KIF9-AS1 promotes nasopharyngeal carcinoma progression by suppressing miR-16

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Abstract. Long non-coding RNAs (lncRNAs) have been reported to serve a crucial role in the progression of nasopharyngeal carcinoma (NPC); however, the underlying molecular mechanisms of lncRNA KIF9-AS1 in the tumorigenesis of NPC remains poorly understood. Reverse transcription-quantitative PCR was used to analyze the expression levels of KIF9-AS1 and microRNA (miR)-16, and Cell Counting Kit-8, wound healing and Transwell assays were used to determine the cell viability, invasion and migration, respectively, of NPC cells. In addition, a dual-luciferase reporter assay was used to analyze the direct interaction between KIF9-AS1 and miR-16. NPC stage was classified according to the seventh edition of the AJCC staging system. The results revealed that KIF9-AS1 expression levels were upregulated in NPC tissues and cell lines. In addition, miR-16 was demonstrated to directly interact with KIF9-AS1 and inhibit KIF9-AS1 expression levels, whereas the miR-16 inhibitor rescued the effects of the KIF9-AS1-knockdown in NPC cells. Furthermore, the expression levels of KIF9-AS1 were upregulated, while those of miR-16 were downregulated in NPC tissues. Notably, the expression levels of KIF9-AS1 were observed to be significantly more upregulated in advanced tumors (III-IV vs. I-II) and patients with high KIF9-AS1 expression levels exhibited a worse prognosis. In conclusion, the findings of the present study suggested that KIF9-AS1 may promote the progression of NPC by targeting miR-16, thus KIF9-AS1 may be a novel molecular target for NPC therapy.

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Introduction

Nasopharyngeal carcinoma (NPC) is a form of head and neck cancer caused by the cancerization of the nasopharynx epithelium, which demonstrates high incidence rates in Southern China, North Africa and Southeast Asia (1,2). NPC is currently classified into three groups, including keratinizing carcinoma (K-NPC), non-keratinizing carcinoma (NK-NPC) and basaloid squamous carcinoma (BSCC) (3). K-NPC has a solely keratinizing phenotype, characterized by variable keratin formation and intercellular junctions (4); NK-NPC presents with a similar phenotype to squamous metaplasia and exhibits ulceration of the overlying epithelium and BSCC presents with a biphasic pattern and stromal hyalinization (5). Significant advances have been made in the treatment of NPC; however, the 5-year survival rate of NPC patients with advanced stage has not significantly improved, and it remains at ~30.3-73.6% worldwide since 2015 (6,7). Therefore, there is a need to determine the important regulators and the underlying mechanisms of NPC progression to improve the prognosis and survival rate of patients with NPC.

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts of >200 nucleotides in length, which have no protein-coding ability (8). The dysregulation of lncRNAs is closely associated with the occurrence and development of various types of cancer, for example the increased expression levels of the lncRNA HULC predicted a poor prognosis and accelerated tumor progression in prostate cancer (9). In addition, lncRNA PICART1 was reported to inhibit the progression of non-small cell lung cancer cells through inactivation of the AKT1 signaling pathway (10), and lncRNA XIST is associated with a poor prognosis and promotes a malignant phenotype in osteosarcoma (11). Recently, KIF9-AS1 was also reported to be a biomarker for inflammatory bowel disease (12). However, to the best of our knowledge, there are currently no relevant studies regarding the role of KIF9-AS1 in NPC.

MicroRNAs (miRNAs/miRs) are a family of small non-coding RNAs of ~22 nucleotides in length, which regulate gene expression by complementary binding or complex mechanisms (13-15). miRNAs have been reported to be aberrantly expressed in NPC, where they have been found to regulate NPC cell proliferation, invasion and metastasis (16).

Therefore, investigations into cancer-associated miRNAs in NPC may help to identify effective novel targets for NPC therapy.

The present study aimed to determine the potential mechanisms of KIF9-AS1 in NPC. These findings may provide novel insights into the progression of NPC and help to develop novel therapeutics for the treatment of NPC.

Materials and methods

Patient studies. The present study was approved by the Ethics Committee of Weifang Traditional Chinese Hospital (Weifang, China; approval no. 2010A121005) and written informed consent was provided by all patients. In total, 25 pairs of NPC and adjacent normal tissues (2-cm adjacent to tumor) were collected from 25 patients with NPC (19 males and 6 females) with a mean age of 41 years (range, 28-63 years) between February 2011 and August 2012 in Weifang Traditional Chinese Hospital (Weifang, China). The total duration of follow-up was 6 years and follow-up was conducted over the phone or in an outpatient clinic. The patients' clinical data are presented in Table I. NPC stage was classified according to the seventh edition of the AJCC staging system (17).

