Am J Transl Res 2019;11(11):7009-7017
www.ajtr.org /ISSN:1943-8141/AJTR0100095

Original Article

LncRNA DLX6-AS1 promotes laryngeal squamous cell carcinoma growth and invasion through regulating miR-376c

Qingjun Yang[1], Jin Sun[2], Yifei Ma[3], Chunjie Zhao[1], Jijun Song[1]

[1]Department of Otolaryngology, Head and Neck Surgery, Zhoukou Central Hospital, Zhoukou 466000, Henan, China; [2]Department of Otolaryngology, Head and Neck Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China; [3]Department of Otorhinolaryngology, Affiliated Hospital of Guizhou Medical University, Guiyang 550004, Guizhou, China

Received July 25, 2019; Accepted September 1, 2019; Epub November 15, 2019; Published November 30, 2019

Abstract: Accumulating evidence showed that lncRNAs play important roles in tumour development. Recently, a novel lncRNA DLX6-AS1 was found to be overexpressed in some tumors such as lung adenocarcinoma, renal cell carcinoma and hepatocellular carcinoma. However, the functional roles of DLX6-AS1 in laryngeal squamous cell carcinoma (LSCC) are still unclear. In the study, we showed that the expression level of DLX6-AS1 was upregulated in the LSCC samples compared to the noncancerous tissues. By using CCK-8 analysis, we demonstrated that knockdown expression of DLX6-AS1 suppressed the Hep2 cell growth. DLX6-AS1 knockdown inhibited the Hep2 cell cycle and invasion. MiR-376c was identified to have the complementary binding sites with the DLX6-AS1. By luciferase reporter assay, we indicated that overexpression of miR-376c inhibited the luciferase activity of wild-type DLX6-AS1, but it failed to suppress luciferase activity of mutated one. DLX6-AS1 knockdown enhanced the expression of miR-376c in the Hep2 cell. Moreover, we showed that the expression level of miR-376c was lower in the LSCC samples than in the noncancerous tissues and the expression of miR-376c was negatively correlated with expression of DLX6-AS1 in LSCC tissues. Ectopic expression of miR-376c suppressed cell proliferation, cycle and invasion of LSCC cell. DLX6-AS1 knockdown suppressed cell proliferation, cycle and invasion via regulating miR-376c expression. These data proved that lncRNA DLX6-AS1 might play as an oncogene in LSCC development and tumorigenesis.

Keywords: Laryngeal squamous cell carcinoma, DLX6-AS1, miR-376c, lncRNA

Introduction

Laryngeal squamous cell carcinoma (LSCC) is 2nd most common neck and head squamous cell carcinoma [1-3]. Therapy options after original diagnosis include chemotherapy, radiation or surgery therapy [4-6]. Patients with LSCC at the early-stage can be effectually treated with multi-modal or single treatment, but most cases diagnosed at the advanced stage die of metastasis and/or recurrence [7-10]. The survival and mortality rate of cases with LSCC has not signicantly improved in recent twenty years and a varity of studes have been show to elucide the mechanism of cancer metastasis and invasion [3, 7, 11-14]. Therefore, it is necessary to study the mechanism of occurrence and development of LSCC and identify risk factors for LSCC case mortality.

Long noncoding RNA (lncRNAs) are defined as a goup of transcripts which are longer than 200 nucleotides without protein coding potential [15-19]. LncRNAs recently attracts more attention due to their important role in several cellular procedures, ranging from post-transcriptional and transcriptional modulation to the govern of subcellular localization, epigenetic modifications and cellular structural integrity [20-24]. LncRNAs has shown to play important roles in several biological processes including cell metastases, proliferation, cycle, apoptosis, invasion and migration [22, 25-27]. Recently, a large number of lncRNAs are deregulated in diverse tumors and the deregulation lncRNAs have been indicated to lead to aberrant expression of gene that contributes to development and progression of tumors including LSCC [28-31]. More recently, a novel lncRNA DLX6-AS1

was found to be overexpressed in some tumors such as lung adenocarcinoma, renal cell carcinoma and hepatocellular carcinoma [32-34]. For example, Li et al [32]. Firstly investigated the role of DLX6-AS1 in lung adenocarcinoma and demonstrated that DLX6-AS1 expression was upregulated in lung adenocarcinoma. Zeng et al [33]. demonstrated that the expression of DLX6-AS1 was upregulated in renal cell carcinoma and ectopic expression of DLX6-AS1 induced the renal cell carcinoma cell proliferation and tumorigenesis via regulating miR-26a/PTEN expression. Zhang and colleagues indicated that DLX6-AS1 expression was upregulated in the hepatocellular carcinoma tissues and knockdown expression of DLX6-AS1 suppressed cell invasion, migration and proliferation of hepatocellular carcinoma cell through regulating miR-203a/MMP-2 pathway [34]. However, the functional roles of DLX6-AS1 in LSCC are still unclear.

In this study, we showed that the expression level of DLX6-AS1 was upregulated in the LSCC samples compared to the noncancerous tissues. In addition, we demonstrated that knockdown expression of DLX6-AS1 suppressed the Hep2 cell growth, cell cycle and invasion.

