European Review for Medical and Pharmacological Sciences 2019; 23: 6562-6569

LncRNA SNHG1 promotes cell proliferation in laryngeal cancer via Notch1 signaling pathway

T. LIU[1], J.-J. ZUO[1], F. LI[1,2], Y.-C. XU[1], A.-Y. ZHENG[1], Z.-Z. TAO[1,2]

[1]Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China
[2]Research Institute of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China

Abstract. – OBJECTIVE: We aimed at elucidating the potential function of long noncoding ribonucleic acids (lncRNAs) small nucleolar RNA host gene 1 (SNHG1) in the progression of laryngeal cancer (LC) and its underlying mechanism.

PATIENTS AND METHODS: Relative level of SNHG1 in LC tissues and controls was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Its expression in LC patients with different tumor stages and statues of lymph node metastasis was examined as well. Correlation between SNHG1 expression and prognosis of LC patients was evaluated by the Kaplan-Meier method. SNHG1 siRNA (si-SNHG1) was constructed for downregulation of SNHG1 expression. Potential effects of downregulated SNHG1 on viability and proliferation of LC cells were detected by cell counting kit-8 (CCK-8) and colony formation assay, respectively. After knockdown of SNHG1, relative levels of Notch1 and hairy, and enhancer of split homolog-1 (Hes1) were determined by qRT-PCR and Western blot. Regulatory effects of SNHG1/Notch1 axis on biological behaviors of LC were finally evaluated.

RESULTS: SNHG1 was upregulated in LC tissues than that of controls. Besides, its level was higher in LC with T3-T4 relative to those of T1-T2. Higher abundance of SNHG1 was identified in LC patients with lymph node metastasis compared with those non-metastatic patients. Survival analysis indicated that LC patients with high-level SNHG1 had worse overall survival. Knockdown of SNHG1 in Tu212 and Hep2 cells downregulated relative levels of Notch1 and Hes1. Moreover, SNHG1 knockdown resulted in decreased viability and proliferative ability of LC cells. Notch1 overexpression could reverse the regulatory effects of SNHG1 on viability and proliferation of LC cells.

CONCLUSIONS: LncRNA SNHG1 is highly expressed in LC tissues. It promotes the proliferation of LC cells by inhibiting Notch1 pathway, thereby promoting the progression of LC.

Key Words:
Laryngeal cancer, SNHG1, Notch1, Proliferation.

Introduction

Laryngeal cancer (LC) is the most common aggressive malignancy of the head and neck. The incidence of LC has increased in recent years[1]. The major features of LC include hoarseness, difficulty in swallowing and breathing, and neck mass[2]. Studies have reported that the etiology of LC involves both genetic factors and environmental factors, but its molecular mechanism is still unclear[3]. At present, therapeutic approaches for LC, including chemotherapy, radiotherapy and surgical intervention have been advanced. However, the prognosis of LC patients is still poor[4,5]. This study focuses on the potential pathogenesis of LC, providing far-reaching clinical significances for LC treatment.

Long noncoding ribonucleic acids (lncRNAs) are a type of RNAs ranging in length from 200 nt to 100,000 nt. They regulate gene expressions at epigenetic, transcriptional, and post-transcriptional levels[6]. Chen et al[7] have shown differentially expressed lncRNAs in different tissues, exerting key regulatory roles in a series of cellular processes, such as metabolism, proliferation, and apoptosis. Many tumor-related lncRNAs have been reported. LncRNA NF-KappaB interacting (LncRNA NKILA), regulates proliferative and invasive abilities of LC cells. LncRNA HOXA11-AS is associated with progression of laryngeal squamous cell carcinoma. Downregulation of lncRNA AFAP1-AS1 inhibits tumor growth by inducing apoptosis and suppressing metastasis in thyroid cancer[8-11]. Cui et al[12] have found that the SNHG1/miR-101-3-p/SOX9/Wnt/β-catenin axis contributes to the aggravation of cancer progression in non-small cell lung cancer. However, the mechanism of SNHG1 in the progression of LC is unclear.

Corresponding Author: Zezhang Tao, MD; e-mail: taozezhang@163.com

The Notch signaling pathway is an intercellular pathway that regulates cell fate[13], proliferation[14], apoptosis[15], stem cell survival and self-renewal[16,17]. The Notch pathway has been reported to exert an important role in tumor cell reprogramming to GSLC phenotype[18,19]. To date, four Notch receptors (Notch1-4) with the corresponding ligand delta-1, delta-3, delta-4, Jagged-1, and Jagged-2, respectively, have been identified[20]. As a transmembrane receptor, Notch1 is capable of regulating the growth and proliferation of glioma[21]. Krikelis et al[22] illustrated the upregulation of Notch-related genes in LC cells.

In this study, we examined SNHG1 level in LC patients and its potential correlation to the prognosis of LC. Subsequently, regulatory effects of SNHG1 on cellular behaviors of LC cells *via* Notch1 pathway were elucidated.

