LINC00839 Knockdown Restrains The Metastatic Behaviors of Nasopharyngeal Carcinoma By Sponging miR-454-3p

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Research

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Abstract

Background

Long intergenic non-coding RNA 00839 (LINC00839) has been verified as a cancer-promoting gene in malignancies. However, the significance of LINC00839 in nasopharyngeal carcinoma (NPC) has yet to be elaborated, as well as its underlying mechanism.

Methods

LINC00839 and miR-454-3p relative expression levels in NPC cells were examined by qRT-PCR. The growth of cells was examined by CCK-8 and colony formation assays. Cell migration and invasion were examined by wound healing and Transwell experiment, respectively. The binding sequence of LINC00839 and miR-454-3p was confirmed by the luciferase reporter gene experiment. The regulatory function of LINC00839 and miR-454-3p on c-Met was investigated by western blot.

Results

Here, we revealed that LINC00839 was elevated in NPC. Both LINC00839 knockdown and upregulation of miR-454-3p suppressed NPC cells proliferation, invasive capacity and EMT in vitro. Besides, LINC00839 was validated as a miR-454-3p “sponge”, and upregulation of LINC00839 could reverse miR-454-3p-mediated functions in NPC C666-1 and SUNE-1 cells. Furthermore, c-Met was determined to be targeted by miR-454-3p. Notably, c-Met was downregulated by LINC00839 knockdown through sponging miR-454-3p. In vivo, LINC00839 knockdown resulted in a slower tumor growth.

Conclusions

Altogether, knockdown of LINC00839 inhibits the aggressive properties of NPC cells *via* sponging miR-454-3p and regulating c-Met.

Introduction

Nasopharyngeal carcinoma (NPC), which derives from nasopharyngeal epithelial cell, is the most prevalent type of head and neck cancer [1]. Due to patients with NPC usually with no specific symptoms in the early stage, most of them have stepped into advanced stages when diagnosed. As for the treatment, radiotherapy is the backbone of therapy for NPC and concurrent chemoradiotherapy has become the choice of treatment for advanced NPC [2]. Therefore, the five-year overall survival of patients with NPC has increased to about 70% [3]. Nevertheless, a complete cure of metastatic NPC remains elusive owing to its local recurrence and metastasis. The mechanism underlying NPC progression, including metastasis has not been completely understood yet.

Long intergenic non-coding RNAs (lincRNAs), more than 200 nucleotides in length, play a pivotal role in carcinogenesis and cancer progression [4]. LncRNAs serve as competing endogenous RNA (ceRNA) to

compete with response elements for binding to miRNAs, thereby regulating mRNAs targeted by miRNAs [5]. In NPC, an increasing body of evidence has manifested that lincRNAs are implicated in tumorigenesis [6, 7]. For instance, lncRNA plasmacytoma variant translocation 1 (PVT1) modulates NPC cells growth by activating the lysine acetyltransferase 2A (KAT2A) acetyltransferase and stabilizing hypoxia-inducible factor-1alpha (HIF-1alpha) [4]. lncRNA Family With Sequence Similarity 225 Member A (FAM225A) promotes NPC tumorigenesis and metastasis via function as ceRNA to sponge miR-590-3p/miR-1275 and upregulating ITGB3 [1]. There are also some reports indicating that several lncRNAs may serve as anti-cancer factors in human NPC and could predict a good prognosis, such as lncRNA-LET, MEG3 and NKILA [8-10].

LINC00839 has been verified as a cancer-promoting factor in multiple malignancies, and its dysregulation is closely correlated with tumor initiation, development and progression. In osteosarcoma, LINC00839 facilitates the malignant development of cancer cell through competitively binding to miRNA-454-3p and enhancing the expression of c-Met [11]. LINC00839 is upregulated in chemo-resistant breast carcinoma cells and tissues, and high level of LINC00839 is associated with a poor prognosis [12]. LINC00839 is elevated in neuroblastoma and identified to be significantly associated with survival [13]. Whereas, the roles of LINC00839 in malignancy of NPC have not been sufficiently elaborated.

In this study, we disclosed the tumor-promoting action of LINC00839 on the malignancy of NPC. A variety of functional experiments were carried out to examine the influence of LINC00839 knockdown on proliferation, invasiveness and EMT of NPC cells in vitro and on tumor growth in vivo. We validated that LINC00839 function as a “sponge” for miR-454-3p to regulate the expression of c-Met.

Materials And Methods

Cell lines

The nasopharyngeal epithelial cell line, NP-69 and NPC cell lines (CNE-2, CNE-1, SUNE-1 and C666-1) were cultured with RPMI-1640 containing 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA). C666-1 and SUNE-1 cells are not misidentification and contamination of human cell lines (ExpASY: SIB Bioinformatics Resource Portal, <https://www.expasy.org/>). All cell lines were obtained from Nanjing KeyGen Biotech Co., Ltd (Nanjing, China) and were cultured in humid chamber at 5% CO₂, 37°C.