Cell culture and transfection. NPC cells (SUNE1 and SUNE2), the human normal nasopharyngeal epithelial cell line NP69 and HEK293T cells were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The cells were cultured in DMEM (Corning Inc.), supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), and maintained in an atmosphere of 5% CO_2 and constant humidity at 37˚C.

For the transfection, short hairpin (sh)RNAs targeting KIF9-AS1 (shKIF9-AS1, 5'-GGAAUGCAGCUGAAAGAUUGC-3') or scrambled negative control (shNC, 5'-AAUUCUCCGAACGUGUCACGU-3') were transfected into SUNE1 and SUNE2 cells. The miR-16 mimic (5'-UAGCAGCACGUAAAUAUUGGUG-3') and the scrambled negative control (NC mimics, 5'-UACACCGAUCGAGUCAGGUUU-3'), and miR-16 inhibitor (5'-CACCAAUAUUUACGUGCUGCUA-3') and the scrambled negative control (NC inhibitor; 5'-UCGAGACACGUACGCAGAAUU-3') were synthesized by Shanghai GeneChem Co., Ltd. Cell transfections were performed using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Subsequent experiments were performed 48 h post-transfection.

Wound healing assay. The transfected SUNE1 and SUNE2 cells were plated into 6-well plates and cultured until 70% confluence for the wound healing assay. Subsequently, a 200-μl pipette tip was used to generate a single wound in the cell monolayer. Cells were washed twice with DMEM and incubated with DMEM supplemented with 1% FBS for 24 h at 37˚C. Images of the migrating cells were acquired at 0 and 24 h using a light microscope (magnification, x200; Leica DMI4000B; Leica Microsystems, Ltd.) and measured using ImageJ software version 1.8 (National Institutes of Health).

Transwell assay. The migration and invasion of the NPC cells was analyzed using Transwell chambers (8.0-μm pore size; EMD Millipore) and Matrigel (Corning Inc.). The transfected SUNE1 and SUNE2 cells were plated in the upper chambers of the Transwell plates in serum-free medium. Transwell membranes were precoated with Matrigel for 1 h at room temperature. A volume of 600 μl DMEM, supplemented with 10% FBS, was plated in the lower chambers. Following incubation for 48 h at 37˚C, the non-invasive cells in the upper chamber were removed and invasive cells in the lower chamber were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet (Beyotime Institute of Biotechnology) both for 20 min at room temperature. For the migration assay, the same assay was performed; however, the Transwell membranes were not coated in Matrigel. Stained cells were counted using a light microscope (magnification, x200; Zeiss GmbH).

Cell Counting Kit-8 (CCK-8) assay. Cell viability was determined using the CCK-8 assay kit (Dojindo Molecular Technologies, Inc.) according to the manufacturer's instructions. Transfected SUNE1 and SUNE2 cells were cultured for 48 h, harvested and plated into 96-well plates at a density of $2x10^3$ cells/well. Cells were incubated with 100 μl DMEM for 48 h at 37˚C and 5% CO_2. Following the incubation, 10 μl CCK-8 solution was added/well and incubated at 37˚C for 2 h. The absorbance of each well was measured at a wavelength of 450 nm using a microplate reader.

Bioinformatic prediction and dual-luciferase reporter assay. StarBase 2.0 (http://starbase.sysu.edu.cn) was used to predict the downstream target of KIF9-AS1. The pmirGLO-KIF9-AS1-wild-type (WT)/mutant (Mut) reporter was purchased from Shanghai GenePharma Co., Ltd. 293T cells were used for the dual-luciferase reporter assay due to the high transfection efficiency (18). Subsequently, 293T cells were co-transfected with the miR-16 mimic and the pmiRGLO-KIF9-AS1-WT/Mut reporter. Transfection was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following incubation for 48 h at 37˚C, the relative luciferase activity was analyzed using a dual-luciferase reporter assay system (Promega Corporation) and normalized to *Renilla* luciferase activity.

Reverse transcription-quantitative PCR. Total RNA was extracted from SUNE1 and SUNE2 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using the Reverse Transcription kit (Takara Bio, Inc.), according to the manufacturer's protocol. qPCR was subsequently performed using the SYBR Select Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) on the ABI 7300 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for qPCR: Initial denaturation at 95˚C for 3 min; 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. The following primer sequences were used for the qPCR: KIF9-AS1, Forward: 5'-CAGCACTGACTACACTGGGA-3' and reverse: 5'-GCCCTCTTCTTCCTCCACAT-3'; miR-16, forward: 5'-TAGCAGCACGTAAATATTGGCG-3' and reverse: 5'-TGCGTGTCGTGGAGTC-3'; GAPDH, forward: 5'-GAGTCAACGGATTTGGTCGT-3' and

Table I. Clinicopathological characteristics of patients with nasopharyngeal carcinoma.

