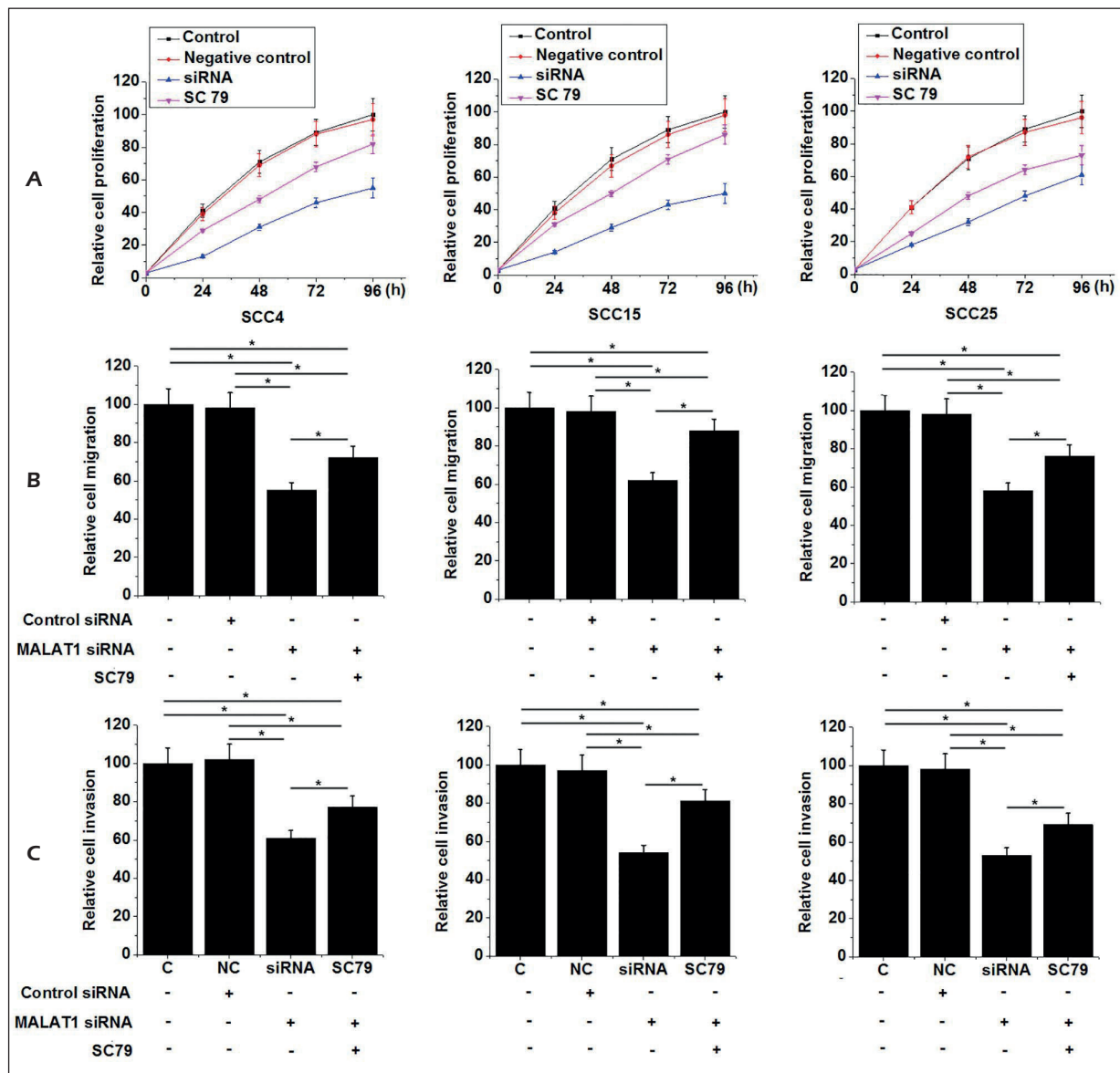


Figure 5. MALAT1 knockdown inhibited the proliferation, migration and invasion of tongue squamous cell carcinoma. **A**, Effects of lncRNA MALAT1 siRNA silencing and Akt activator on proliferation of tongue squamous cell carcinoma cells. **B**, Effects of lncRNA MALAT1 siRNA silencing and Akt activator on migration of tongue squamous cell carcinoma cells. **C**, Effects of lncRNA MALAT1 siRNA silencing and Akt activator on invasion of tongue squamous cell carcinoma cells.

Discussion

LncRNA MALAT1 is oncogenic lncRNA that shows altered expression patterns in various types of malignancies. The study of pancreatic cancer reported that the expression level of MALAT1 was significantly higher in tumor tissues than that in adjacent healthy tissues. In addition, MALAT1 expression was upregulated in pancreatic cancer cell lines than that in normal pancreatic tissue cell lines⁹. The upregulation of MALAT1 expression

was also observed in the development of clear cell renal cell carcinoma, and the increased expression level of MALAT1 was proved to be closely correlated with the progression of tumor¹⁰. In this work, the expression of MALAT1 was remarkably upregulated in tumor tissues compared with adjacent healthy tissues in 61 out of 72 patients, while the expression of MALAT1 was markedly downregulated in tumor tissues than that in adjacent healthy tissues in only 2 patients. In addition, the serum level of MALAT1 was significantly

higher in patients with different stages of tongue squamous cell carcinoma than that in healthy controls. The expression level of MALAT1 was further increased with the increased stage of primary tumor. These results suggest that the upregulation of MALAT1 expression is very likely to be involved in the development and progression of tongue squamous cell carcinoma.