Patients and Methods

Sample Collection

LC tissues and paracancerous tissues (>3 cm away from the tumor edge) were surgically resected from 42 LC patients admitted in Renmin Hospital of Wuhan University from December 2016 to September 2018. Tissue samples were immediately preserved in liquid nitrogen. Enrolled LC patients did not receive preoperative anti-tumor treatments and were pathologically diagnosed with LC. This research was approved by the Renmin Hospital of Wuhan University Ethics Committee. Each patient or their guardian was informed consent.

Cell Culture

Tu212 and Hep2 cell lines were provided by Cell Bank, Chinese Academy of Science (Shanghai, China). Cells were cultured in RIPA-1640 (Roswell Park Memorial Institute (RPMI)-1640, Gibco, Grand Island, NY, USA) containing 10% FBS (fetal bovine serum: Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin, and preserved in a 37°C, 5%CO_2 incubator. Corresponding treatment was performed at the cell confluence of 60%.

Cell Transfection

Until 60% of confluence, cell transfection was performed using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). Transfection vectors used in this study included si-SNHG1, si-NC, complementary deoxyribonucleic acid (cDNA) pcDNA-Notch1 and pcDNA-NC. The medium was replaced at 6 h.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from LC cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was subjected to determination of purity and concentration using a spectrophotometer. The complementary deoxyribonucleic acid (cDNA) was obtained by reverse transcription of RNA and amplified by PCR. Primer sequence were as follows: SNHG1, F: 5′-CCGCTCGAGATTTAGGTGACACTATAGAAGTTCTCATTTTTCTACTGCTCG-3′, R: 5′-ATAGTTTAGCGGCCGCTTTTTTTTTTTTTTTTATGTAATCAATCATTTTAT-3′; Notch1, F: 5′-ATGACTGCCCAGGAAACAAC-3′, R: 5′-GTCCAGCCATTGACACACAC-3′; Hes-1, F: 5′-CAACACGACACCGGACAAAC-3′, R: 5′-CGGAGGTGCTTCACTGTCAT-3′; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), F: 5′-CGGAGTCAACGGATTTGGTCGT-3′, R: 5′-GGGAAGGATCTGTCTCTGACC-3′.

Cell Viability Determination

Cells were seeded in a 96-well plate with 5 replicate wells per group. At the appointed time points, 10 μL of cell counting kit-8 (CCK-8) solution (Dojindo, Kumamoto, Japan) was applied per well. After incubation for 2 h, the recorded absorbance at 450 nm using a microplate reader was used for plotting the growth curve.

Colony Formation Assay

Cells were prepared for suspension and seeded in the 6-well plate (Corning, Corning, NY, USA) with 2.5×10^3 cells per well. Each group had 3 replicate wells. Cells were incubated for 7 days. After fixation in 95% ethanol and dye with 1% violet crystal for 20 min, colonies were captured for counting under a microscope.

Western Blot

Proteins were extracted from cells and loaded for electrophoresis. After transferring on a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), it was blocked in 5% skim milk for 2 h, incubated with primary antibodies at 4°C overnight and secondary antibodies (Abcam, Cambridge, MA, USA) for 2 h. Bands were exposed by enhanced chemiluminescence

(ECL) and analyzed by Image Software (Version X; Silver Springs, MD, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 16.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Data were represented as mean ± SD (standard deviation). The *t*-test was used to analyze intergroup differences. The Kaplan-Meier method was introduced for evaluating the overall survival and differences between curves were analyzed by the log-rank test. $p<0.05$ indicated a significant difference.

Results

Highly Expressed SNHG1 in LC Predicted a Poor Prognosis

Compared to paracancerous tissues, SNHG1 was highly expressed in LC as qRT-PCR data revealed (Figure 1A). Besides, SNHG1 expression remained higher in LC with T3-T4 relative to those with T1-T2 (Figure 1B). In comparison to non-metastatic LC patients, those with lymph node metastasis presented a higher level of SNHG1 (Figure 1C). The Kaplan-Meier analysis indicated that LC patients with high-level SNHG1 had worse survival than those with a low level (Figure 1D). The above data indicated the involvement of SNHG1 in LC, which may be related to poor prognosis of LC.

SNHG1 Knockdown Inhibited the Growth of LC Cells

It is reported that SNHG1 is highly expressed in colorectal cancer cells and promotes the tumorigenesis by upregulating CCND2[23]. To further investigate the role of SNHG1 in LC, we silenced the expression of SNHG1 in Tu212 and Hep2 cells by transfection of si-SNHG1 (Figure 2A). Knockdown of SNHG1 markedly suppressed viability

