LncRNA SNHG10 is downregulated in non-small cell lung cancer and predicts poor survival

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**Abstract**

LncRNA SNHG10 has been characterized as an oncogenic IncRNA in liver cancer. By analyzing TCGA dataset we observed the downregulation of SNHG10 in non-small cell lung cancer (NSCLC), indicating its involvement in this disease. We then analyzed the role of SNHG10 in NSCLC. Tumor and paired non-tumor tissues were harvested from 62 NSCLC patients. The expression of SNHG10 and miR-21 in tissues were determined by RT-qPCR. Overexpression of SNHG10 or miR-21 in NSCLC cells was achieved and the interaction between them was evaluated. Cell proliferation was determined by CCK-8 assay. In this study we found that SNHG10 was downregulated in cancer tissues of NSCLC patients. High expression levels of SNHG10 predicted favorable survival of NSCLC patients. The expression levels of miR-21 were increased in NSCLC and inversely correlated with SNHG10. In NSCLC cells, overexpression of SNHG10 led to increased miR-21 gene methylation and decreased the expression levels of miR-21. In cell proliferation assay, overexpression of SNHG10 attenuated the enhancing effect of overexpression of miR-21 on cell proliferation. SNHG10 was downregulated in non-small cell lung cancer and predicted poor survival. It may downregulate miR-21 through methylation to suppress cancer cell proliferation.

**Introduction**

Non-small cell lung cancer (NSCLC) is the major subtype of lung cancer and is the main cause of cancer-related deaths worldwide [1]. NSCLC has two major subtypes including lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) [2]. Despite of the advances have been made on the treatment and diagnosis of NSCLC, only less than 15% of NSCLC patients can survive for more than 5 years [3]. Therefore, more effective therapeutic approaches are needed. Smoking is the major risk factor for NSCLC [4]. However, never-smokers also develop NSCLC [5], suggesting the involvement of other factors, such as genetic factors, in the molecular pathogenesis of NSCLC [6].

It has been well established that molecular players participate in nearly all aspects of the occurrence and development of NSCLC [7,8]. Increased understanding of the molecular mechanism of NSCLC provides novel targets for the development of anti-cancer approaches, such as targeted therapy [9,10]. LncRNAs are emerging critical players in cancer biology and they participate in cancer mainly by regulating the expression of cancer-related genes [11,12]. Therefore, LncRNAs are potential targets for cancer targeted therapy [13]. SNHG10 has been characterized as an oncogenic IncRNA in liver cancer [14]. However, we observed the downregulation of SNHG10 in NSCLC and its inversely correlation with miR-21 by exploring the TCGA dataset. It is known that miR-21 is a key oncogenic miRNA in cancer [15]. This study was therefore performed to investigate the role of SNHG10 and miR-21 in NSCLC.

**Materials And Methods**

**Patients and follow-up**
This study enrolled a total of 62 NSCLC patients (30 cases of LUAD and 32 cases of LUSC) between May 2013 and January 2015 at Taihe Hospital. This study was approved by the ethics committee of this hospital. All patients were confirmed by histopathological biopsy. No patients received any therapy for any clinical disorders within 3 months before this study. Other severe clinical disorders were excluded from these patients. Based on AJCC staging system, there were 28 cases at stage I or II, and 34 cases at stage III or IV. Informed consent was signed by all patients. From the day of admission, the 62 patients were followed up for 5 years. The patients were visited every month through phone call. Patients died of causes unrelated with NSCLC were excluded from this study. All patients completed the follow-up.

**Tissue collections**

All patients were subjected to biopsy prior to therapy. During biopsy, NSCLC and paired non-tumor tissues were obtained from each patient. All tissues were confirmed by histopathological exam. Tissues were immediately subjected to RNA extraction after collections.