Clinicopathological features	Value
Mean age (range), years	41 (28-63)
Sex (male/female), n	19/6
Degree of differentiation (undifferentiated/differentiated), n	17/8
Histology (squamous/others), n	25/0
Lymph node metastasis (+/-), n	18/7
Distal metastasis (+/-), n	0/25
Clinical TNM stage (I-II/III-IV), n	9/16

TNM, tumor-node-metastasis.

reverse: 5'-TTGATTTTGGAGGGATCTCG-3'; U6, forward: 5'-CTCGCTTCGGCAGCACATATACTA-3' and reverse: 5'-ACGAATTTGCGTGTCATCCTTGCG-3'. The relative expression levels of the mRNAs were calculated using the $2^{-\Delta\Delta Cq}$ method (19) and normalized to GAPDH and U6, the endogenous controls.

Xenograft experiment. Eight male BALB/c nude mice (6-weeks old; ~20 g) were acquired from the Laboratory Animal Center of Nanjing Medical University and randomly divided into two groups with four in each group. The mice were maintained under specific pathogen-free conditions, with free access to water and food, at room temperature of 26-28˚C and humidity of 60±10%, and under a 12 h light/dark cycle. Each group of mice was injected subcutaneously into the right flank of nude mice with SUNE1 cells ($5x10^6$) transfected with shKIF9-AS1 or shNC. After 35 days, the mice were sacrificed by cervical dislocation after deep anesthesia (confirmed by normal rectal temperature, respiratory rate and sleeping state) with 2% isoflurane (Baxter Healthcare Corporation). The length (L), width (W) and weight of tumors were measured and tumor volume was calculated by the following formula: $V = ½ x L x W^2$. Animal experimental protocols were approved by the Animal Welfare Committee of Weifang Traditional Chinese Hospital (Weifang, China), and all methods were conducted according to the guidelines (20).

Statistical analysis. Statistical analysis was performed using SPSS version 16.0 (SPSS, Inc.). Data are represented as the mean ± standard deviation of three independent experimental repeats. Comparisons among multiple groups were performed using one-way analysis of variance, followed by Tukey's post hoc test. Comparison between NPC and adjacent normal tissue samples from patients with NPC was performed using a paired Student's t-test, while comparisons between the experimental and control groups was performed using an unpaired Student's t-test. The correlation between the mRNA expression levels was analyzed using Pearson's correlation coefficient. The overall survival rates and the survival curve were determined using the Kaplan-Meier method, followed by the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

KIF9-AS1 expression levels are increased in NPC cell lines, which facilitates NPC progression. To investigate the molecular mechanisms of KIF9-AS1 in NPC, KIF9-AS1 expression levels were analyzed using RT-qPCR. The results indicated that the expression levels of KIF9-AS1 were increased in the NPC cell lines (SUNE1 and SUNE2) compared with the human normal nasopharyngeal epithelial cell line NP69 (Fig. 1A). RT-qPCR was subsequently used to determine the transfection efficiency of the shRNA; KIF9-AS1 expression levels were markedly decreased in the shKIF9-AS1 group compared with the shNC group (Fig. 1B). The CCK-8 assay revealed that SUNE1 and SUNE2 cell viability was decreased following the suppression of KIF9-AS1 expression levels compared with shNC-treated cells at 72 h (Fig. 1C). In addition, cell migration and invasion were both attenuated by the knockdown of KIF9-AS1 (Fig. 1D-F). Xenograft tumor experiments were performed to determine the role of KIF9-AS1 in NPC *in vivo*, and results showed that the tumor volume and weight were markedly decreased in the shKIF9-AS1 group compared with the control (Fig. 1G-I). These results suggested that KIF9-AS1 may serve as an oncogenic factor during NPC progression.

miR-16 is a direct target of KIF9-AS1 in NPC cells. Using the StarBase bioinformatics analysis software, KIF9-AS1 was revealed to bind to miR-16 via complementary base pairing (Fig. 2A). A dual-luciferase reporter assay subsequently demonstrated that the miR-16 mimic decreased the relative luciferase activity of 293T cells transfected with the WT KIF9-AS1-fused luciferase gene, whereas no significant differences were observed in the relative luciferase activity in cells harboring Mut KIF9-AS1 (Fig. 2B). Furthermore, RT-qPCR revealed that the miR-16 mimic significantly decreased the expression levels of KIF9-AS1 compared with NC mimics (Fig. 2C). Taken together, these findings suggested that miR-16 may inhibit the expression levels of KIF9-AS1 through direct binding.