Materials and methods

Human LSCC tissues and cell cultured and transfection

Human LSCC tissues and their pair noncancerous samples utilized in our study were collected from Zhoukou Central Hospital, Zhoukou, China under resections. No systemic or local therapies were performed in these cases before operation. All these samples were snap-frozen in the liquid nitrogen and then stored until RNA was extracted. Informed consent was collected from patients and our study was approved with clinical Ethics Committee of Zhoukou Centre Hospital. Hep2 (LSCC cell line) was collected from Shanghai Chinese Academy of Science (Shanghai, China). Hep2 cell was cultured in the RPMI1640 (Gibco) supplemented with FBS, penicillin and streptomycin. miR-376c mimic and scramble, miR-376c inhibitor and control, si-DLX6-AS1 and si-control were synthesized from GenePharma (Shanghai, China) and then transfected into Hep2 cell with using Lipofectamine 3000 following to instruction.

RNA extraction and real-time PCR

Total RNA of cells or samples was separated by using TRIzol kit (Invitrogen, CA, USA) following to standard protocol. qRT-PCR assay was done to analyze the expression of DLX6-AS1 and miR-376c by using SYBR Green mix (Applied Biosystems) on the iQ5 PCR Detection System (Bio-Rad, USA). The primers were shown as follows: DLX6-AS1, forward primers 5'-CAAATGCTACCATCCAGCC-3' and reverse primers 5'-TCTGGCTTCCCTTAACCAAA-3'; U6, forward primers 5'-CTCGCTTCGGCAGCACA-3' and reverse primers 5'-AAC GCT TCA CGA ATT TGC GT-3'; miR376c, forward primers 5'-GCGCAACATAGAGGAAA-3' and reverse primers 5'-GGTGCAGGGTCCGAGGT-3'.

Cell proliferation, cycle and invasion assay

Cell Counting Kit-8 (CCK8, Dojindo, Kumamoto, Japan) was used to detect the cell growth. Cell was cultured in the 96 well plates and was incubated with 10 μl CCK8 for 3 hours at the set time points. The absorbance at 450 nm was determined by a microplate reader. For cell cycle, Hep2 cells were fixed with ethanol (70%) and treated with 50 mg/ml RNase A and then stained with 25 mg/ml PI (propidium iodide). Populations in the differenct phase were determined via flow cytometry (Beckman Coulter, CA, USA). For cell invasion assay, transwell chamber was used. The upper chamber was coated matrigel for filtering and the culture medium was added to lower chamber. The Hep2 cell was cultured in the medium (serum-free) and placed in the upper chamber. After 48 hours, no-invasive cell was removed and invasive cells were fixed and staining with crystal violet (0.1%).

Statistical analysis

Results were presented as mean values ± SD (standard deviation). Statistical assay was carried out by using Student's t test for two groups and one-way ANOVA was utilized to analyze the differences between more than two groups. P less than 0.05 were considered to be statistically significant.

Results

DLX6-AS1 expression was upregulated in LSCC samples

To study the potential roles of lncRNA DLX6-AS1 in the development of LSCC, we firstly

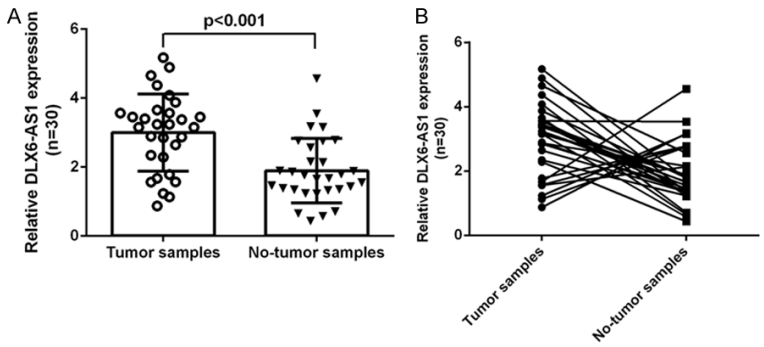

Figure 1. DLX6-AS1 expression was upregulated in LSCC samples. A. By using qRT-PCR assay, the expression level of DLX6-AS1 was higher in the LSCC samples than in the noncancerous tissues. B. The DLX6-AS1 expression was upregulated in 23 patients (23/30, 77%) compared to adjacent noncancerous tissues.

detected the DLX6-AS1 expression in LSCC samples and their pair noncancerous tissues. By using qRT-PCR assay, the expression level of DLX6-AS1 was higher in the LSCC samples than in the noncancerous tissues (**Figure 1A**). The DLX6-AS1 expression was upregulated in 23 patients (23/30, 77%) compared to adjacent noncancerous tissues (**Figure 1B**).

DLX6-AS1 knockdown inhibited cell proliferation, cycle and invasion of LSCC cell

Then, in LSCC cell line Hep2 transfected with si-DLX6-AS1, the expression level of DLX6-AS1 was downregulated compared to the si-control group (**Figure 2A**). By using CCK-8 analysis, knockdown expression of DLX6-AS1 suppressed the Hep2 cell growth (**Figure 2B**). Moreover, DLX6-AS1 knockdown decreased the S stage of Hep2 cell and increased the G0-G1 stage (**Figure 2C**). Furthermore, we showed that DLX6-AS1 knockdown suppressed the Hep2 cell invasion (**Figure 2D** and **2E**).