**Cells and transfections**

To match the patients included in this study, the LUSC cell line KLN 205 and LUAD cell line HCC827 were used. Cell culture medium was composed of FBS (10%) and RPMI-1640 medium (90%). A 5% CO\textsubscript{2} incubator was used to cultivate both cell lines at 37 °C. SNHG10 expressing vector was constructed using pcDNA3.1 (Invitrogen) as the backbone vector. Mimic of miR-21 and negative control (NC) miRNA were purchased from Sigma-Aldrich. KLN 205 and HCC827 cells were collected at about 85% confluence and vectors (1 µg) or miRNAs (40 nM) were transfected into cells (10\textsuperscript{8}) using lipofectamine 2000 (Invitrogen). Untransfected cells were used as the control (C) cells. Cells were also transfected with either NC miRNA or empty vector to serve as NC group. Cells were cultivated for further 48 h prior to the following experiments.

**RNA isolation and DNA removal**

Isolation of RNA from tissues and *in vitro* cultivated cells was performed using Ribozol (Invitrogen). DNase I was used to incubate with RNA samples at 37 °C for 2 h to completely digest genomic DNA.

**RT-qPCR**

RNA samples were reverse transcribed into cDNA samples using a Reverse Transcription System (A5001, Promega Corporation). With cDNA samples as template, qPCRs were carried out to determine the expression of SNHG10 using SYBR Green Master Mix (Bio-Rad). The internal control of SNHG10 was 18S rRNA. Addition of poly (A) was added to mature miRNAs, following by miRNA reverse transcriptions and miRNA qPCRs to determine the expression of miR-21 with U6 as internal control. Three replicates were set for each experiment and Ct values were calculated using the 2^{-\Delta\Delta CT} method.

**Methylation-specific PCR (MSP)**
KLN 205 and HCC827 cells transfected with empty vector or SNHG10 expression vector were used to extract genomic DNAs using Genomic DNA Extraction Kit (ab156900, Abcam). DNA samples were converted using DNA Methylation-Gold™ kit (ZYMO RESEARCH). After that, PCRs were performed using Taq 2X master mix (NEB) to evaluate the methylation of miR-21.

**Cell Counting Kit-8 (CCK-8) assay**

Cells with transfections were subjected to cell proliferation analysis using CCK-8 kit (Dojindo). Cells were washed with ice-cold PBS, followed by cell counting. After that, 3,000 cells in 0.1 ml medium were transferred to each well of a 96-well plate, followed by cell culture at 37 °C. OD values (450 nm) were measured every 24 h for a total of 4 d. At 4 h before the measurement of OD values, CCK-8 solution was added into each well to reach 10%.

**Statistical analysis**

Mean and mean ± SD values were calculated. Paired t test was used to compare paired tissues. ANOVA Tukey’s test was used to compare multiple groups. Correlations were analyzed by linear regression. To analyze survival, the 62 patients were divided into high and low SNHG10 level groups (n = 31, with median expression level of SNHG10 in NSCLC tissues as cutoff value). Survival curves were plotted and compared by log-rank test. \( P < 0.05 \) was statistically significant.

**Results**

**Downregulation of SNHG10 is correlated with the poor survival of NSCLC patients**

TCGA dataset was explored to analyze the expression of SNHG10 in NSCLC. It was observed that SNHG10 was downregulated in both LUAD (4.04 vs. 8.20) and LUSC (5.71 vs. 8.29) in comparison to that in tumor tissues. To confirm the downregulation of SNHG10 in NSCLC, expression of SNHG10 in paired tissues was determined by RT-qPCR. Compared to non-tumor tissues, NSCLC tissues exhibited significantly lower expression levels of SNHG10 (Fig. 1A, \( p < 0.001 \)). Survival curve analysis showed that, compared to patients in low SNHG10 level group, patients in high SNHG10 level group showed higher overall survival rate (Fig. 1B).