Cell transfection

MiR-454-3p mimic and negative control (miR-NC), shRNA negative control (sh-Ctrl) and shRNA against LINC00839 (sh-LINC00839 #1 and sh-LINC00839 #2) were synthesized by GenePharma (Shanghai, China). The pcDNA3.1 control plasmid (pc-vector) and plasmid for mediating LINC00839 overexpression (pc-LINC00839) were purchased from GenePharma. pcDNA3.1 plasmid or miRNA mimic was transfected into C666-1 and SUNE-1 cells using Lipofectamine 3000 (Thermo Fisher Scientific). To stably obtain sh-LINC00839 stable transfected cell, lentivirus carrying sh-LINC00839 or sh-Ctrl packaged in HEK-293T cell

and secreted into culture medium. SUNE-1 cells were infected by lentivirus using polybrene (Sigma, Shanghai, China) and selected by 1.5 µg/mL puromycin (Sigma) for 14 days.

Quantitative real-time PCR analysis (qPCR)

Total RNAs were isolated using a TRIZOL kit (Thermo Fisher Scientific). 1 µg of RNA was used for cDNA synthesis with a reverse transcriptase PCR kit (Thermo Fisher Scientific). qPCR was conducted using a SYBR Green qPCR Master Mix kit (TAKARA). The relative expression of miR-454-3p was measured using an All-in-One miRNA qRT-PCR Detection Kit (GeneCopoeia, Rockville, Montgomery, USA). The primer sequences are shown below. miR-454-3p forward primer: 5'-ACCCTATCAATATTGTCTCTGC-3', Reverse primer: 5'-GCGAGCACAGAATTAATACGAC-3'; U6 forward primer: 5'-CGCGCGTCGTGAAGCGTTC-3', Reverse primer: 5'-GTGCAGGGTCCGAGGT-3'; ZO-1 forward primer: 5'-GCCGCTAAGAGCACAGCAA-3', Reverse primer: 5'-TCCCCACTCTGAAAATGAGGA-3'; Vimentin forward primer: 5'-GACGCCATCAACACCGAGTT-3', Reverse primer: 5'-CTTTGTCGTTGGTTAGCTGGT-3'; GAPDH forward primer: 5'-TGTGGGCATCAATGGATTTGG-3', Reverse primer: 5'-ACACCATGTATTCCGGGTCAAT-3'. U6 was used for miR-454-3p normalization and GAPDH for mRNA normalization. The relative expression of RNA was calculated using the $2^{-\Delta\Delta C_t}$ method.

Cell proliferation

miR-454-3p mimic or sh-LINC00839 transfected SUNE-1 or C666-1 cells were seeded into 96 well culture plates (1×10^4). At 24, 48, 72 or 96 hours, 20 µl of MTT (5 mg/ml) were added into plates. Following the 4 hours incubation, supernatant in each well was gently removed and 200 µl of dimethyl sulfoxide (DMSO) was added. The absorbance was measured at 450 nm.

Clone-formation assay

SUNE1 or C666-1 cells (1000 cells/well) were seeded into six-well plates. After 14 days, cell colonies in plates were fixed using methanol and stained by 0.1% crystal violet (Sigma).

Migration assay

After transfection, SUNE1 or C666-1 cells were seeded into 6-well plates to incubate until 80% confluence. Then, the scratch was generated with a 200 µL pipette tip and cultured for 24 hours. The healing distance was captured by a microscope. The percentage of migration = (width at 0 hour – width at 24 hours)/width at 0 hour $\times$ 100%.

Cell invasion

The membrane in Transwell chamber (8 µm pore size, Corning Costar, NY, USA) was coated with 40 µl of Matrigel (BD). After transfection, 200 µl of SUNE1 or C666-1 cells were plated into the upper chamber and 600 µl of medium was added into the lower chamber. After 24 hours, the invading cells on the lower surface of membrane were stained by 0.1% crystal violet and calculated.

Immunoblotting

Total proteins were extracted using RIPA lysis buffer (Biyuntian, Nanjing, China). 35 µg of proteins were separated using 10% SDS-PAGE and transferred onto PVDF membrane (Millipore, Braunschweig, Germany). After blocking with 5% skim milk, membrane was incubated with c-Met, E-cadherin, N-cadherin or GAPDH antibody (1:1000, Santa Cruz Biotechnology, CA, USA) at 4°C overnight. After washed with TBST for three times, membrane was incubated with HRP-linked secondary antibody (1:10000, Biyuntian) for 2 hours. The bands were assessed using an ECL kit (Millipore).