DLX6-AS1 decreased expression of miR-376c in trophoblastic cells

Bioinformatics analyse was analyzed to study molecular mechanism through which DLX6-AS1 modulates LSCC cell behavior. MiR-376c was identified to have the complementary binding sites with the DLX6-AS1 (**Figure 3A**). Then, in LSCC cell line Hep2 transfected with miR-376c mimic, the expression level of miR-376c was upregulated compared to the scramble group (**Figure 3A**). By luciferase reporter assay, we indicated that overexpression of miR-376c inhibited the luciferase activity of wild-type DLX6-AS1, but it failed to suppress luciferase activity of mutated one (**Figure 3C**). DLX6-AS1 knockdown enhanced the expression of miR-376c in the Hep2 cell (**Figure 3D**).

miR-376c expression was downregulated in LSCC samples

To study the potential roles of miR-376c in the development of LSCC, we then detected the miR-376c expression in LSCC samples and their pair noncancerous tissues. By using qRT-PCR assay, the expression level of miR-376c was lower in the LSCC samples than in the noncancerous tissues (**Figure 4A**). The miR-376c expression was downregulated in 23 patients (23/30, 77%) compared to adjacent noncancerous tissues (**Figure 4B**). In addition, we indicated that the expression of miR-376c was negatively correlated with expression of DLX6-AS1 in LSCC tissues (**Figure 4C**).

Ectopic expression of miR-376c suppressed cell proliferation, cycle and invasion of LSCC cell

By using CCK-8 analysis, ectopic expression of miR-376c inhibited the Hep2 cell growth (**Figure 5A**). Moreover, miR-376c overexpression decreased the S stage of Hep2 cell and increased the G0-G1 stage (**Figure 5B**). Furthermore, we showed that overexpression of miR-376c suppressed the Hep2 cell invasion (**Figure 5C** and **5D**).

DLX6-AS1 knockdown suppressed cell proliferation, cycle and invasion via regulating miR-376c expression

Then, in LSCC cell line Hep2 transfected with miR-376c inhibitor, the expression level of miR-376c was downregulated compared to the control group (**Figure 6A**). The inhibition effect of DLX6-AS1 on cell proliferation was effectively recoveried through miR-376c knockdown (**Figure 6B**). In addition, we showed that the inhibition effect of DLX6-AS1 on cell cycle was resuced by miR-376c knockdown (**Figure 6C**).

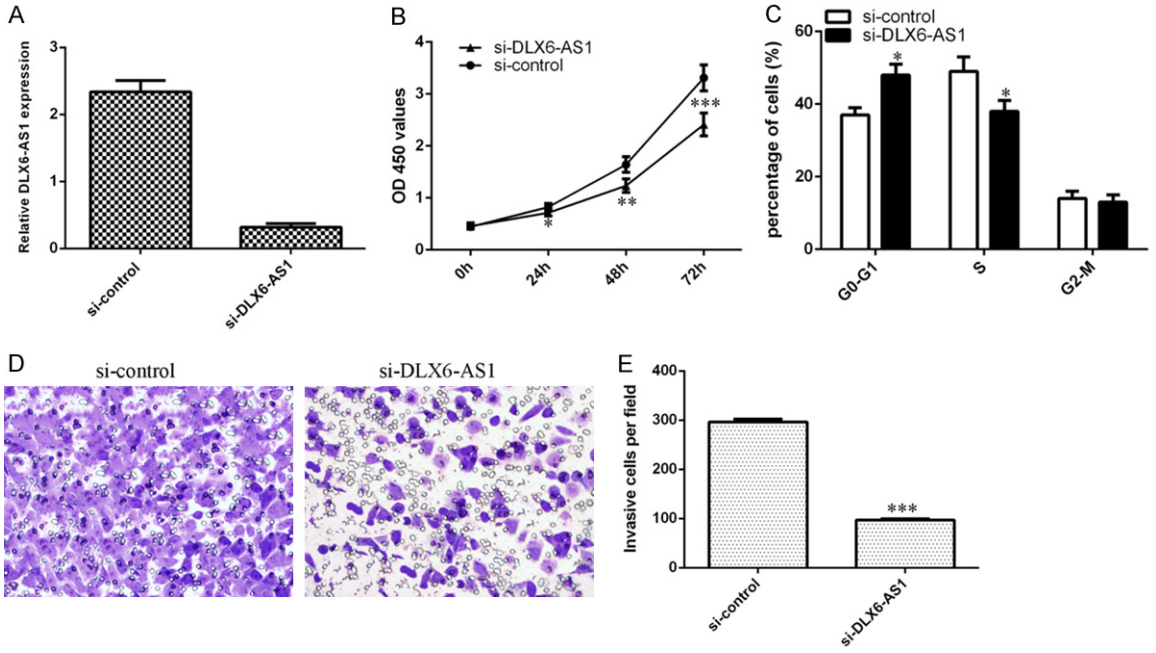

Figure 2. DLX6-AS1 knockdown inhibited cell proliferation, cycle and invasion of LSCC cell. A. The expression of DLX6-AS1 was detected in the LSCC cell line Hep2 cell by using qRT-PCR analysis. B. By using CCK-8 analysis, knockdown expression of DLX6-AS1 suppressed the Hep2 cell growth. C. DLX6-AS1 knockdown decreased the S stage of Hep2 cell and increased the G0-G1 stage. D. DLX6-AS1 knockdown suppressed the Hep2 cell invasion. E. The relative invasive cells were shown. *P<0.05, **P<0.01 and ***P<0.001.

