microRNA-577 inhibits cell proliferation and invasion in non-small cell lung cancer by directly targeting homeobox A1

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Abstract. An increasing number of studies have indicated that the dysregulation of microRNAs (miRNAs/miR) is closely associated with non-small cell lung cancer (NSCLC) development and progression by acting as tumor suppressors or oncogenes. Therefore, an in-depth understanding of the biological roles of miRNAs in NSCLC may provide novel therapeutic methods for the treatment of patients with this disease. In the present study, reverse transcription-quantitative polymerase chain reaction was used to detect miR-577 expression in NSCLC tissues and cell lines. Cell Counting Kit-8 and Transwell invasion assays were performed to determine the effects of miR-577 on NSCLC cell proliferation and invasion. Luciferase reporter assays were used to demonstrate the relationship between miR-577 and homeobox A1 (HOXA1) in NSCLC cells. The results revealed that miR-577 was markedly downregulated in NSCLC tissues and cell lines. Additionally, restoration of miR-577 expression significantly decreased the proliferation and invasion of NSCLC cells. Furthermore, miR-577 negatively regulated HOXA1 expression in NSCLC cells by directly binding to its 3'-untranslated region. HOXA1 was significantly upregulated in NSCLC tissues, and its upregulation was inversely correlated with miR-577. Notably, restored HOXA1 expression abrogated the reduced proliferation and invasion of NSCLC cells caused by miR-577 overexpression. Taken together, these results indicated that miR-577 may have served tumor suppressive roles in NSCLC by directly targeting HOXA1. Therefore, this miRNA may be developed as a potential therapeutic target for the therapy of patients with NSCLC.

Introduction

Lung cancer ranks as the third most common human malignancy and the leading cause of cancer-associated mortality worldwide (1). Lung cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) subtypes, based on the pathological characteristics (2). NSCLC is the main type of lung cancer, accounting for approximately 85% of lung cancer cases (3). It may be further classified into three major histotypes: Adenocarcinoma, squamous cell carcinoma and large cell carcinoma (4). In recent decades, the incidence of NSCLC has markedly increased in many countries, including China (5,6). Despite considerable advancement in several treatments, patients with NSCLC diagnosed at the advanced stage have extremely poor prognosis, with a 5-year survival rate of <5% (7,8). Rapid tumor growth, recurrence and metastasis are the major factors responsible (9). The poor outcomes of NSCLC highlight the urgent need to better understand the molecular mechanisms underlying NSCLC occurrence and development, which may facilitate the identification of effective therapeutic techniques.

microRNAs (miRNAs/miRs) have emerged as a group of endogenous, non-coding and short RNA molecules that function as regulators of gene expression by base pairing with a partially complementary site in the 3'-untranslated regions (3'-UTRs) of their target genes, to induce mRNA degradation or repress mRNA translation (10,11). Approximately one-third to one-half of all human protein-coding genes are directly or indirectly modulated by miRNAs (12), which indicates that miRNAs may be closely associated with a variety of disorders, including NSCLC (13). Several studies have reported that numerous miRNAs are dysregulated in NSCLC. For example, miR-183 (14), miR-215 (15) and miR-615 (16) are downregulated in NSCLC, whereas miR-9 (17), miR-106b (18) and miR-875 (19) are upregulated. aberrantly expressed miRNAs may function as tumor-suppressors or oncogenes in NSCLC initiation and progression, depending on the characteristics of their target genes (20). Hence, miRNAs have potential as targets in NSCLC diagnosis, treatment and prognosis.

miR-577 has been reported to be abnormally expressed in several tumor types (21-24). However, the expression pattern, roles and underlying mechanisms of miR-577 in NSCLC have not been clarified. In the present study, miR-577 expression...
was detected in NSCLC tissues and cell lines and the effects of miR-577 on the proliferation and invasion of NSCLC cells were examined in vitro. In addition, the underlying mechanisms of miR-577 in NSCLC cells were investigated. It was found that miR-577 was downregulated in NSCLC, and miR-577 inhibited the NSCLC cell proliferation and invasion by directly targeting homeobox A1 (HOXA1). The present study may provide an effective target for the therapy of patients with lung cancer.