**MiR-21 was upregulated in NSCLC and inversely correlated with SNHG10**

The expression of miR-21 in paired NSCLC and non-tumor tissues from the 62 patients were determined by RT-qPCR. Compared to non-tumor tissues, NSCLC tissues exhibited significantly higher expression levels of miR-21 (Fig. 2A, \( p < 0.001 \)). Correlation analysis showed that the expression levels of SNHG10 and miR-21 were inversely and significantly correlated across NSCLC tissues (Fig. 2B), but not non-tumor tissues (Fig. 2C).

**SNHG10 downregulated miR-21 in NSCLC cell through methylation**
SNHG10 expression vector or miR-21 mimic was transfected into KLN 205 and HCC827 cells. Transfections were confirmed by RT-qPCR (Fig. 3A, \( p < 0.05 \)). Overexpression of SNHG10 resulted in downregulation of miR-21 in these cells (Fig. 3B, \( p < 0.05 \)). In contrast, overexpression of miR-21 did not affect the expression of SNHG10 (Fig. 3C, \( p > 0.05 \)). MSP was performed to evaluate the effects of overexpression of SNHG10 on miR-21. Compared to cells transfected with empty pcDNA 3.1 vector, cells transfected with SNHG10 expression vector showed significantly increased methylation of miR-21 (Fig. 3D).

**Overexpression of SNHG10 attenuated the enhancing effect of overexpression of miR-21 on cell proliferation**

The roles of SNHG10 and miR-21 in regulating the proliferation of KLN 205 and HCC827 cells were evaluated by CCK-8. It was observed that, compared to control cells, decreased proliferation of cells was observed after the overexpression of SNHG10, while increased proliferation of cells was observed after the overexpression of miR-21. Moreover, overexpression of SNHG10 attenuated the enhancing effect of overexpression of miR-21 on cell proliferation (Fig. 4, \( p < 0.05 \)).

**Discussion**

In the present study, we aimed to investigate the role and underlying mechanism of SNHG10 in NSCLC. Clinical data showed that SNHG10 was downregulated in NSCLC and predicted poor survival of NSCLC patients. Additionally, miR-21 was up-regulated and negatively correlated with SNHG10 in NSCLC. In two NSCLC cell lines, we revealed that SNHG10 reduced miR-21 via methylation. Moreover, SNHG10 inhibited the proliferation of NSCLC cells by targeting miR-21. Therefore, SNHG10 is a tumor suppressor in NSCLC.

Increasing numbers of IncRNAs have been found to be involved in the development of cancer. For example, IncRNA DANCR can enhance cancer cell migration and invasion in gastric cancer [16]. LncRNA XIST can induce proliferation in pancreatic cancer cells [17]. A recent study reported that SNHG10 is an oncogenic IncRNA in liver cancer. It is reported that SNHG10 was upregulated in liver cancer and formed a positive feedback loop with its homolog SCARNA13, thereby promoting cancer metastasis [14]. Interestingly, SNHG10 was remarkably downregulated in NSCLC according to our analyses of TCGA dataset. We confirmed this finding by determining the expression of SNHG10 in paired NSCLC and non-tumor tissues. In two NSCLC cell lines, overexpression of SNHG10 resulted in decreased proliferation of NSCLC cells. Therefore, SNHG10 is likely a tumor suppressor IncRNA in NSCLC, and SNHG10 may play different roles in different types of cancer, suggesting that NSCLC and liver cancer may have different molecular pathogenesis.

Even with active treatments, such as surgical resection and chemotherapy, the overall survival of NSCLC is still poor [18,19]. In this study we showed that overexpression of SNHG10 suppressed the proliferation of NSCLC and high expression levels of SNHG10 were correlated with the favorable survival of NSCLC patients. Therefore, overexpression of SNHG10 may serve as a target for the treatment of NSCLC. In
addition, measuring the expression levels of SNHG10 before therapy may assist the prognosis of NSCLC, thereby guiding the determination of treatments and improve patients’ survival.