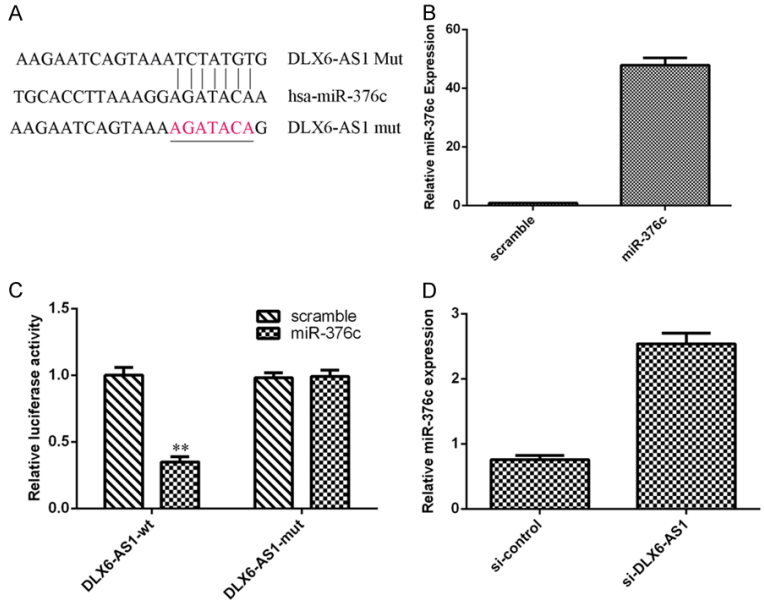

Figure 3. DLX6-AS1 decreased expression of miR-376c in trophoblastic cells. A. MiR-376c was identified to have the complementary binding sites with the DLX6-AS1 by using Bioinformatics analyse. B. The expression level of DLX6-AS1 was detected in Hep2 cell by using qRT-PCR analysis. C. Overexpression of miR-376c inhibited the luciferase activity of wild-type DLX6-AS1, but it failed to suppress luciferase activity of mutated one by using luciferase reporter assay. D. DLX6-AS1 knockdown enhanced the expression of miR-376c in the Hep2 cell. **P<0.01.

Moreover, the inhibition function of DLX6-AS1 on cell invasion was also recoveried by miR-376c knockdown (**Figure 6D**).

Discussion

Increasing evidences have shown to that lncRNAs act essential roles in tumour development and have been proved to be important regulators of signaling pathways underling the carcinogenesis [18, 35-37]. In the study, we showed that the expression level of DLX6-AS1 was upregulated in the LSCC samples compared to the noncancerous tissues. By using CCK-8 analysis, we demonstrated that knockdown expression of DLX6-AS1 suppressed the Hep2 cell growth. DLX6-AS1 knockdown inhibited the Hep2 cell cycle and invasion. MiR-

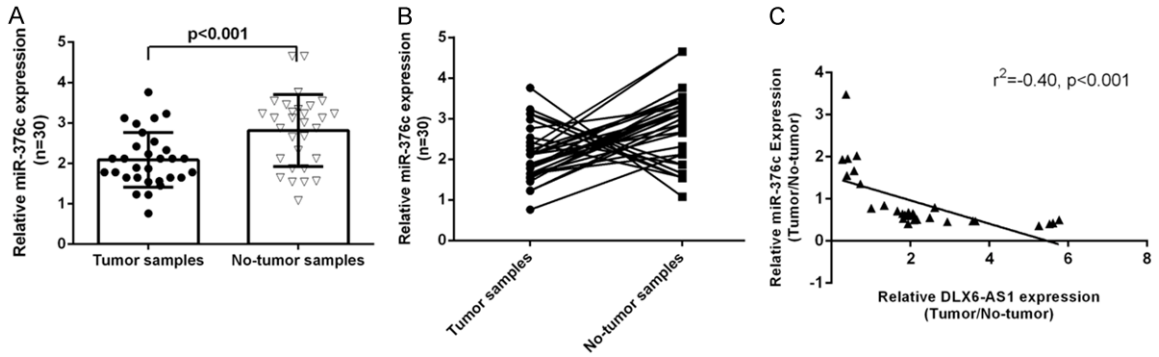

Figure 4. miR-376c expression was downregulated in LSCC samples. A. By using qRT-PCR assay, the expression level of miR-376c was lower in the LSCC samples than in the noncancerous tissues. B. The miR-376c expression was downregulated in 23 patients (23/30, 77%) compared to adjacent noncancerous tissues. C. The expression of miR-376c was negatively correlated with expression of DLX6-AS1 in LSCC tissues.