Materials and methods

Ethical statement and clinical specimens. The present study was approved by the Ethical Committee of China-Japan Union Hospital of Jilin University (approval no. 20140311). All patients enrolled in the research provided written consent and were informed of the study’s purpose. In total, 35 pairs of NSCLC and adjacent non-tumor tissues were collected from patients (21 males and 14 females; age range, 42-69) who received surgical resection at China-Japan Union Hospital of Jilin University between March 2014 and April 2017. None of the patients underwent any pre-operative chemotherapy or radiotherapy treatment. Patients who had been treated with pre-operative chemotherapy or radiotherapy were excluded from this study. All tissue specimens were rapidly frozen in liquid nitrogen and stored at -80°C.

Cell lines. A nontumorigenic bronchial epithelium cell line (BEAS2B) and four NSCLC cell lines (NCI-H460, SK-MES-1, NCI-H522 and A549) were purchased from the American Type Culture Collection (Manassas, VA, USA). BEAS2B cells were cultured in LHC9 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). All NSCLC cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.). Four NSCLC cell lines (NCI-H522 and A549) were purchased from the American Type Culture Collection (Manassas, VA, USA). BEAS2B and four NSCLC cell lines (NCI-H460, SK-MES-1, NCI-H522 and A549) were purchased from the American Type Culture Collection (Manassas, VA, USA). BEAS2B cells were cultured in LHC9 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). All NSCLC cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and 1% penicillin/streptomycin mixture. Cells were cultured in a humidified atmosphere at 37°C containing 5% CO₂.

Transfection. Cells were plated into 6-well culture plates with a density of 7x10⁵ cells/well 1 day before transfection and maintained in an incubator at 37°C containing 5% CO₂. miR-577 mimics and miRNA mimics negative control (miR-NC) were chemically synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China) and transfected into cells at a final concentration of 100 nM. The miR-577 mimics sequence was 5'-UGA AUA AAG UGG UGG AGC-3' and the miR-NC sequence was 5'-UUC UCC GGA CAC GUG UAC-3'. HOXA1 overexpression plasmid pcDNA3.1-HOXA1 (pc-HOXA1) and empty pcDNA3.1 plasmid were provided by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Cells were transfected with miRNA mimics (100 pmol) or plasmid (4 μg) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer’s protocol. Following a 6 h incubation at 37°C with 5% CO₂, the culture medium was removed and replaced with fresh DMEM containing 10% FBS.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from tissue specimens or cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer’s instructions. The concentration of total RNA was determined with a NanoDrop 2000/2000c spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). The All-in-One™ miRNA qRT-PCR Detection kit (GeneCopoeia, Inc., Rockville, MD, USA) was used to detect miR-577 expression, and was carried out according to the manufacturer’s instructions. To analyze HOXA1 mRNA expression, reverse transcription was conducted using PrimeScript 1st Strand cDNA Synthesis kit (Takara Biotechnology Co., Ltd., Dalian, China). The synthesized complementary DNA (cDNA) was then subjected to qPCR using SYBR Premix Ex Taq™ (Takara Biotechnology Co., Ltd.), and qPCR was performed according to the manufacturer’s instructions. The relative expression of miR-577 and HOXA1 was calculated using the 2⁻ΔΔCq method (25) and was normalized to U6 snRNA and GAPDH mRNA, respectively. The primers were designed as follows: miR-577, 5'-TCCGCT AGATGAAATATTGG-3' (forward) and 5'-GTGCAGGCT CGGAGGTT-3' (reverse); U6, 5'-CCTTCCTCGCCGACACA-3' (forward) and 5'-AACGCTTCAAGAATTTCGGT-3' (reverse); HOXA1, 5'-CTCTGGGATACCCATCATTAGC-3' (forward) and 5'-GCACGACTGGAAGTTGATACCC-3' (reverse); and GAPDH, 5'-CTGCTTCGCTACTGACGACC-3' (forward) and 5'-AAGGTTCTGTTAGGGAATG-3' (reverse).