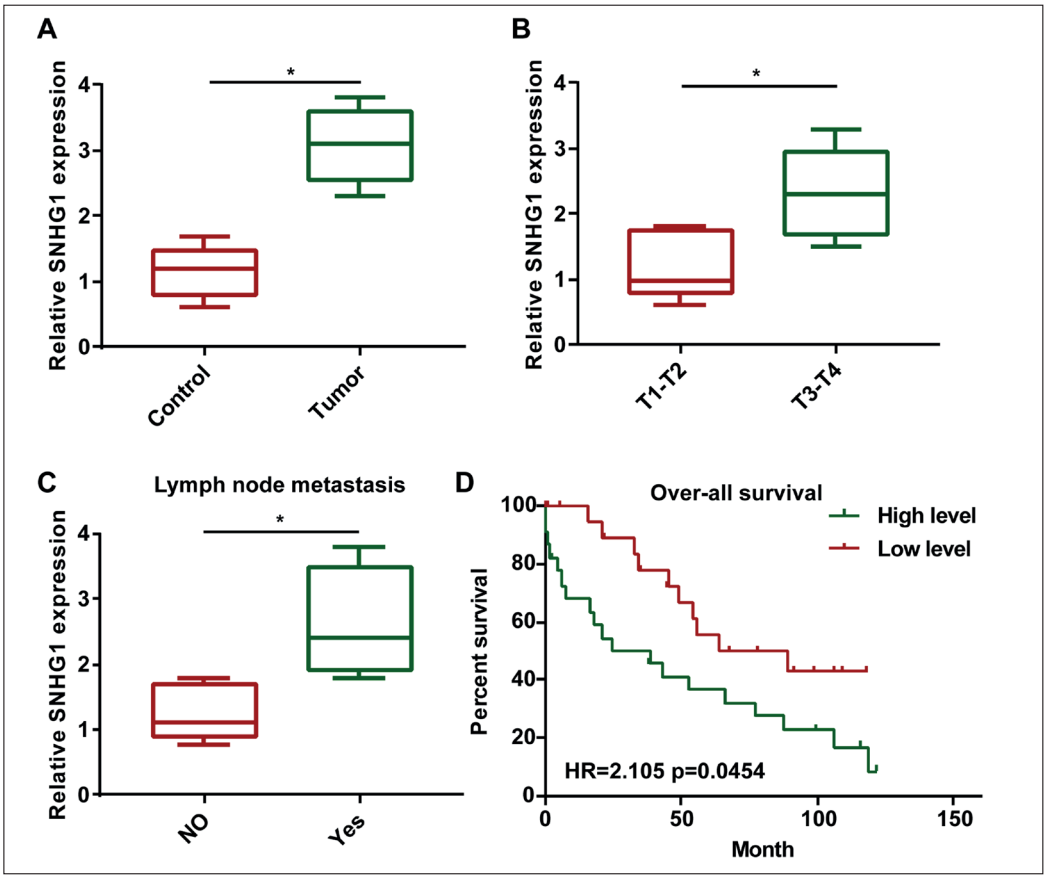

Figure 1. Upregulation of SNHG1 in LC predicted a poor prognosis ***A,*** SNHG1 was highly expressed in LC compared to paracancerous tissues. ***B,*** SNHG1 expression remained higher in LC with T3-T4 relative to those with T1-T2. ***C,*** LC patients with lymph node metastasis presented a higher level of SNHG1 compared with non-metastatic ones. **D,** Kaplan-Meier analysis indicated that LC patients with high-level SNHG1 had worse survival than those with low level.