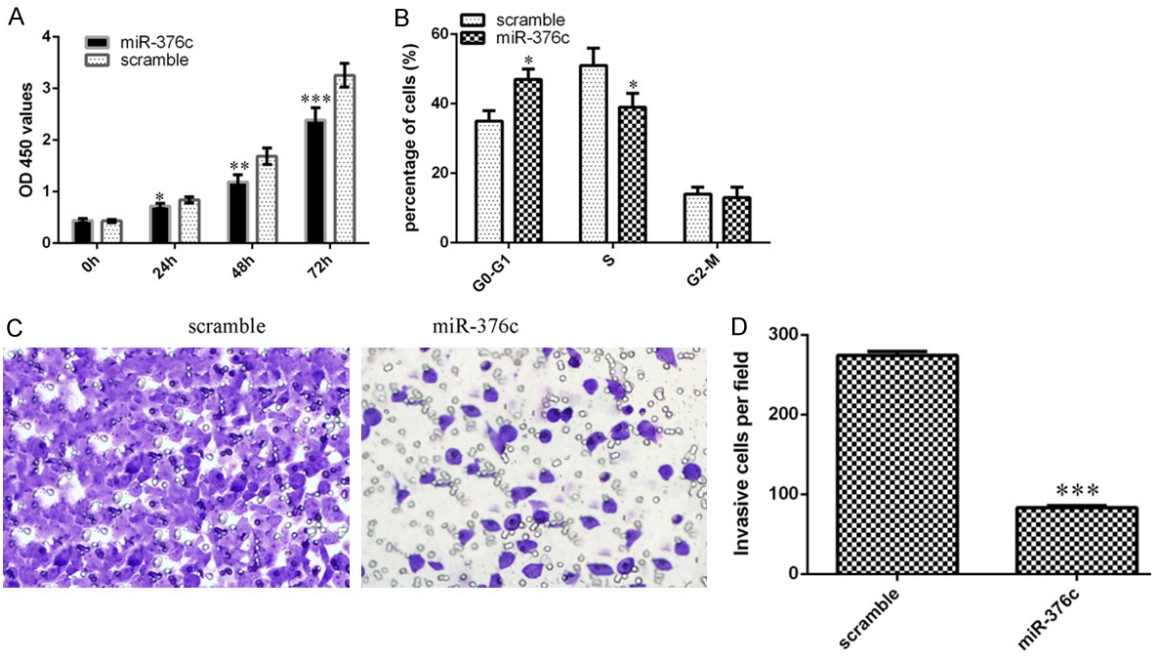

Figure 5. Ectopic expression of miR-376c suppressed cell proliferation, cycle and invasion of LSCC cell. A. Ectopic expression of miR-376c inhibited the Hep2 cell growth by using CCK-8 analysis. B. miR-376c overexpression decreased the S stage of Hep2 cell and increased the G0-G1 stage. C. Overexpression of miR-376c suppressed the Hep2 cell invasion. D. The relative invasive cells were shown. *P<0.05, **P<0.01 and ***P<0.001.

376c was identified to have the complementary binding sites with the DLX6-AS1. By luciferase reporter assay, we indicated that overexpression of miR-376c inhibited the luciferase activity of wild-type DLX6-AS1, but it failed to suppress luciferase activity of mutated one. DLX6-AS1 knockdown enhanced the expression of miR-376c in the Hep2 cell. Moreover, we showed that the expression level of miR-376c was lower in the LSCC samples than in the noncancerous tissues and the expression of miR-376c was negatively correlated with expression of DLX6-AS1 in LSCC tissues. Ectopic expression of miR-376c suppressed cell proliferation, cycle and invasion of LSCC cell. DLX6-AS1 knockdown suppressed cell proliferation, cycle and invasion via regulating miR-376c expression. These data proved that lncRNA DLX6-AS1 might play as an oncogene in LSCC development and tumorigenesis.

DLX6-AS1 was found to locate on the chr7: 96955141-97014065 and overexpressed in some tumors such as lung adenocarcinoma,