KIF9-AS1 regulates NPC progression via miR-16. The expression levels of miR-16 were upregulated in SUNE1 cells transfected with the miR-16 mimic (Fig. 3A). Moreover, the CCK-8, wound healing and Transwell assays revealed that the miR-16 mimic inhibited the cell viability, migration and invasion of NPC cells, respectively (Fig. 3B-E). These findings indicated that miR-16 inhibited the development and progression of NPC cells. In addition, as miR-16 was observed to interact with KIF9-AS1, the study aimed to determine whether miR-16 was involved in KIF9-AS1-regulated NPC tumor progression. RT-qPCR results indicated that miR-16 expression was decreased in SUNE1 cells transfected with miR-16 inhibitor compared with NC inhibitor (Fig. 3F). Subsequently, the miR-16 inhibitor was co-transfected into KIF9-AS1-knockdown cells and the results indicated that the introduction of the miR-16 inhibitor was able to decrease the expression levels of miR-16 in SUNE1 cells transfected with shKIF9-AS1 (Fig. 3G). CCK-8 assays further revealed that the knockdown of KIF9-AS1 decreased the cell viability of SUNE1 cells, which was abolished by miR-16 inhibitor

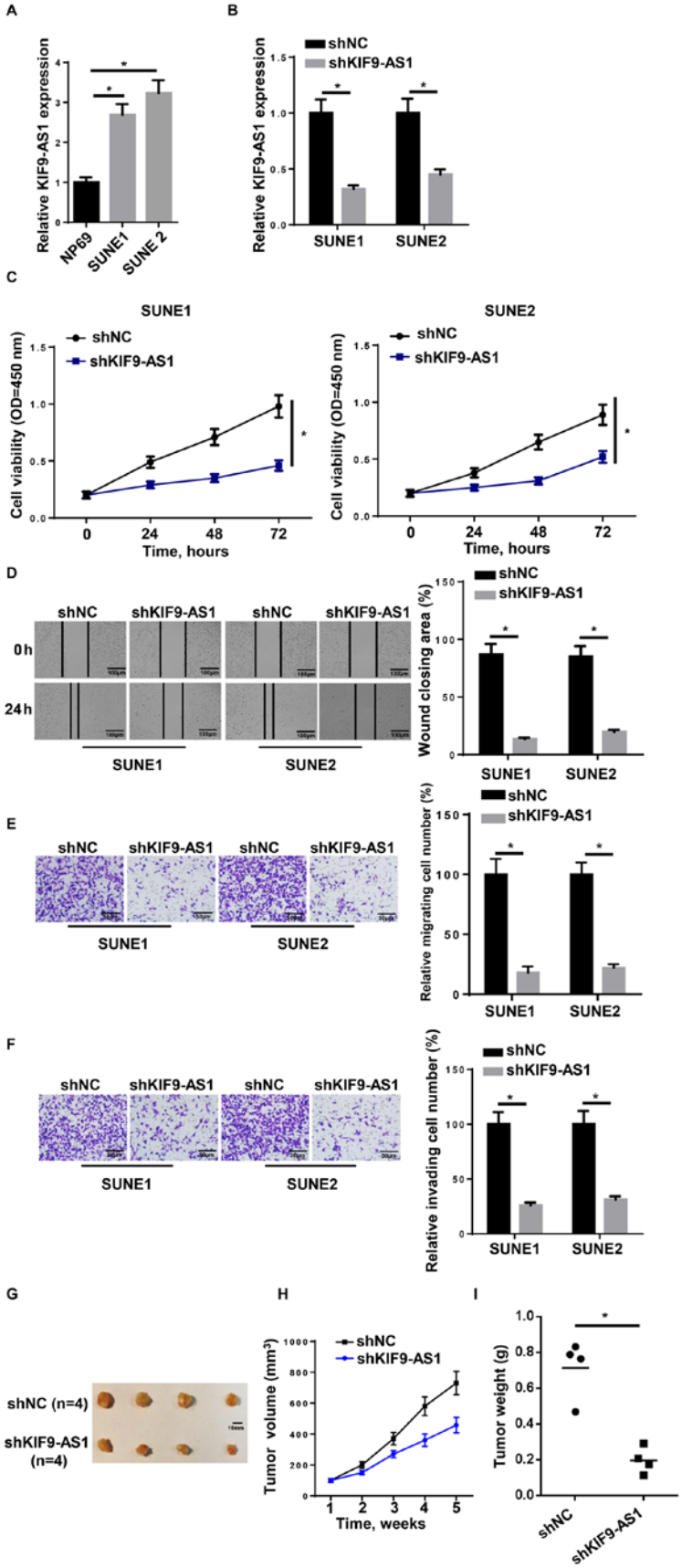

Figure 1. KIF9-AS1 exhibits higher expression in NPC cell lines, and facilitates NPC progression. (A) RT-qPCR showed the relative KIF9-AS1 expression in normal nasopharyngeal epithelial cell line (NP69) and NPC cell lines (SUNE1 and SUNE2). (B) RT-qPCR showed the relative KIF9-AS1 expression in SUNE1 and SUNE2 cell lines transfected with shNC and shKIF9-AS1. (C) Cell Counting Kit-8 assay showed the