Cell Counting Kit-8 (CCK-8) assay. Cells were harvested and plated into 96-well plates at a density of 3x10⁴ cells/well 24 h after transfection. Cells were incubated at 37°C with 5% CO₂, and proliferation was detected at different time points (0, 24, 48 and 72 h). CCK-8 reagent (10 μl; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added into each well for a further 2 h at 37°C in a humidified incubator. The optical density of each well was measured at 450 nm using a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Transwell invasion assay. Transwell inserts (24-well insert; Corning Incorporated, Corning, NY, USA) containing 8 μm pore size membranes were employed to determine NSCLC cell invasion capacity. Transwell inserts were coated with Matrigel® (BD Biosciences, San Jose, CA, USA) and dried overnight under aseptic conditions. Transfected cells were harvested 24 h after transfection, suspended into DMEM without FBS and plated into the upper Transwell inserts at a density of 5x10⁴ cells/insert. DMEM containing 10% FBS was used as a chemoattractant in the lower compartment of Transwell inserts. Transfected cells were then incubated at 37°C with 5% CO₂ for 24 h. The non-invaded cells remaining on the upper side of the membranes were wiped off with a cotton swab. Invaded cells were fixed with 4% paraformaldehyde at 37°C for 30 min and stained with 0.1% crystal violet at 37°C for 30 min. Images of five randomly-selected fields of view for per Transwell insert were captured under an inverted microscope (x200 magnification; CKX41; Olympus Corporation, Tokyo, Japan). The invasive ability was quantified by counting the average number (mean) of invaded cells in the images.

Bioinformatics prediction and luciferase reporter assay. TargetScan 7.2 (www.targetscan.org) and miRDB 5.0
miR-577 was used to search for the potential targets of miR-577. These indicated that the 3'-UTR of HOXA1 contained the putative miR-577 binding site. The 3'-UTR of HOXA1 containing the wild-type (Wt) or mutant (Mut) miR-577-binding sequences was generated (Shanghai GenePharma Co., Ltd.). The chemically synthesized Wt and Mut fragments were inserted into pMIR-GLOTM Luciferase vector (Promega Corporation, Madison, WI, USA) and defined as pMIR-Wt-HOXA1-3'-UTR and pMIR-Mut-HOXA1-3'-UTR, respectively. Cells were plated into 24-well plates at a density of 1.0x10^4 cells per well. Luciferase reporter plasmids were introduced into cells in 24-well plates using Lipofectamine® 2000 and co-transfected with miR-577 mimics or miR-NC. After a 48 h culture, luciferase activity was measured using a Dual-Luciferase Reporter Assay system (Promega Corporation) as per the manufacturer's protocol, and was normalized to Renilla luciferase activity. Western blot analysis. Total protein was extracted from tissue specimens or cells using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). Following protein extraction, a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Haimen, China). The total protein was subjected to 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Beyotime Institute of Biotechnology). Membranes were blocked in 5% fat-free milk in Tris-buffered saline-0.1% Tween-20 (TBST). The membranes were incubated overnight at 4°C with primary antibodies against HOXA1 (cat. no. ab168179; 1:1,000 dilution; Abcam, Cambridge, UK) or GAPDH (cat. no. ab110305; 1:1,000 dilution; Abcam). Following extensive washing with TBST, the membranes were incubated at room temperature for 2 h with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (cat. no. ab205719; 1:5,000 dilution; Abcam). An enhanced chemiluminescence detection kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to visualize protein signals. Protein expression was quantified using Quantity One software version 4.62 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. All data were expressed as the mean ± standard deviation from at least three independent experiments. Two-tailed Student's t-test was used to analyze the difference between two groups. The difference between multiple groups was investigated using one-way analysis of variance with Student-Newman-Keuls as a post-hoc test. Spearman's correlation analysis was performed to explore the relationship between miR-577 and HOXA1 mRNA in NSCLC tissues. P<0.05 was considered to indicate a statistically significant difference.