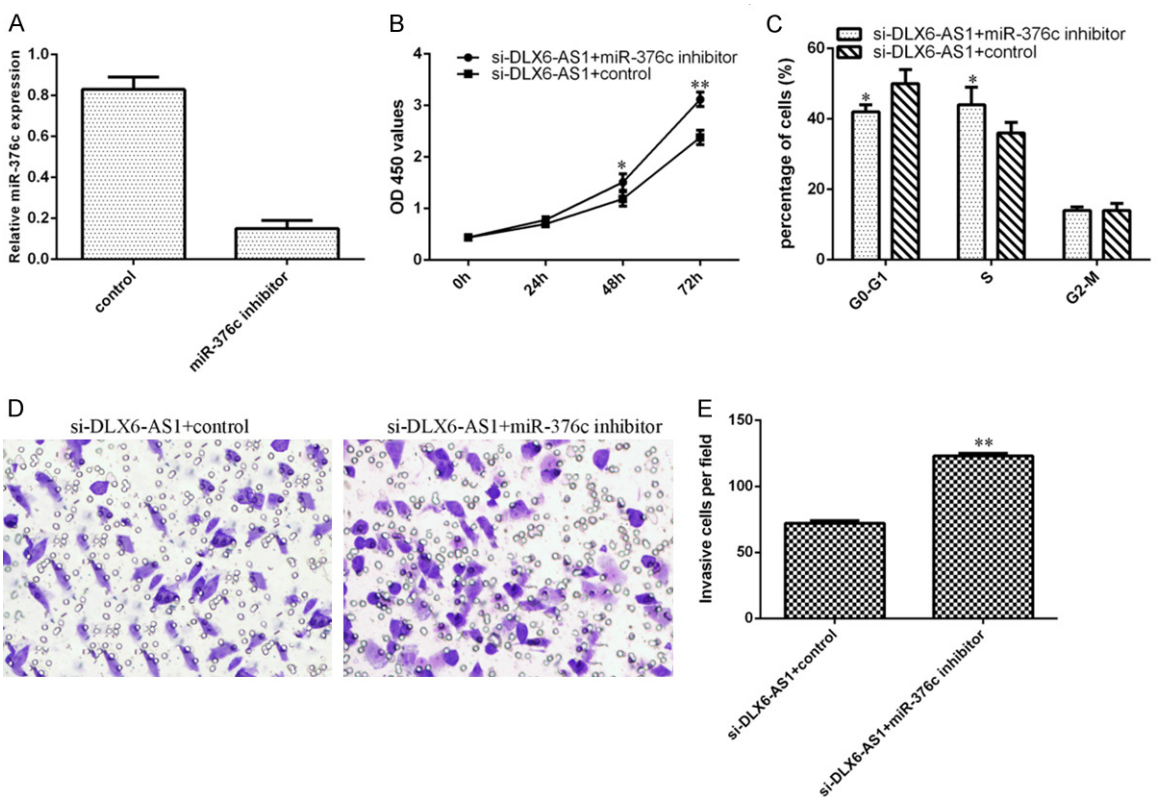

Figure 6. DLX6-AS1 knockdown suppressed cell proliferation, cycle and invasion via regulating miR-376c expression. A. The expression of miR-376c was measured in the Hep2 cell by using qRT-PCR analysis. B. The inhibition effect of DLX6-AS1 on cell proliferation was effectively recoveried through miR-376c knockdown. C. The inhibition effect of DLX6-AS1 on cell cycle was resuced by miR-376c knockdown. D. The inhibition function of DLX6-AS1 on cell invasion was also recoveried by miR-376c knockdown. E. The relative invasive cells were shown. *P<0.05, **P<0.01.

renal cell carcinoma and hepatocellular carcinoma [32]. For instance, Li and colleagues firstly investigated the role of DLX6-AS1 in lung adenocarcinoma and found that the expression of DLX6-AS1 was upregulated in lung adenocarcinoma [32]. Zeng et al [33]. demonstrated that DLX6-AS1 expression was upregulated in renal cell carcinoma and ectopic expression of DLX6-AS1 induced the renal cell carcinoma cell proliferation and tumorigenesis through regulating miR-26a/PTEN expression. Zhang and colleagues demonstrated that DLX6-AS1 expression was upregulated in hepatocellular carcinoma samples and knockdown expression of DLX6-AS1 decreased cell invasion, migration and proliferation of hepatocellular carcinoma cell via regulating miR-203a/MMP-2 pathway [34]. However, the functional roles of DLX6-AS1 in LSCC are still unclear. In our study, we firstly detected the DLX6-AS1 expression in LSCC samples and their pair noncancerous tissues. Our data showed that the expression level of DLX6-AS1 was higher in the LSCC samples than in the noncancerous tissues. The DLX6-AS1 expression was upregulated in 23 patients (23/30, 77%) compared to adjacent noncancerous tissues. DLX6-AS1 knockdown inhibited cell proliferation, cycle and invasion of LSCC cell.

Recently, accumulating references indicated that one potential mechanism of lncRNA was to interact with miRNAs as the sponge and modulate their activity and expression [24, 38]. For example, Yang and their colleagues showed that LOC554202 promoted LSCC cell proliferation, cell cyle and cell invasion via suppressing the miR-31 expression [31]. Shen and their colleagues indicated that lncRNA AC026166.2-001 suppressed that LSCC cell growth and migration through modulating miR-24-3p/p27 expression [39]. Wang and colleagues demonstrated that lncRNA NEAT1 enhanced the LSCC progression via modulating miR-107/CDK6

pathway expression [40]. Moreover, Yan and their colleagues indicated that DLX6-AS1 expression was upregulated in the preeclampsia and regulated trophoblasts invasion and migration via modulating miR-376c/GADD45A expression [41]. In our study, we used Bioinformatics analyse to found that miR-376c was identified to have the complementary binding sites with the DLX6-AS1. By using qRT-PCR assay, the expression level of miR-376c was lower in the LSCC samples than in the noncancerous tissues. Ectopic expression of miR-376c suppressed cell proliferation, cycle and invasion of LSCC cell. Furthermore, we showed that DLX6-AS1 knockdown suppressed cell proliferation, cycle and invasion via regulating miR-376c expression.

In conclusion, we indicated that the expression level of DLX6-AS1 was upregulated in the LSCC samples compared to the noncancerous tissues. DLX6-AS1 knockdown suppressed cell proliferation, cycle and invasion via regulating miR-376c expression. These data suggested that lncRNA DLX6-AS1 might play as an oncogene in LSCC development and tumorigenesis.

Disclosure of conflict of interest

None.

Address correspondence to: Jijun Song, Department of Otolaryngology, Head and Neck Surgery, Zhoukou Central Hospital, Zhoukou 466000, Henan, China. E-mail: songliju1n@126.com

References

[1] Yungang W, Xiaoyu L, Pang T, Wenming L and Pan X. miR-370 targeted FoxM1 functions as a tumor suppressor in laryngeal squamous cell carcinoma (LSCC). Biomed Pharmacother 2014; 68: 149-154.

[2] Wang F, Song G, Liu M, Li X and Tang H. miRNA-1 targets fibronectin1 and suppresses the migration and invasion of the HEp2 laryngeal squamous carcinoma cell line. FEBS Lett 2011; 585: 3263-3269.

[3] Zhao XD, Zhang W, Liang HJ and Ji WY. Overexpression of miR -155 promotes proliferation and invasion of human laryngeal squamous cell carcinoma via targeting SOCS1 and STAT3. PLoS One 2013; 8: e56395.