Ago2-RNA immunoprecipitation (RIP) assay

After transfection of miR-454-3p, C666-1 and SUNE1 cell were collected, and cell lysate was prepared. Magna RIP™ RNA-binding protein immunoprecipitation kit (Millipore) was utilized to measure the mRNA level of LINC00839 from the samples bound to the Ago2 or IgG antibody. After washing, the retrieved RNAs were subjected to qPCR analysis.

Luciferase reporter assay

The LINC00839 3'-UTR containing putative wild type binding sites for miR-454-3p was inserted into pGL3 luciferase reporter (Promega, Madison, WI, USA) to generate pGL3-LINC00839-wt. The mutated miR-454-3p-binding sequence was constructed using a QuikChange Site-Directed Mutagenesis Kit (Stratagene, USA) to construct pGL3-LINC00839-mut. C666-1 and SUNE1 cells were co-transfected with miR-454-3p mimic plus pGL3-LINC00839-wt or miR-454-3p plus pGL3-LINC00839-mut. After 48 hours, the luciferase activity was measured by a luciferase reporter assay kit (Promega). The c-Met 3'-UTR containing putative wild type binding sites or mutated binding sequence for miR-454-3p was inserted into pGL3 luciferase reporter to generate pGL3-c-Met-wt or pGL3-c-Met-mut, respectively. SUNE-1 and C666-1 cells were co-transfected with miR-454-3p mimic plus pGL3-c-Met-wt or miR-454-3p plus pGL3-c-Met-mut. After 48 hours, the luciferase activity was measured by a luciferase reporter assay kit (Promega).

Tumor growth in vivo

sh-Ctrl or sh-LINC00839 stable transfected SUNE-1 (100 µL, 2×10^6) cells were subcutaneously inoculated into BALB/c nude mice (n=3 in each group). The width and length of tumor were recorded every week. Tumor volume = length $\times$ width² $\times$ 0.5. After five weeks, nude mice were sacrificed. Tumor tissues was subjected for immunohistochemical staining assay. The animal experiment was in accordance with the Guide for the Care and Use of Laboratory Animals and approved by Weifang Hospital of traditional Chinese Medicine.

Statistical analysis

Data are presented as Mean $\pm$ SD. Statistical differences were assessed by Student's t-test or one-way ANOVA analysis followed by Dunnett's test. *P* value less than 0.05 was considered as statistical difference.

Results

Knockdown of LINC00839 inhibits NPC cells growth in vitro

Two NPC-related GEO microarray chip data (GSE53819 and GSE64634) were screened for the identification of differentially expressed lincRNAs. Total 343 differentially expressed lincRNAs in GSE53819 and 789 differentially expressed in GSE64634 were observed. Volcano plot displayed the differential expressed lincRNAs in GSE53819 and GSE64634 (Fig. 1A-1B). 13 upregulated lincRNAs in GSE53819 and 7 upregulated lincRNAs in GSE64634 were observed. Volcano plot displayed the differential expressed lincRNAs in GSE53819 and GSE64634. The results displayed that solely the LINC00839 was found at the intersection, with its high level in NPC recorded in the two GEO datasets (Fig. 1C). To verify the expression pattern of LINC00839 in NPC, qPCR was performed to measure LINC00839 levels in NPC cell lines. As presented in Fig. 1D, when compared with in normal nasopharyngeal epidermal cell line NP-69, LINC00839 expression was markedly elevated in NPC cell lines (SUNE-1, CNE-1, C666-1, and CNE-2). Then, SUNE-1 and C666-1 cells were transfected with sh-LINC00839 #1 or sh-LINC00839 #2. The transfection efficiency was determined by qPCR (Fig. 1E). We noted that sh-LINC00839 #2 exhibited higher shRNA transfection efficiency when compared with the sh-LINC00839 #1. Therefore, we chose to perform in vitro experiments with sh-LINC00839 #2 transfected cell line. LINC00839 knockdown attenuated cell proliferation after transfection (Fig. 1F-1G). Similarly, LINC00839 knockdown attenuated the colony formation of C666-1 and SUNE-1 cells in vitro (Fig. 1H). These results demonstrate LINC00839 is upregulated in NPC and LINC00839 knockdown inhibits NPC cells growth in vitro.

Knockdown of LINC00839 inhibits NPC cell EMT in vitro

Besides, the capacities of cell migration, invasion and EMT were examined. Wound healing and Transwell assays showed C666-1 and SUNE-1 cell migration (Fig. 2A-2B) and invasive ability (Fig. 2C-2D) was attenuated in sh-LINC00839 group. Therefore, we further investigated the influence of LINC00839 knockdown on EMT in NPC cells in vitro. The result was that SUNE-1 and C666-1 cells knocked down LINC00839 exhibited lower level of N-cadherin (mesenchymal cell marker) and higher level of E-cadherin (epithelial cell marker) (Fig. 2E). In addition, we analyzed the mRNA levels of different EMT biomarkers to identify the progression of EMT. As illustrated in Fig. 2F, knock-down of LINC00839 decreased the mRNA of Vimentin (mesenchymal cell marker) and raised the level of ZO-1 (epithelial cell marker). These data suggested that silencing of LINC00839 diminishes the invasion and EMT of NPC cells.