cell proliferation rate of SUNE1 and SUNE2 cell lines transfected with shNC and shKIF9-AS1 at different time points of 0, 24, 48 and 72 h. (D) Wound healing assay of SUNE1 and SUNE2 cell lines transfected with shNC and shKIF9-AS1. (E and F) Transwell assay of SUNE1 and SUNE2 cell lines transfected with shNC and shKIF9-AS1. (G-I) Xenograft tumor assay showed knockdown of KIF9-AS1 markedly suppressed tumor growth. Scale bar, 10 mm. The data are presented as mean ± SD. *P<0.05. NPC, nasopharyngeal carcinoma; sh, short hairpin; NC, negative control; RT-q, reverse transcription-quantitative.

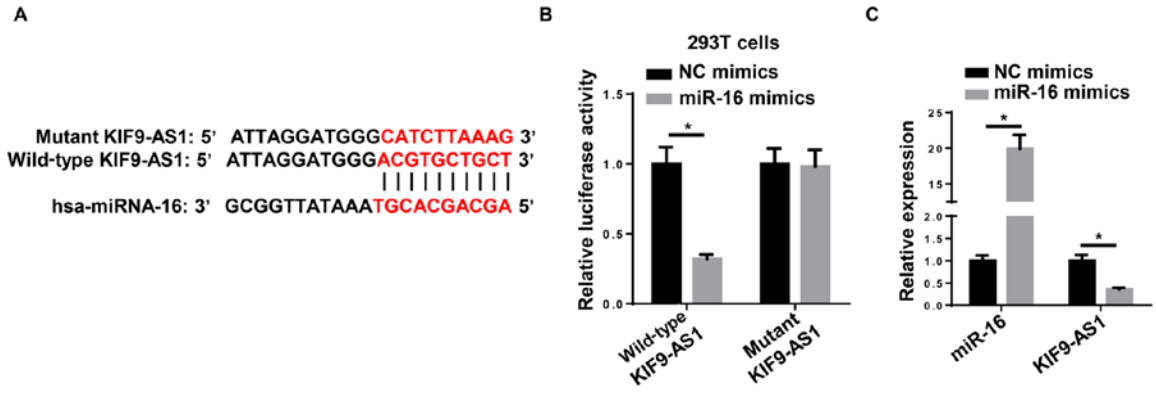

Figure 2. miR-16 is a direct target of KIF9-AS1 in NPC cells. (A) Bioinformatic prediction of the binding site (red) of miR-16 by KIF9-AS1 using starBase software. (B) Dual-luciferase reporter assay showed luciferase activity in 293T cells transfected with NC mimics or miR-320b mimics. (C) RT-qPCR showed miR-16 and KIF9-AS1 expression levels in SUNE1 cell line transfected with NC mimics or miR-16 mimics. The data were presented as mean ± SD. *P<0.05. NPC, nasopharyngeal carcinoma; miR, microRNA; NC, negative control.

(Fig. 3H). In addition, the wound healing and Transwell assays demonstrated that knockdown of KIF9-AS1 attenuated the migration and invasion of SUNE1 cells compared with the cells transfected with shNC; however, the miR-16 inhibitor was able to partially rescue the shKIF9-AS1-induced NPC cell phenotype (Fig. 3I-K). Altogether, these data suggested that miR-16 may be crucial for KIF9-AS1-mediated migration and invasion of NPC cells.