[4] Lee KM, Choi EJ and Kim IA. MicroRNA-7 increases radiosensitivity of human cancer cells with activated EGFR-associated signaling. Radiother Oncol 2011; 101: 171-176.

[5] Li X, Wang HL, Peng X, Zhou HF and Wang X. miR-1297 mediates PTEN expression and contributes to cell progression in LSCC. Biochem Biophys Res Commun 2012; 427: 254-260.

[6] Gao C, Li X, Tong B, Wu K, Liu Y, Anniko M and Duan M. Up-regulated expression of Dicer reveals poor prognosis in laryngeal squamous cell carcinoma. Acta Otolaryngol 2014; 134: 959-963.

[7] Tian L, Zhang J, Ge J, Xiao H, Lu J, Fu S, Liu M and Sun Y. MicroRNA-205 suppresses proliferation and promotes apoptosis in laryngeal squamous cell carcinoma. Med Oncol 2014; 31: 785.

[8] Li W, Ma H and Sun J. MicroRNA34a/c function as tumor suppressors in Hep2 laryngeal carcinoma cells and may reduce GALNT7 expression. Mol Med Rep 2014; 9: 1293-1298.

[9] Guo Y, Fu W, Chen H, Shang C and Zhong M. miR-24 functions as a tumor suppressor in Hep2 laryngeal carcinoma cells partly through down-regulation of the S100A8 protein. Oncol Rep 2012; 27: 1097-1103.

[10] Long XB, Sun GB, Hu S, Liang GT, Wang N, Zhang XH, Cao PP, Zhen HT, Cui YH and Liu Z. Let-7a microRNA functions as a potential tumor suppressor in human laryngeal cancer. Oncol Rep 2009; 22: 1189-1195.

[11] Minor J, Wang X, Zhang F, Song J, Jimeno A, Wang XJ, Lu X, Gross N, Kulesz-Martin M, Wang D and Lu SL. Methylation of microRNA-9 is a specific and sensitive biomarker for oral and oropharyngeal squamous cell carcinomas. Oral Oncol 2012; 48: 73-78.

[12] Tian L, Li M, Ge J, Guo Y, Sun Y, Liu M and Xiao H. MiR-203 is downregulated in laryngeal squamous cell carcinoma and can suppress proliferation and induce apoptosis of tumours. Tumour Biol 2014; 35: 5953-5963.

[13] Shen Z, Zhan G, Ye D, Ren Y, Cheng L, Wu Z and Guo J. MicroRNA-34a affects the occurrence of laryngeal squamous cell carcinoma by targeting the antiapoptotic gene survivin. Med Oncol 2012; 29: 2473-2480.

[14] Yu X, Wu Y, Liu Y, Deng H, Shen Z, Xiao B and Guo J. miR-21, miR-106b and miR-375 as novel potential biomarkers for laryngeal squamous cell carcinoma. Curr Pharm Biotechnol 2014; 15: 503-508.

[15] Huan JL, Xing L, Lin QH, Xui H and Qin XJ. Long noncoding RNA CRNDE activates Wnt/beta-catenin signaling pathway through acting as a molecular sponge of microRNA-136 in human breast cancer. Am J Transl Res 2017; 9: 1977-1989.

[16] Li J, Lian YF, Yan CS, Cai ZL, Ding J, Ma ZH, Peng P and Wang KM. Long non-coding RNA FOXP4-AS1 is an unfavourable prognostic fac-