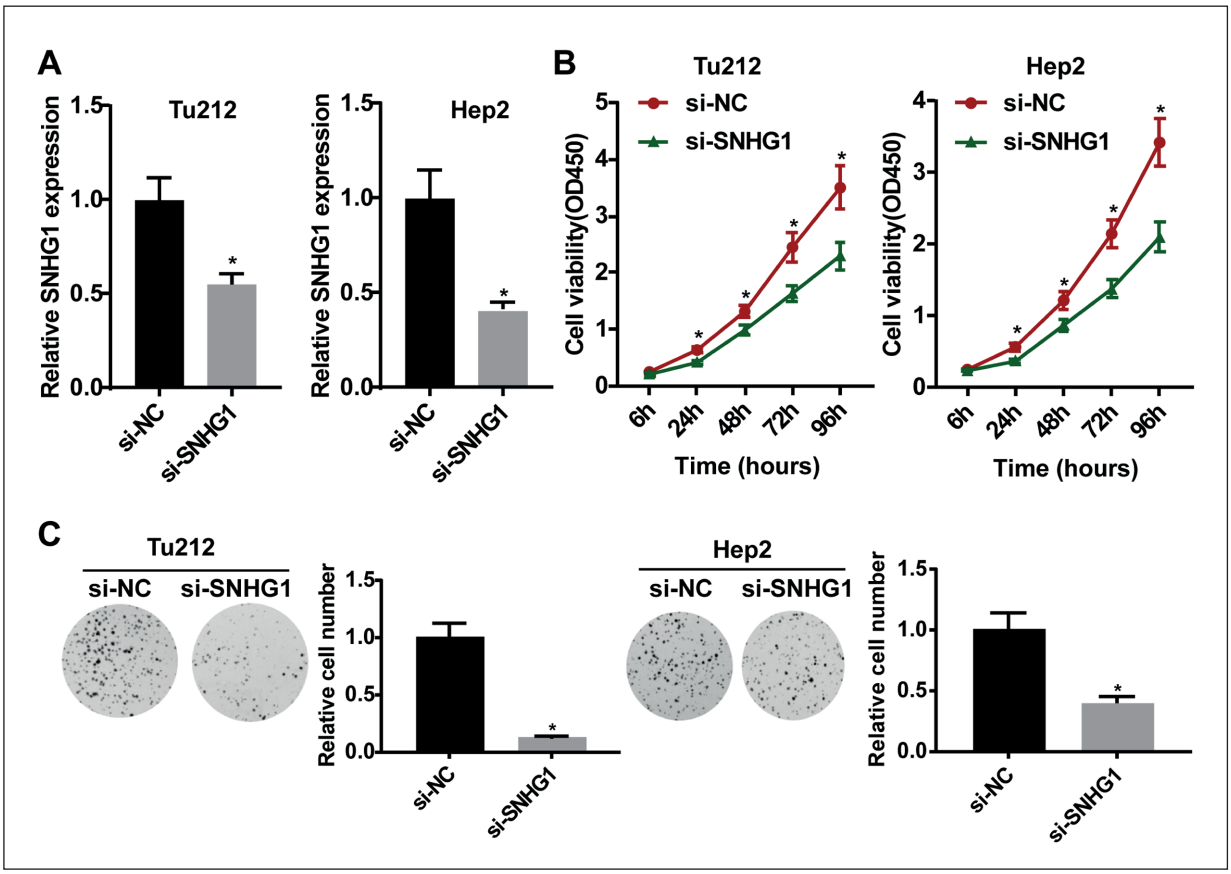

Figure 2. SNHG1 knockdown inhibited the growth of LC cells ***A***, Transfection of si-SNHG1 markedly downregulated SNHG1 level in Tu212 and Hep2 cells. ***B***, CCK-8 assay showed that knockdown of SNHG1 markedly suppressed viability of LC cells. ***C***, Colony formation assay showed that knockdown of SNHG1 inhibited proliferative ability in Tu212 and Hep2 cells.

of LC cells (Figure 2B). Meanwhile, colony formation assay revealed the inhibited proliferative ability in Tu212 and Hep2 cells transfected with si-SNHG1 (Figure 2C). Hence, the knockdown of SNHG1 was proved to inhibit the growth of LC cells.

SNHG1 Knockdown Inhibited the Notch1 Pathway

SNHG1 has been identified to promote the proliferative, migratory and invasive abilities of pituitary tumor cells by activating TGFBR2/SMAD3 and RAB11A/Wnt/β-catenin pathway[24]. Here, we found that SNHG1 knockdown suppressed the mRNA levels of Notch1 and Hes1 in TU212 and Hep2 cells (Figure 3A, 3C). Western blot also showed the downregulated protein levels of Notch1 and Hes1 due to SNHG1 knockdown (Figure 3B, 3D). We believed that SNHG1 negatively regulated expressions of Notch1 and Hes1 in LC cells.

SNHG1 Regulated Proliferative Ability of LC Cells Via the Notch1 Pathway

We speculated the involvement of Notch1 pathway in SNHG1-regulated progression of LC. Firstly, we found the downregulated mRNA levels of Notch1 and Hes, due to SNHG1 knockdown that were partially reversed after co-transfection of si-SNHG1 and pcDNA-Notch1 (Figure 4A, 4C). Similar trends were yielded at the protein levels of Notch1 and Hes1 as well (Figure 4B, 4D). CCK-8 assay showed that Notch1 overexpression reversed the inhibited viability in LC cells with SNHG1 knockdown (Figure 4E). The above results demonstrated that SNHG1 promoted LC cells to proliferate by mediating Notch1 and Hes1.

Discussion

The key role of lncRNA in cancer progression has been extensively studied[25]. Dysregulated ln-