KIF9-AS1 and miR-16 expression levels are dysregulated in patients with NPC. To confirm the roles of KIF9-AS1 and miR-16 in NPC progression, the expression levels of KIF9-AS1 and miR-16 were analyzed in patients with NPC. Patient characteristics are presented in Table I. A total of eight patients had differentiated NPC, 18 patients were documented to have lymph node metastasis and 16 patients were in TNM stage III-IV. The expression levels of KIF9-AS1 were increased in NPC tumors compared with the adjacent normal tissues, whereas the expression levels of miR-16 were decreased in the NPC tumor samples (Fig. 4A and B). Moreover, a negative correlation was identified between the expression levels of KIF9-AS1 and miR-16 (Fig. 4C). In addition, it was observed that the expression levels of KIF9-AS1 were increased in high-stage tumors (III-IV) compared with low-stage tumors (I-II) (Fig. 4D). Meanwhile, Kaplan-Meier analysis reported that patients with high KIF9-AS1 expression levels were associated with a poorer prognosis in NPC (hazard ratio, 0.234; 95% confidence interval, 0.06350-0.8655; Fig. 4E). These results indicated that KIF9-AS1 expression levels may be upregulated in high-stage tumors and associated with a less favorable overall survival rate of patients with NPC.

Discussion

NPC is regarded as one of the most common head and neck malignancies, with an incidence rate of 2.8/100,000 person-years in men and 1.9/100 000 person-years in women in China (21). Despite significant advances in the treatment of NPC, the incomplete understanding of the pathogenesis of NPC has prevented the development of effective targeted therapies.

An increasing number of studies have demonstrated that the abnormal expression levels of lncRNAs serve as a prognostic biomarker for different types of cancer, including NPC (22-24). lncRNAs have been discovered to serve roles in inhibiting or promoting the development of cancer by influencing carcinogenic molecules to regulate cancer-related progression. For example, lncRNA ZFAS1 was reported to accelerate the proliferation of NPC cells via the activation of the PI3K/AKT signaling pathway (25). Jia *et al* (15) also demonstrated that lncRNA PXN-AS1-L promoted the proliferation, migration and invasion of NPC cells through upregulating the expression levels of suppressor APC domain-containing protein 2. Therefore, determining the involvement of cancer-specific lncRNAs may prove beneficial for identifying novel targets for anticancer treatment. Wang *et al* (11) suggested that lncRNA KIF9-AS1 may belong to a novel group of non-coding RNAs, which are associated with inflammation and tumorigenesis (12). Thus, the present study aimed to determine the expression levels of KIF9-AS1 in NPC tissues and cell lines. The results from the RT-qPCR analysis revealed that the expression levels of KIF9-AS1 were significantly increased in NPC tissues and cell lines. Furthermore, the clinical significance of KIF9-AS1 was investigated in patients with NPC; the expression levels of KIF9-AS1 in stage I-IV tumors demonstrated a gradually upregulated trend, and Kaplan-Meier survival analysis indicated that patients with NPC and high KIF9-AS1 expression levels had a shorter OS time compared with those patients with low KIF9-AS1 expression levels. Thus, these results suggested that KIF9-AS1 may be a potential target for the treatment of NPC.

Competing endogenous RNA (ceRNA) networks have regulatory functions in numerous types of human malignancies (26,27). For example, lncRNA CR749391 was reported to promote the progression of gastric cancer by sponging miR-181a (28); lncRNA HCG18 promotes the development of NPC by functioning as a ceRNA of miR-16 to upregulate G1/S-specific cyclin-D1 expression levels (29) and lncRNA AFAP1-AS1 was observed to function as a ceRNA of miR-423-5p to promote NPC metastasis by activating the Rho/Rac signaling pathway (30). Previous studies have also