tor and regulates proliferation and apoptosis in colorectal cancer. Cell Prolif 2017; 50.
[17] Zhang XY, Tang XY, Ma LJ, Guo YL, Li XS, Zhao LM, Tian CJ, Cheng DJ, Chen ZC and Zhang LX. Schisandrin B down-regulated lncRNA BCYRN1 expression of airway smooth muscle cells by improving miR-150 expression to inhibit the proliferation and migration of ASMC in asthmatic rats. Cell Prolif 2017; 50.
[18] Gao D, Lv AE, Li HP, Han DH and Zhang YP. LncRNA MALAT-1 elevates HMGB1 to promote autophagy resulting in inhibition of tumor cell apoptosis in multiple myeloma. J Cell Biochem 2017; 118: 3341-3348.
[19] Huang JK, Ma L, Song WH, Lu BY, Huang YB, Dong HM, Ma XK, Zhu ZZ and Zhou R. LncRNA-MALAT1 promotes angiogenesis of thyroid cancer by modulating tumor-associated macrophage FGF2 protein secretion. J Cell Biochem 2017; 118: 4821-4830.
[20] Liao YW, Shen LF, Zhao HT, Liu Q, Fu J, Guo Y, Peng RJ and Cheng L. LncRNA CASC2 interacts with miR-181a to modulate glioma growth and resistance to TMZ through PTEN pathway. J Cell Biochem 2017; 118: 1889-1899.
[21] Wang XB, Lv GH, Li J, Wang B, Zhang QS and Lu C. LncRNA-RP11-296A18.3/miR-138/HIF1A pathway regulates the proliferation ECM synthesis of human nucleus pulposus cells (HNPCs). J Cell Biochem 2017; 118: 4862-4871.
[22] Wang XM, Lu XB, Geng ZS, Yang GY and Shi Y. LncRNA PTCSC3/miR-574-5p governs cell proliferation and migration of papillary thyroid carcinoma via Wnt/-catenin signaling. J Cell Biochem 2017; 118: 4745-4752.
[23] Yu QY, Zhou XF, Xia Q, Shen J, Yan J, Zhu JT, Li X and Shu M. Long non-coding RNA CCAT1 that can be activated by c-Myc promotes pancreatic cancer cell proliferation and migration. Am J Transl Res 2016; 8: 5444-5454.
[24] Liu J, Song ZW, Feng C, Lu YL, Zhou Y, Lin Y and Dong CY. The long non-coding RNA SUMO1P3 facilitates breast cancer progression by negatively regulating miR-320a. Am J Transl Res 2017; 9: 5594-5602.
[25] Tan XY, Huang ZG and Li XG. Long non-coding RNA MALAT1 interacts with miR-204 to modulate human hilar cholangiocarcinoma proliferation, migration, and invasion by targeting CXCR4. J Cell Biochem 2017; 118: 3643-3653.
[26] Li JQ, Hu SY, Wang ZY, Lin J, Jian S, Dong YC, Wu XF, Dai L and Cao LJ. Long non-coding RNA MEG3 inhibits microRNA-125a-5p expression and induces immune imbalance of Treg/Th17 in immune thrombocytopenic purpura. Biomed Pharmacother 2016; 83: 905-911.
[27] Peng W, Si S, Zhang Q, Li C, Zhao F, Wang F, Yu J and Ma R. Long non-coding RNA MEG3 functions as a competing endogenous RNA to regulate gastric cancer progression. J Exp Clin Cancer Res 2015; 34: 79.
[28] Zhu H, Zhou X, Chang H, Li H, Liu F, Ma C and Lu J. CCAT1 promotes hepatocellular carcinoma cell proliferation and invasion. Int J Clin Exp Pathol 2015; 8: 5427-5434.
[29] Deng L, Yang SB, Xu FF and Zhang JH. Long noncoding RNA CCAT1 promotes hepatocellular carcinoma progression by functioning as let-7 sponge. J Exp Clin Cancer Res 2015; 34: 18.
[30] He X, Tan X, Wang X, Jin H, Liu L, Ma L, Yu H and Fan Z. C-Myc-activated long noncoding RNA CCAT1 promotes colon cancer cell proliferation and invasion. Tumour Biol 2014; 35: 12181-12188.
[31] Yang S, Wang J, Ge W and Jiang Y. Long noncoding RNA LOC554202 promotes laryngeal squamous cell carcinoma progression through regulating miR-31. J Cell Biochem 2018; 119: 6953-6960.
[32] Li J, Li P, Zhao W, Yang R, Chen S, Bai Y, Dun S, Chen X, Du Y, Wang Y, Zang W, Zhao G and Zhang G. Expression of long non-coding RNA DLX6-AS1 in lung adenocarcinoma. Cancer Cell Int 2015; 15: 48.
[33] Zeng X, Hu Z, Ke X, Tang H, Wu B, Wei X and Liu Z. Long noncoding RNA DLX6-AS1 promotes renal cell carcinoma progression via miR-26a/PTEN axis. Cell Cycle 2017; 16: 2212-2219.
[34] Zhang L, He X, Jin T, Gang L and Jin Z. Long non-coding RNA DLX6-AS1 aggravates hepatocellular carcinoma carcinogenesis by modulating miR-203a/MMP-2 pathway. Biomed Pharmacother 2017; 96: 884-891.
[35] Fu X, Zhang L, Dan L, Wang K and Xu Y. LncRNA EWSAT1 promotes ovarian cancer progression through targeting miR-330-5p expression. Am J Transl Res 2017; 9: 4094-4103.
[36] Hu XG, Jiang HJ and Jiang XJ. Downregulation of lncRNA ANRIL inhibits proliferation, induces apoptosis, and enhances radiosensitivity in nasopharyngeal carcinoma cells through regulating miR-125a. Cancer Biol Ther 2017; 18: 331-338.
[37] Liu B, Shen ED, Liao MM, Hu YB, Wu K, Yang P, Zhou L and Chen WD. Expression and mechanisms of long non-coding RNA genes MEG3 and ANRIL in gallbladder cancer. Tumor Biology 2016; 37: 9875-9886.
[38] He JH, Han ZP, Liu JM, Zhou JB, Zou MX, Lv YB, Li YG and Cao MR. Overexpression of long noncoding RNA MEG3 inhibits proliferation of hepatocellular carcinoma Huh7 cells via negative modulation of miRNA-664. J Cell Biochem 2017; 118: 3713-3721.

[39] Shen Z, Hao W, Zhou C, Deng H, Ye D, Li Q, Lin L, Cao B and Guo J. Long non-coding RNA AC026166.2-001 inhibits cell proliferation and migration in laryngeal squamous cell carcinoma by regulating the miR-24-3p/p27 axis. Sci Rep 2018; 8: 3375.

[40] Wang P, Wu T, Zhou H, Jin Q, He G, Yu H, Xuan L, Wang X, Tian L, Sun Y, Liu M and Qu L. Long noncoding RNA NEAT1 promotes laryngeal squamous cell cancer through regulating miR-107/CDK6 pathway. J Exp Clin Cancer Res 2016; 35: 22.

[41] Tan Y, Xiao D, Xu Y and Wang C. Long non-coding RNA DLX6-AS1 is upregulated in pre-eclampsia and modulates migration and invasion of trophoblasts through the miR-376c/GADD45A axis. Exp Cell Res 2018; 370: 718-724.