Results
miR-577 is downregulated in NSCLC tissues and cell lines. To determine the expression pattern of miR-577 in NSCLC, total RNA was isolated from 35 pairs of NSCLC tissues and adjacent non-tumor tissues, and RT-qPCR analysis was conducted. The data indicated that miR-577 expression was downregulated in NSCLC tissues, compared with non-tumor tissues (P<0.05; Fig. 1A). To confirm this observation, the expression of miR-577 in NSCLC cell lines was also detected. Compared with BEAS-2B, all four NSCLC cell lines (H460, SK-MES-1, H522 and A549) had decreased miR-577 expression, compared with that in BEAS-2B cells (P<0.05; Fig. 1B). miR-577 expression in H460 and A549 cells was the lowest among the four NSCLC cell lines; therefore, these two NSCLC cell lines were selected for subsequent functional experiments.

miR-577 restricts proliferation and invasion of NSCLC cells. To elucidate the functions of miR-577 in NSCLC, miR-577 mimics were transfected to increase miR-577 expression in H460 and A549 cells. RT-qPCR results confirmed that miR-577 expression was significantly upregulated in H460 and A549 cells transfected with miRNA mimics (P<0.05; Fig. 2A). The effect of miR-577 overexpression on NSCLC cell proliferation was determined by CCK-8 assay. Ectopic miR-577 expression evidently decreased the proliferative ability of H460 and A549 cells, compared with the miR-NC groups (P<0.05; Fig. 2B). Transwell invasion assays were then performed to detect invasion of H460 and A549 cells transfected with miR-577 mimics or miR-NC. miR-577 overexpression significantly inhibited H460 and A549 cell invasion (P<0.05; Fig. 2C). These results indicated that miR-577 may be a tumor suppressor in NSCLC.
HOXA1 is a direct target gene of miR-577 in NSCLC cells. To examine the mechanisms by which miR-577 affected NSCLC cell proliferation and invasion, bioinformatics analysis was performed to predict the putative targets of miR-577. The miRNA target prediction algorithms (TargetScan and miRDB) indicated that the 3'-UTR of HOXA1 contained the putative miR-577-binding site (Fig. 3A). To determine whether HOXA1 was a direct target of miR-577, luciferase reporter assays were performed in H460 and A549 cells following co-transfection with miR-577 mimics or miR-NC and pMIR-Wt-HOXA1-3'-UTR or pMIR-Mut-HOXA1-3'-UTR. miR-577 overexpression suppressed the luciferase activity of pMIR-Wt-HOXA1-3'-UTR in H460 and A549 cells (P<0.05). There was no decrease in the luciferase activity in the pMIR-Mut-HOXA1-3'-UTR transfected group (Fig. 3B). Next, the mRNA and protein expression of HOXA1 in H460 and A549 cells was measured, following transfection with miR-577 mimics or miR-NC. The results revealed that miR-577 mimic transfection in H460 and A549 cells significantly reduced the mRNA (P<0.05; Fig. 3C) and protein (P<0.05; Fig. 3D) expression of HOXA1. Taken together, these results demonstrated that HOXA1 was a direct target of miR-577 in NSCLC cells.

HOXA1 is overexpressed in NSCLC tissues and inversely correlated with miR-577 level. To further evaluate the relationship between miR-577 and HOXA1 in NSCLC, HOXA1 expression was detected in 35 pairs of NSCLC tissues and adjacent non-tumor tissues. The mRNA expression of HOXA1 was notably higher in NSCLC tissues, compared with that in non-tumor tissues (P<0.05; Fig. 4A). In addition, the protein expression of HOXA1 in several pairs of NSCLC tissues and adjacent non-tumor tissues was determined by western blot analysis. The results indicated that HOXA1 protein expression...
Figure 3. HOXA1 is a direct target gene of miR-577 in NSCLC cells. (A) The putative Wt and Mut miR-577 binding sites in the 3′-UTR of HOXA1. (B) Luciferase activity was detected in H460 and A549 cells that were co-transfected with miR-577 mimics or miR-NC and pMIR-Wt-HOXA1-3′-UTR or pMIR-Mut-HOXA1-3′-UTR. (C and D) The mRNA and protein expression of HOXA1 was determined by (C) reverse transcription-quantitative polymerase chain reaction and (D) western blot analysis in H460 and A549 cells following transfection with miR-577 mimics or miR-NC. *P<0.05 vs. miR-NC. HOXA1, homeobox A1; NSCLC, non-small cell lung cancer; miR, microRNA; NC, negative control; UTR, untranslated region; Wt, wild-type; Mut, mutated.