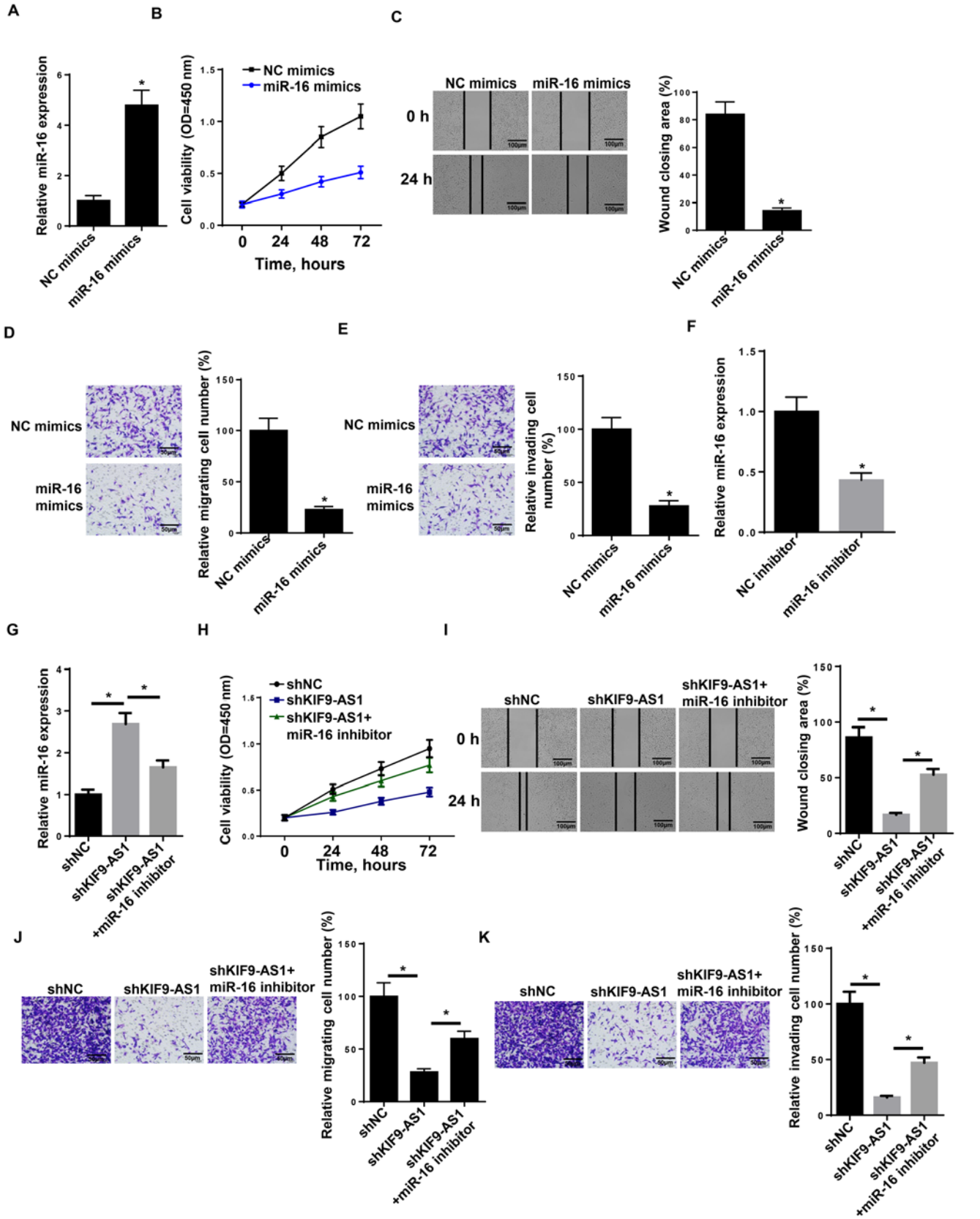

Figure 3. KIF9-AS1 regulates nasopharyngeal carcinoma progression via miR-16. (A) RT-qPCR showed the relative miR-16 expression in SUNE1 cell lines transfected with miR-16 mimics. (B) CCK-8 assay showed the cell proliferation rate of SUNE1 cells transfected with miR-16 mimics at different time points of 0, 24, 48 and 72 h. (C) Wound healing assay of SUNE1 cells transfected with miR-16 mimics. (D and E) Transwell assay of SUNE1 cells transfected with miR-16 mimics. (F) RT-qPCR showed the relative miR-16 expression in SUNE1 cell lines transfected with NC inhibitor and miR-16 inhibitor. (G) RT-qPCR shows the relative miR-16 expression in SUNE1 cell lines transfected with shNC, shKIF9-AS1 and shKIF9-AS1+ miR-16 inhibitor. (H) CCK-8 assay showed the cell proliferation rate of SUNE1 cells transfected with shNC, shKIF9-AS1 and shKIF9-AS1+ miR-16 inhibitor at different time points of 0, 24, 48, and 72 h. (I) Wound healing assay of SUNE1 cells transfected with shNC, shKIF9-AS1 and shKIF9-AS1+ miR-16 inhibitor. (J and K) Transwell assay of SUNE1 cells transfected with shNC, shKIF9-AS1 and shKIF9-AS1+ miR-16 inhibitor. The data were presented as mean ± SD. *P<0.05. RT-q, reverse transcription-quantitative; miR, microRNA; CCK-8, Cell Counting Kit-8; sh, short hairpin; NC, negative control.