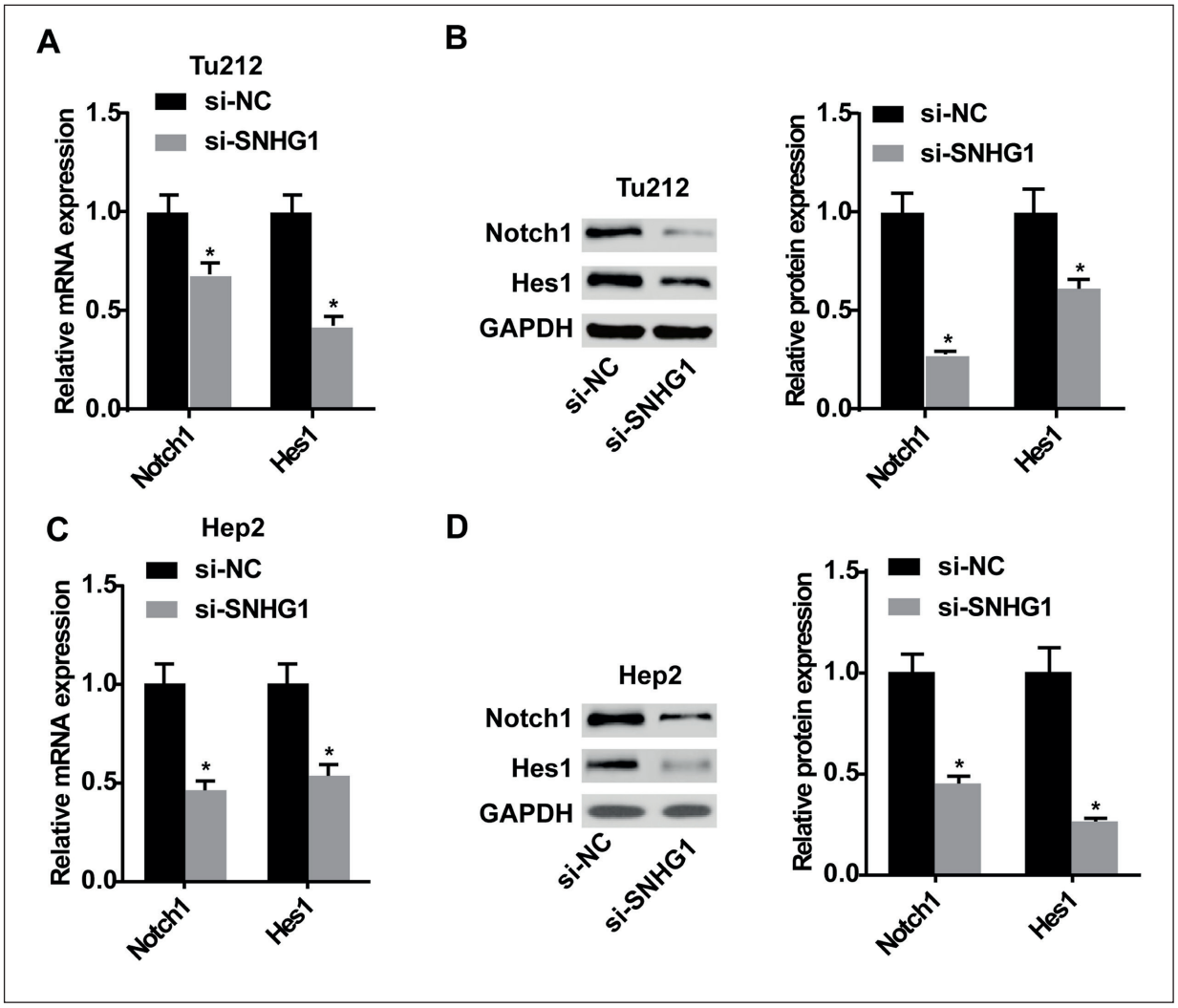

Figure 3. SNHG1 knockdown inhibited the Notch1 pathway ***A, B,*** SNHG1 knockdown suppressed the mRNA and protein levels of Notch1 and Hes1 in TU212 cells. ***C, D,*** SNHG1 knockdown suppressed the mRNA and protein levels of Notch1 and Hes1 in Hep2 cells.

cRNAs are found to be associated with many cancers[23]. A large number of lncRNAs present vital functions in tumor-related pathways, thus mediating the occurrence and progression of tumors by various aspects[26-28]. Previous studies have found that SNHG1 is upregulated in tumors, including colorectal cancer and lung cancer[29,30]. In this paper, SNHG1 was highly expressed in LC patients, especially in those with worse tumor stage. LC patients with high-level SNHG1 were tended to suffer worse prognosis.

A growing number of evidence has proved the carcinogenic role of SNHG1 in tumors. SNHG1 is identified to be upregulated in lung cancer cells. *In vitro* experiments showed that SNHG1 knockdown inhibits lung cancer cells to proliferate[31]. Zhang et al[32] suggested that SNHG1 accelerates the proliferation of liver cancer by inhibiting expressions of p53 and its target genes. Besides, high level of SNHG1 indicates a poor prognosis of liver cancer. The specific mechanism of SNHG1 in LC, however, remains unclear[33]. This study showed that SNHG1 knockdown inhibited relative levels of Notch1 and Hes1. Meanwhile, the viability of LC cells was suppressed by SNHG1 knockdown.