MiR-21 is a well-characterized oncogenic miRNA that promotes tumorigenesis in many cancers, such as cervical, breast and gastric cancers [20-22]. In NSCLC, miR-21 is a serum biomarker for detection of early-stage NSCLC, and has been found to enhance cancer progression by targeting its targets genes, like SOCS1, PTEN, SOX7 [23-25]. However, the upstream regulators of miR-21 have not been well studied. In this study, we found that miR-21 was negatively correlated with SNHG10 in NSCLC tissues. Moreover, SNHG10 was directly regulated by SNHG10 through methylation, and it was involved in the inhibitory effect of SNHG10 on NSCLC cell proliferation. Hence, SNHG10 is an upstream regulator of miR-21 and can inhibits its oncogenic function in NSCLC. It is worth noting that SNHG10 and miR-21 were only closely correlated across NSCLC tissues, but not non-tumor tissues. Therefore, certain pathological factors may mediate the interaction between them, and further studies are needed.

**Conclusion**

In conclusion, SNHG10 is downregulated in NSCLC, and miR-21 was upregulated in NSCLC. SNHG10 predicts the prognosis of NSCLC and it can downregulate miR-21 through methylation to suppress the proliferation of cancer cells.

**Declarations**

**Ethics approval and consent to participate**

The present study was approved by the Ethics Committee of Tianhe Hospital. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All patients and healthy volunteers provided written informed consent before their inclusion within the study.

**Consent for publication**

All subjects participating in the image acquisition signed the consent form.

**Data availability statement**

The data used to support the findings of this study are included within the article.

**Competing interests**

The authors declare that there is no conflict of interests.

**Funding**

None
Authors' contributions

W.F. designed and directed this study. L.M. and W.L.L performed the experiments and wrote the manuscript. C.C.H participated in revision of the manuscript. S.S.M. provided assistance for data analysis. All authors read and approved the manuscript for publication.

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None

Abbreviations

NSCLC: non-small cell lung cancer; LUSC: lung squamous cell carcinoma; LUAD: lung adenocarcinoma; MSP: Methylation-specific PCR; CCK-8: Cell Counting Kit-8 assay

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Figures

Figure 1

Downregulation of SNHG10 is correlated with the poor survival of NSCLC patients. Expression of SNHG10 in paired tissues was determined by RT-qPCR. Levels of SNHG10 expression were compared between NSCLC and non-tumor tissues. Mean values were compared (A). ***, p < 0.001. To analyze survival, the 62 patients were divided into high and low SNHG10 level groups (n = 31, with median expression level of SNHG10 in NSCLC tissues as cutoff value). Survival curves were plotted and compared by log-rank test (B).
Figure 2

MiR-21 was upregulated in NSCLC and inversely correlated with SNHG10. Expression of miR-21 in paired NSCLC and non-tumor tissues from the 62 patients was determined by RT-qPCR. Levels of miR-21 expression were compared between NSCLC and non-tumor tissues. Mean values were compared (A). ***, p < 0.001. Linear regression was performed to analyze the correlations between SNHG10 and miR-21 across NSCLC tissues (B) and non-tumor tissues (C).
SNHG10 downregulated miR-21 in NSCLC cell through methylation SNHG10 expression vector or miR-21 mimic was transfected into KLN 205 and HCC827 cells. Transfections were confirmed by RT-qPCR (A). The effects of SNHG10 overexpression on miR-21 (B), and the effects of miR-21 overexpression on SNHG10 (C) were also analyzed by RT-qPCR. MSP was performed to analyze the effects of SNHG10 overexpression on miR-21 (D). Mean±SD values were presented and compared. M, methylation; U, un-methylation; *, p < 0.05.

Figure 3
Figure 4

SNHG10 overexpression attenuated the enhancing effect of miR-21 overexpression on cell proliferation. The roles of SNHG10 and miR-21 in regulating the proliferation of KLN 205 and HCC827 cells were analyzed by CCK-8. Mean ± SD values were presented and compared. *, p < 0.05.