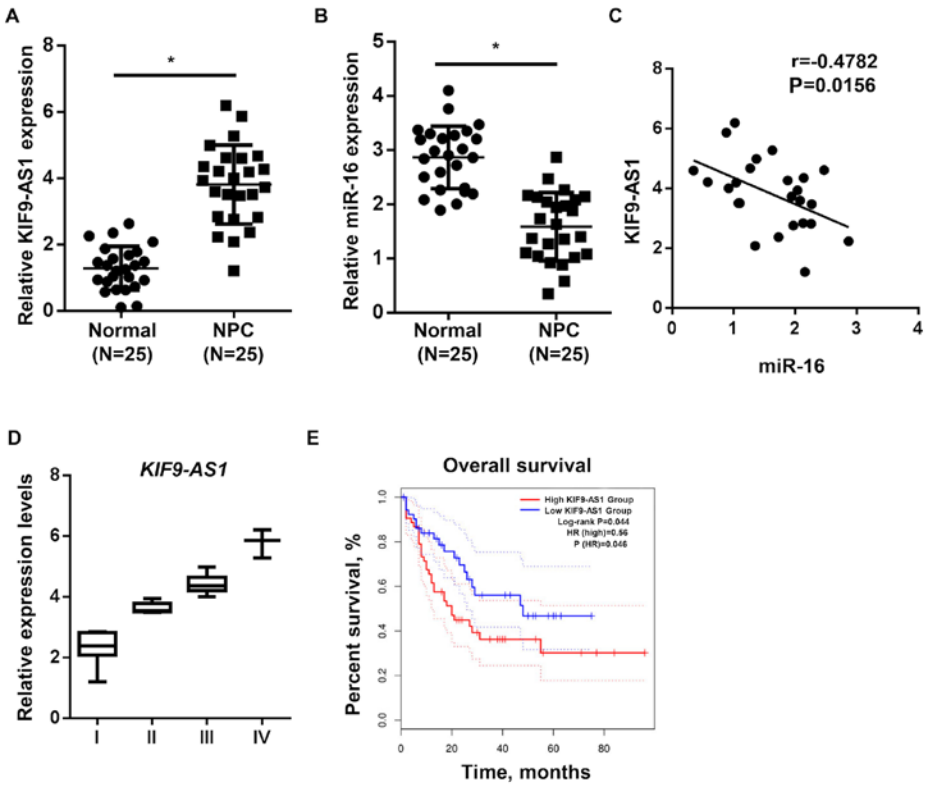

Figure 4. KIF9-AS1 and miR-16 are dysregulated in patients with NPC. (A) RT-qPCR showed KIF9-AS1 expression in NPC tissues and adjacent normal tissues, n=25. (B) RT-qPCR showed miR-16 expression in NPC tissues and adjacent normal tissues, n=25. (C) Pearson's correlation analysis showed a negative correlation between KIF9-AS1 and miR-16 in patients with NPC, n=25. (D) RT-qPCR showed KIF9-AS1 levels in tumors (TNM I-IV) of patients with NPC. (E) Kaplan-Meier survival analysis showed a correlation between KIF9-AS1 expression and prognosis of NPC patients. The data are presented as mean ± SD. *P<0.05. NPC, nasopharyngeal carcinoma; HR, hazard ratio.

indicated that miR-16 is associated with cancer regulation, including cervical cancer, bladder cancer and neuroblastoma (31-33). Consistent with these findings, the present study also observed an interaction between KIF9-AS1 and miR-16 through a series of experiments. In addition, the co-transfection with an miR-16 inhibitor partially reversed the shKIF9-AS1-mediated decrease in the viability, migratory and invasive abilities of NPC cells. The expression levels of miR-16 were also decreased in NPC tissues compared with those in the corresponding normal nasopharyngeal tissues. In addition, the expression levels of miR-16 were negatively correlated with the expression levels of KIF9-AS1. Altogether, these findings suggested that KIF9-AS1 may promote the development and progression of NPC by inhibiting the expression levels of miR-16.

In conclusion, the present study reported that KIF9-AS1 promoted the progression of NPC by targeting miR-16. These findings not only strengthen our understanding of NPC initiation and progression, but they also inform the development of novel biomarkers and therapeutic methods for NPC. However, the limitations of the present study need to be addressed in future studies. Firstly, other miRNAs or downstream effectors that are crucial to KIF9-AS1-regulated phenotypes of NPC need to be identified. Secondly, the present findings should be validated using additional NPC cell lines to ensure a more in-depth analysis. Thirdly, both SUNE1 and SUNE2 should be used to strengthen the preciseness of the experiment.

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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 upon reasonable request.

Authors' contributions

HY and SY designed the present study. HY, SW and SY performed all the experiments, analyzed the data and prepared the figures. HY and SY drafted the initial manuscript. SY reviewed and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Weifang Traditional Chinese Hospital (Weifang, China; approval no. 2010A121005) and written informed consent was provided by all patients prior to the study start. Animal

experimental protocols were approved by the Animal Welfare Committee of Weifang Traditional Chinese Hospital (Weifang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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