The Notch pathway has been reported[34] to exert anti-tumor or tumorigenic effects in various human malignancies. Li et al[35] have reported that the Notch pathway inhibits tumorigenesis of cervical cancer. Notch1 is a key factor of the Notch pathway. The classical target genes

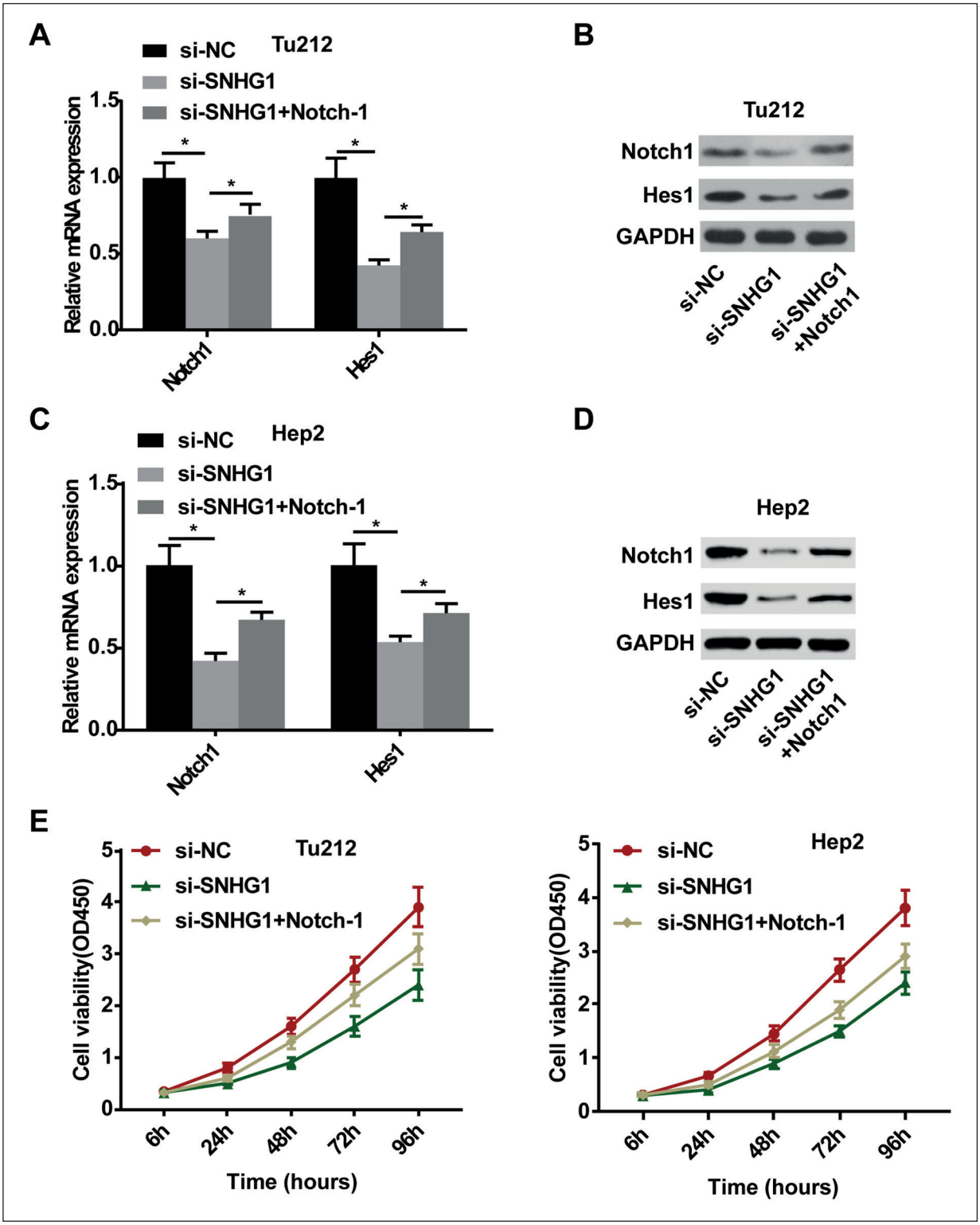

Figure 4. SNHG1 regulated the proliferative ability of LC cells *via* the Notch1 pathway ***A, B,*** Downregulated mRNA and protein levels of Notch1 and Hes1 due to SNHG1 knockdown were partially reversed after co-transfection of si-SNHG1 and pcDNA-Notch1 in TU212 cells. ***C, D,*** Downregulated mRNA and protein levels of Notch1 and Hes1 due to SNHG1 knockdown were partially reversed after co-transfection of si-SNHG1 and pcDNA-Notch1 in Hep2 cells. ***E,*** CCK-8 assay showed that Notch1 overexpression reversed the inhibited viability in LC cells with SNHG1 knockdown.

of Notch1 include hairy enhancer of split (Hes) and Hes related with YRPW motif (Hey)[36]. Yuan et al[37] have found that proliferation and metastasis of NSCLC cells are regulated by Notch1/Hes1. In the present study, knockdown of SNHG1 in LC cells downregulated Notch1 expression. Overexpression of Notch1 reversed the decreased viability due to knockdown of SNHG1. It is indicated that SNHG1 regulated the proliferative ability of LC cells by mediating Notch1 and Hes1.