In spite of the great progress that has been made in the clinical application of radiotherapy, chemotherapy and targeted therapy, surgical resection is still the exclusively radical treatment for patients with tongue squamous cell carcinoma. However, the prognosis of this disease after a proper surgical operation is still very limited due to the existing of local invasion and metastasis, which is also a major cause of postoperative recurrence¹¹. Therefore, early diagnosis and treatment is the key to improve the survival of patients with tongue squamous cell carcinoma. The development of human diseases is usually accompanied by the changes of certain substances in the blood, and the detection of those substances in the blood can usually provide references for the diagnosis and prognosis for certain diseases^{12,13}. In this work, ROC curve analysis showed that serum MALAT1 can be used to effectively and accurately predict tongue squamous cell carcinoma. In addition, the overall survival rate of patients with high expression level of MALAT1 was significantly lower than that of patients with low expression level of MALAT1. Tobacco consumption and alcohol abuse are two major risk factors for tongue squamous cell carcinoma. However, tongue squamous cell carcinoma can also be caused by other factors such as viral infection, e.g. Epstein-Barr virus and human papillomavirus infection⁴. In this work, serum MALAT1 showed no significant correlations with patients' living habits including diet structure, smoking and alcohol consumption. These data suggest that serum MALAT1 is an accurate and reliable diagnostic as well as a prognostic biomarker for tongue squamous cell carcinoma.

The activation of the PI3K/Akt pathway is critical for the development of various types of cancers including tongue squamous cell carcinoma^{14,15}, and the inhibition of the PI3K/Akt activation may inhibit the development of certain malignancies¹⁶. Previous studies^{17,18} have shown that lncRNA MALAT1 can regulate the activation of PI3K/Akt to achieve its biological functions. The expression of matrix metalloproteinase-9 (MMP-9) is also crucial to the

metastasis of certain malignancies such as breast cancer cells¹⁹. In this work, MALAT1 knockdown showed no significant effects on the expression of Akt, but reduced the phosphorylation level of Akt and expression level of MMP-9 in all three tongue squamous cell carcinoma cell lines. At cellular level, MALAT1 promotes tumor development by promoting the proliferation, migration and invasion of tumor cells²⁰, while MALAT1 knockdown inhibits tumor growth and metastasis²¹. In this study, MALAT1 knockdown markedly inhibit the proliferation, migration and invasion of tongue cancer cells, but this inhibitory effect was significantly reduced by the PI3K activator treatment. These data suggest that the downregulation of lncRNA MALAT1 can inhibit the proliferation, migration and invasion of tongue cancer cells by inactivating the PI3K/Akt pathway and downregulating MMP-9.

Conclusions

We showed that the expression level of MALAT1 was significantly higher in tumor tissues than that in adjacent healthy tissues among most patients. Serum MALAT1 was significantly higher in tongue cancer patients than that in healthy controls. MALAT1 knockdown significantly inhibits the proliferation, migration and invasion of tongue cancer cells, and reduced the phosphorylation level of Akt and expression level of MMP-9. PI3K activator treatment reduced the inhibitory effects of MALAT1 knockdown on the proliferation, migration and invasion of tongue cancer cells. Therefore, we conclude that lncRNA MALAT1 expression inhibition can inhibit the proliferation, migration and invasion of tongue cancer cells by inactivating the PI3K/Akt pathway and downregulating MMP-9, and MALAT1 may serve as a target for the treatment of tongue squamous cell carcinoma. However, this study is somehow limited by the small sample size. Future studies with bigger sample size are still needed to further verify the conclusions of this work.

Acknowledgements

We thank the financial support from the Technology Project of Guangdong Province (No. 2016A020215203).

Conflict of Interests

The authors declare that they have no conflict of interest.