Figure 4. HOXA1 is upregulated in NSCLC tissues, and its expression is inversely correlated with miR-577. (A) Reverse transcription-quantitative polymerase chain reaction and (B) western blot analysis were applied to determined HOXA1 mRNA and protein expression in NSCLC tissues and adjacent non-tumor tissues. (C) Protein expression was quantified by densitometry. *P<0.05 vs. non-tumor tissues. (D) Relationship between miR-577 and HOXA1 mRNA expression in NSCLC tissues was determined by Spearman’s correlation analysis. r=-0.5896, P=0.0002. HOXA1, homeobox A1; NSCLC, non-small cell lung cancer; miR, microRNA.
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was upregulated in NSCLC tissues, compared with the adjacent non-tumor tissues (P<0.05; Fig. 4B and C). Furthermore, an inverse association between miR-577 and HOXA1 mRNA expression in NSCLC tissues was observed (r=-0.5896, P=0.0002; Fig. 4D).

**Restored HOXA1 expression prevents the inhibitory effects of miR-577 overexpression in NSCLC cells.** Given that HOXA1 was identified as a direct target of miR-577, whether HOXA1 was required for the suppressive roles of miR-577 on NSCLC cells was further clarified. HOXA1 overexpression plasmid pcDNA3.1-HOXA1 (pc-HOXA1) was used to restore HOXA1 expression in H460 and A549 cells. HOXA1 expression was significantly increased in pc-HOXA1-transfected H460 and A549 cells, compared with cells transfected with empty pcDNA3.1 plasmid (P<0.05; Fig. 5A). Next, rescue experiments were performed by co-transfecting miR-577 mimics and pc-HOXA1 or pcDNA3.1 into H460 and A549 cells. Following transfection, the decreased HOXA1 protein level in H460 and A549 cells caused by miR-577 overexpression was recovered by pc-HOXA1 (P<0.05; Fig. 5B). Similarly, CCK-8 and Transwell invasion assays confirmed that HOXA1 restoration abolished the inhibitory effects of miR-577 mimics on H460 and A549 cell proliferation (P<0.05; Fig. 5C and D) and invasion (P<0.05; Fig. 5E). These results suggested that miR-577 served a tumor suppressive role in NSCLC, at least partially through targeting HOXA1.

**Discussion**

An increasing number of studies have indicated the presence of aberrant miRNA expression in NSCLC (26-28). miRNA dysregulation is closely associated with NSCLC oncogenesis and development, by acting as tumor suppressors or oncogenes (14,16,29). Therefore, an in-depth understanding of the biological roles of miRNAs in NSCLC may provide novel therapeutic methods for the management of patients with this disease. In the present study, it was demonstrated that miR-577 expression was significantly reduced in NSCLC tissues and cell lines. The restoration of miR-577 expression significantly decreased the proliferation and invasion of NSCLC cells. Notably, miR-577 negatively regulated HOXA1 expression by directly binding to its 3'-UTR. Furthermore, HOXA1 expression was upregulated in NSCLC tissues, and the upregulation...
of HOXA1 was inversely correlated with miR-577. HOXA1 restoration prevented the inhibitory effects of miR-577 over-expression on NSCLC cell proliferation and invasion. These results provided novel insights into NSCLC development and invasion, and may aid in the identification of therapeutic strategies.