Conclusions

We found that lncRNA SNHG1 is highly expressed in LC tissues. It promotes the proliferation of LC cells by inhibiting Notch1 pathway, thereby promoting the progression of LC. SNHG1 may be a potential therapeutic and prognostic target for LC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Li Y, Tao C, Dai L, Cui C, Chen C, Wu H, Wei Q, Zhou X. MicroRNA-625 inhibits cell invasion and epithelial-mesenchymal transition by targeting SOX4 in laryngeal squamous cell carcinoma. Biosci Rep 2019; 39: BSR20181882.
2) Tan J, Jing YY, Han L, Zheng HW, Shen JX, Zhang LH, Yu LS. MicroRNA-203 inhibits invasion and induces apoptosis of laryngeal cancer cells via targeting LASP1. Eur Rev Med Pharmacol Sci 2018; 22: 6350-6357.
3) Wang L, Sun J, Cao H. MicroRNA-384 regulates cell proliferation and apoptosis through directly targeting WISP1 in laryngeal cancer. J Cell Biochem 2019; 120: 3018-3026.
4) Smith JD, Birkeland AC, Rosko AJ, Hoesli RC, Foltin SK, Swiecicki P, Mierzwa M, Chinn SB, Shuman AG, Malloy KM, Casper KA, McLean SA, Wolf GT, Bradford CR, Prince ME, Brenner JC, Spector ME. Mutational profiles of persistent/recurrent laryngeal squamous cell carcinoma. Head Neck 2019; 41: 423-428.
5) Miyamaru S, Minoda R, Kodama N. Long-term changes in vocal function after supracricoid partial laryngectomy with cricohyoidoepiglottopexy for laryngeal cancer. Head Neck 2019; 41: 139-145.
6) Zhao L, Cao H, Chi WW, Meng WX, Cui WN, Guo W, Wang BS. Expression profile analysis identifies the long non-coding RNA landscape and the potential carcinogenic functions of LINC00668in laryngeal squamous cell carcinoma. Gene 2019; 687: 47-55.
7) Chen J, Wu D, Zhang Y, Yang Y, Duan Y, An Y. LncRNA DCST1-AS1 functions as a competing endogenous RNA to regulate FAIM2 expression by sponging miR-1254 in hepatocellular carcinoma. Clin Sci (Lond) 2019; 133: 367-379.
8) Ahmad P, Sana J, Slavik M, Slampa P, Smilek P, Slaby O. MicroRNAs involvement in radioresistance of head and neck cancer. Dis Markers 2017; 2017: 8245345.
9) Arshad H, Jayaprakash V, Gupta V, Cohan DM, Ambujakshan D, Rigual NR, Singh AK, Hicks WJ. Survival differences between organ preservation surgery and definitive radiotherapy in early supraglottic squamous cell carcinoma. Otolaryngol Head Neck Surg 2014; 150: 237-244.
10) Cai C, He H, Duan X, Wu W, Mai Z, Zhang T, Fan J, Deng T, Zhong W, Liu Y, Zhong W, Zeng G. miR-195 inhibits cell proliferation and angiogenesis in human prostate cancer by downregulating PRR11 expression. Oncol Rep 2018; 39: 1658-1670.
11) Chen EG, Zhang JS, Xu S, Zhu XJ, Hu HH. Long non-coding RNA DGCR5 is involved in the regulation of proliferation, migration and invasion of lung cancer by targeting miR-1180. Am J Cancer Res 2017; 7: 1463-1475.
12) Cui Y, Zhang F, Zhu C, Geng L, Tian T, Liu H. Upregulated lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/beta-catenin signaling pathway. Oncotarget 2017; 8: 17785-17794.
13) Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res 2004; 6: R605-R615.
14) Cohen B, Shimizu M, Izrailit J, Ng NF, Buchman Y, Pan JG, Dering J, Reedijk M. Cyclin D1 is a direct target of JAG1-mediated Notch signaling in breast cancer. Breast Cancer Res Treat 2010; 123: 113-124.
15) Meurette O, Stylianou S, Rock R, Collu GM, Gilmore AP, Brennan K. Notch activation induces Akt signaling via an autocrine loop to prevent apoptosis in breast epithelial cells. Cancer Res 2009; 69: 5015-5022.
16) D'Angelo RC, Ouzounova M, Davis A, Choi D, Tchuenkam SM, Kim G, Luther T, Quraishi AA, Senbabaoglu Y, Conley SJ, Clouthier SG, Hassan KA, Wicha MS, Korkaya H. Notch reporter activity in breast cancer cell lines identifies a subset of cells with stem cell activity. Mol Cancer Ther 2015; 14: 779-787.
17) Harrison H, Farnie G, Howell SJ, Rock RE, Stylianou S, Brennan KR, Bundred NJ, Clarke RB. Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. Cancer Res 2010; 70: 709-718.

18) Charles N, Ozawa T, Squatrito M, Bleau AM, Brennan CW, Hambardzumyan D, Holland EC. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell 2010; 6: 141-152.

19) Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, Koh C, Zhang J, Li YM, Maciaczyk J, Nikkhah G, Dimeco F, Piccirillo S, Vescovi AL, Eberhart CG. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. Stem Cells 2010; 28: 5-16.

20) Purow BW, Haque RM, Noel MW, Su Q, Burdick MJ, Lee J, Sundaresan T, Pastorino S, Park JK, Mikolaenko I, Maric D, Eberhart CG, Fine HA. Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. Cancer Res 2005; 65: 2353-2363.

21) Chernaya G, Mikhno N, Khabalova T, Svyatchenko S, Mostovich L, Shevchenko S, Gulyaeva L. The expression profile of integrin receptors and osteopontin in thyroid malignancies varies depending on the tumor progression rate and presence of BRAF V600E mutation. Surg Oncol 2018; 27: 702-708.

22) Krikelis D, Kotoula V, Bobos M, Fountzilas E, Markou K, Karasmanis I, Angouridakis N, Vlachtsis K, Kalogeras KT, Nikolaou A, Fountzilas G. Protein and mRNA expression of notch pathway components in operable tumors of patients with laryngeal cancer. Anticancer Res 2014; 34: 6495-6503.

23) Wang F, Zu Y, Zhu S, Yang Y, Huang W, Xie H, Li G. Long noncoding RNA MAGI2-AS3 regulates CCDC19 expression by sponging miR-15b-5p and suppresses bladder cancer progression. Biochem Biophys Res Commun 2018; 507: 231-235.

24) Wang H, Wang G, Gao Y, Zhao C, Li X, Zhang F, Jiang C, Wu B. Lnc-SNHG1 activates the TGFBR2/SMAD3 and RAB11A/Wnt/beta-catenin pathway by sponging MiR-302/372/373/520 in invasive pituitary tumors. Cell Physiol Biochem 2018; 48: 1291-1303.

25) Yang S, Chen J, Yu Y, Li D, Huang M, Yuan L, Yin G. Long noncoding RNA ROR as a novel biomarker for progress and prognosis outcome in human cancer: a meta-analysis in the Asian population. Cancer Manag Res 2018; 10: 4641-4652.

26) Leng Q, Lin Y, Zhan M, Jiang F. An integromic signature for lung cancer early detection. Oncotarget 2018; 9: 24684-24692.

27) Han W, Du X, Liu M, Wang J, Sun L, Li Y. Increased expression of long non-coding RNA SNHG16 correlates with tumor progression and poor prognosis in non-small cell lung cancer. Int J Biol Macromol 2019; 121: 270-278.

28) Lin Y, Leng Q, Zhan M, Jiang F. A plasma long non-coding RNA signature for early detection of lung cancer. Transl Oncol 2018; 11: 1225-1231.

29) Zhao Y, Qin ZS, Feng Y, Tang XJ, Zhang T, Yang L. Long non-coding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) promote cell proliferation in colorectal cancer by affecting P53. Eur Rev Med Pharmacol Sci 2018; 22: 976-984.

30) Li Z, Lu Q, Zhu D, Han Y, Zhou X, Ren T. Lnc-SNHG1 may promote the progression of non-small cell lung cancer by acting as a sponge of miR-497. Biochem Biophys Res Commun 2018; 506: 632-640.

31) You J, Fang N, Gu J, Zhang Y, Li X, Zu L, Zhou Q. Noncoding RNA small nucleolar RNA host gene 1 promote cell proliferation in nonsmall cell lung cancer. Indian J Cancer 2014; 51 Suppl 3: e99-e102.

32) Zhang M, Wang W, Li T, Yu X, Zhu Y, Ding F, Li D, Yang T. Long noncoding RNA SNHG1 predicts a poor prognosis and promotes hepatocellular carcinoma tumorigenesis. Biomed Pharmacother 2016; 80: 73-79.

33) Zhang H, Zhou D, Ying M, Chen M, Chen P, Chen Z, Zhang F. Expression of long non-coding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) exacerbates hepatocellular carcinoma through suppressing miR-195. Med Sci Monit 2016; 22: 4820-4829.

34) Ding X, Ding C, Wang F, Deng W, Yu M, Meng Q, Sun P. Effects of NOTCH1 signaling inhibitor gamma-secretase inhibitor II on growth of cancer stem cells. Oncol Lett 2018; 16: 6095-6099.

35) Li S, Ren B, Shi Y, Gao H, Wang J, Xin Y, Huang B, Liao S, Yang Y, Xu Z, Li Y, Zeng Q. Notch1 inhibition enhances DNA damage induced by cisplatin in cervical cancer. Exp Cell Res 2019; 376: 27-38.

36) Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol 2003; 194: 237-255.

37) Yuan Q, Chen X, Han Y, Lei T, Wu Q, Yu X, Wang L, Fan Z, Wang S. Modification of alpha2,6-sialylation mediates the invasiveness and tumorigenicity of non-small cell lung cancer cells in vitro and in vivo via Notch1/Hes1/MMPs pathway. Int J Cancer 2018; 143: 2319-2330.