References

- 1) NARUSE T, YANAMOTO S, YAMADA SI, TAKAHASHI H, MATSUSHITA Y, IMAYAMA N, IKEDA H, SHIRAISHI T, FUJITA S, IKEDA T, ASAHINA I, UMEDA M. Immunohistochemical study of vascular endothelial growth factor-C/vascular endothelial growth factor receptor-3 expression in oral tongue squamous cell carcinoma: correlation with the induction of lymphangiogenesis. *Oncol Letter* 2015; 10: 2027-2034.
- 2) FANG Z, WU L, WANG L, YANG Y, MENG Y, YANG H. Increased expression of the long non-coding RNA UCA1 in tongue squamous cell carcinomas: a possible correlation with cancer metastasis. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2014; 117: 89-95.
- 3) DIBBLE EH, ALVAREZ AC, TRUONG MT, MERCIER G, COOK EF, SUBRAMANIAM RM. 18F-FDG metabolic tumor volume and total glycolytic activity of oral cavity and oropharyngeal squamous cell cancer: adding value to clinical staging. *J Nucl Med* 2012; 53: 709-715.
- 4) JALOULI J, JALOULI MM, SAPKOTA D, IBRAHIM SO, LARSSON PA, SAND L. Human papilloma virus, herpes simplex virus and epstein barr virus in oral squamous cell carcinoma from eight different countries. *Anticancer Res* 2012; 32: 571-580.
- 5) HUANG S. Gene expression profiling, genetic networks, and cellular states: an integrating concept for tumorigenesis and drug discovery. *J Mol Med (Berl)* 1999; 77: 469-480.
- 6) MATTICK JS, MAKUNIN IV. Non-coding RNA. *Hum Mol Genet* 2006; 15: R17-R29.
- 7) CUI Z, REN S, LU J, WANG F, XU W, SUN Y, WEI M, CHEN J, GAO X, XU C, MAO JH, SUN Y. The prostate cancer-up-regulated long noncoding RNA PlncRNA-1 modulates apoptosis and proliferation through reciprocal regulation of androgen receptor. *Urol Oncol* 2013; 31: 1117-1123.
- 8) GUTSCHNER T, HÄMMERLE M, EISSMANN M, HSU J, KIM Y, HUNG G, REVENKO A, ARUN G, STENTRUP M, GROSS M, ZORNIG M, MACLEOD AR, SPECTOR DL, DIEDERICH S. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer Res* 2013; 73: 1180-1189.
- 9) LIU JH, CHEN G, DANG YW, LI CJ, LUO DZ. Expression and prognostic significance of lncRNA MALAT1 in pancreatic cancer tissues. *Asian Pac J Cancer Prev* 2014; 15: 2971-2977.
- 10) ZHANG HM, YANG FQ, CHEN SJ, CHE J, ZHENG JH. Upregulation of long non-coding RNA MALAT1 correlates with tumor progression and poor prognosis in clear cell renal cell carcinoma. *Tumour Biol* 2015; 36: 2947-2955.
- 11) WANG B, ZHANG S, YUE K, WANG XD. The recurrence and survival of oral squamous cell carcinoma: a report of 275 cases. *Chin J Cancer* 2013; 32: 614-618.
- 12) HORI SS, GAMBHIR SS. Mathematical model identifies blood biomarker-based early cancer detection strategies and limitations. *Sci Transl Med* 2011; 3: 109ra116-109ra116.
- 13) KLEIN RJ, HALLDÉN C, CRONIN AM, PLONER A, WIKLUND F, BJARTELL AS, STATTIN P, XU J, SCARDINO PT, OFFIT K, VICKERS AJ, GRONBERG H, LILJA H. Blood biomarker levels to aid discovery of cancer-related single-nucleotide polymorphisms: kallikreins and prostate cancer. *Cancer Prev Res* 2010; 3: 611-619.
- 14) OSAKI M, OSHIMURA M, ITO H. PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis* 2004; 9: 667-676.
- 15) PAN ST, QIN Y, ZHOU ZW, HE ZX, ZHANG X, YANG T, YANG YX, WANG D, QIU JX, ZHOU SF. Plumbagin induces G2/M arrest, apoptosis, and autophagy via p38 MAPK- and PI3K/Akt/mTOR-mediated pathways in human tongue squamous cell carcinoma cells. *Drug Des Devel Ther* 2015; 9: 1601-1626.
- 16) ZHAO H, YANG J, FAN T, LI S, REN X. RhoE functions as a tumor suppressor in esophageal squamous cell carcinoma and modulates the PTEN/PI3K/Akt signaling pathway. *Tumour Biol* 2012; 33: 1363-1374.
- 17) DONG Y, LIANG G, YUAN B, YANG C, GAO R, ZHOU X. MALAT1 promotes the proliferation and metastasis of osteosarcoma cells by activating the PI3K/Akt pathway. *Tumour Biol* 2015; 36: 1477-1486.
- 18) XU S, SUI S, ZHANG J, BAI N, SHI Q, ZHANG G, GAO S, YOU Z, ZHAN C, LIU F, PANG D. Downregulation of long noncoding RNA MALAT1 induces epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer. *Int J Clin Exp Pathol* 2015; 8: 4881-4891.
- 19) YAO J, XIONG S, KLOS K, NGUYEN N, GRIJALVA R, LI P, YU D. Multiple signaling pathways involved in activation of matrix metalloproteinase-9 (MMP-9) by heregulin-beta1 in human breast cancer cells. *Oncogene* 2001; 20: 8066-8074.
- 20) YANG MH, HU ZY, XU C, XIE LY, WANG XY, CHEN SY, LI ZG. MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9. *Biochim Biophys Acta* 2015; 1852: 166-174.
- 21) WANG X, LI M, WANG Z, HAN S, TANG X, GE Y, ZHOU L, ZHOU C, YUAN Q, YANG M. Silencing of long noncoding RNA MALAT1 by miR-101 and miR-217 inhibits proliferation, migration, and invasion of esophageal squamous cell carcinoma cells. *J Biol Chem* 2015; 290: 3925-3935.