miR-577 expression has been examined in several types of human cancer. For example, miR-577 expression is downregulated in breast cancer, and this downregulation is significantly correlated with tumor size, stage and lymphatic metastasis (21). In addition, miR-577 expression is reduced in hepatocellular carcinoma tissues and cell lines, and low miR-577 expression is associated with tumor size and metastasis (22). miR-577 is also downregulated in colorectal cancer (23), papillary thyroid carcinoma (24), glioblastoma (30) and gastric cancer (31). However, miR-577 expression is upregulated in esophageal squamous cell carcinoma (32). These findings indicate that the expression pattern of miR-577 in human cancers is tissue specific. Hence, miR-577 may be an effective diagnostic biomarker for these malignant tumors.

miR-577 serves as a tumor suppressor in human cancer types. For instance, miR-577 overexpression inhibits epithelial-mesenchymal transition and invasion in breast cancer cells (21). In hepatocellular carcinoma, the upregulation of miR-577 suppresses cell proliferation, promotes cell apoptosis and induces cell cycle arrest at the G1/G0 phase (22). In colorectal cancer, miR-577 expression restoration attenuates cell growth, induces G1/G0 cell cycle arrest in vitro and inhibits tumor growth in vivo (23). In papillary thyroid carcinoma, miR-577 expression restricts cell growth, migration and invasion in vitro (24). In glioblastoma, ectopic miR-577 overexpression impedes cell viability and growth (30). In gastric cancer, miR-577 overexpression represses cell proliferation by affecting the G1 to S phase transition (31). Nevertheless, miR-577 plays oncogenic roles in esophageal squamous cell carcinoma and promotes cell proliferation and colony formation (32). These conflicting pieces of evidence indicate that the biological roles of miR-577 exhibit evident tissue specificity and suggest that miR-577 may be a valuable therapeutic target for treating patients with these cancers.

Various genes have been demonstrated to be the direct targets of miR-577, including Ras-related protein Rab-25 in breast cancer (21), β-catenin in hepatocellular carcinoma (22), heat shock protein27 in colorectal cancer (23), sphingosine kinase 2 in papillary thyroid carcinoma (24), E2F transcription factor 3 in gastric cancer (31) and testis specific 10 in esophageal squamous cell carcinoma (32). In the present study, HOXA1, mapped to the short arm of chromosome 7 at band 15.2 (7p15.2), was validated as a direct target gene of miR-577 in NSCLC cells. It belongs to the homeodomain-containing transcription factor (HOXA) family and serves crucial roles in early developmental patterns and organogenesis (33,34). Previous studies have shown that HOXA1 is markedly upregulated in NSCLC tissues (35) and has oncogenic function in the carcinogenesis and progression of NSCLC (36-38). Herein, it was found that miR-577 directly targeted HOXA1 to inhibit the proliferation and invasion of NSCLC cells. The present study, together with previous findings, suggested that the identified miR-577/HOXA1 axis may represent a promising therapeutic target for patients with NSCLC.

In conclusion, miR-577 expression was decreased in NSCLC tissues and cell lines. Functional analyses indicated that miR-577 was able to inhibit the proliferation and invasion of NSCLC cells. Furthermore, HOXA1 was identified as a direct target gene of miR-577 in NSCLC, and it was required for the inhibitory effects of miR-577 on NSCLC cells. These results may help to further understand the mechanisms underlying the occurrence and development of NSCLC, and provided evidence for the miR-577/HOXA1 axis as a potential therapeutic target for the treatment of patients with this malignancy. However, the sample size of the present study was small, and the relationship between miR-577 and the clinicopathological characteristics of NSCLC patients was not investigated. More samples will be collected to resolve this in future experiments.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

HN designed the research. LM, DN and HN performed functional experiments. All authors read and approved the final draft.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University (approval no. 20140311), and was performed in accordance with the Declaration of Helsinki and the guidelines of the Ethics Committee of China-Japan Union Hospital of Jilin University. Written informed consent was provided by all patients for the use of their clinical tissues.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