LINC00839 acts as a “sponge” for miR-454-3p

With the research on online bioinformatics database (starBase v2.0), we found miR-454-3p was predicted to have complementary base pairing with LINC00839 (Fig. 3A). To verify the target binding of miR-454-3p and LINC00839, we constructed luciferase reporter vectors containing the predicted wild-type (wt) or mutant (mut) binding sites of miR-454-3p on LINC00839 (named as LINC00839-wt and LINC00839-mut). As show in Fig. 3B, the luciferase activity was reduced when co-transfected with miR-454-3p mimic and

LINC00839-wt whereas there was no difference in the LINC00839-mut group. Ago2-RIP assay further showed LINC00839 enrichment was much higher in miR-454-3p overexpression groups than that in the miR-NC group (Fig. 3C). Moreover, LINC00839 silencing significantly increased the expression of miR-454-3p (Fig. 3D). qPCR analysis declared a downregulation of miR-454-3p in NPC cell lines in comparison with NP-69 cells (Fig. 3E). SUNE-1 and C666-1 cells were transfected with miR-NC or miR-454-3p mimic (miR-454-3p). As shown in Fig. 3F, miR-454-3p was dramatically upregulated in C666-1 and SUNE-1 cells. Subsequently, we observed that miR-454-3p mimic decreased the colony formation of NPC cells (Fig. 3G). In terms of the capacity of cell invasion, Transwell assays showed that invasive cells were declined in miR-454-3p-overexpressed NPC cells (Fig. 3H). These findings suggest that miR-454-3p is downregulated in NPC and miR-454-3p represses the growth and invasiveness of NPC cells in vitro.

c-Met is targeted by miR-454-3p

According to *in silico* data on TargetScan Human (TargetScan Human release 7.2), the putative target genes of miR-454-3p were investigated and bioinformatics analysis indicated that human c-Met was a potential target of miR-454-3p (Fig. 4A). To confirm this prediction, c-Met 3'-UTR wild type (c-Met-wt) and mutant (c-Met-mut) reporter plasmids were constructed, and luciferase reporter gene assay was carried out. The luciferase activity was remarkably reduced in c-Met-wt group when transfected with miR-454-3p mimic; however, there was no difference in c-Met-mut group (Fig. 4B). In addition, the regulatory effect of LINC00839 and miR-454-3p on c-Met expression was determined by western blot. The result showed that c-Met expression was inhibited when cells were transfected with miR-454-3p or sh-LINC00839 (Fig. 4C). In co-transfection group, re-introducing LINC00839 (pc-LINC00839) almost restored c-Met expression level in miR-454-3p mimic transfected NPC cells (Fig. 4D). Altogether, these findings demonstrate that LINC00839 serves as a "sponge" to competitively interact with miR-454-3p in NPC cells and regulate the expression of c-Met.

LINC00839 overexpression abates the effects of miR-454-3p

To further explore the mechanism of LINC00839 in affecting the malignancy of NPC, rescue experiments were launched to clarify the effect of LINC00839 on mediating the biological action of miR-454-3p in NPC cells in vitro. SUNE-1 and C666-1 cells co-transfected with pc-LINC00839 and miR-454-3p. Transfection cells were divided into three groups: miR-NC, miR-454-3p mimic and pc-LINC00839 + miR-454-3p (Fig. 4D). In miR-454-3p mimic group, cells exhibited lower cell colony formation, which was rescued by upregulation of LINC00839 (Fig. 5A and 5B). Cell invasion ability was rescued in pc-LINC00839 + miR-454-3p group, compared to cells transfected with miR-454-3p group (Fig. 5C and 5D). These results reveal a novel LINC00839/miR-454-3p axis in NPC development.

LINC00839 knockdown alleviates NPC cells growth in vivo

Finally, SUNE-1 cells were stably expressed sh-LINC00839 by lentivirus infection (Fig. 6A), and were xenogenic transplanted into nude mice (n = 3). After 5 weeks, injection of SUNE-1 cells induced tumor growth in both groups whereas the tumor growth rate was slowed down because of LINC00839 silencing

(Fig. 6B-6C). Additionally, tumors evidently weigh less due to knockdown of LINC00839 (Fig. 6D). Furthermore, the expression of c-Met was extremely downregulated and miR-454-3p was highly boosted in experimental group (Fig. 6E-F). Besides, the expression of N-cadherin was abundantly attenuated by sh-LINC00839 whereas the expression of E-cadherin was raised as demonstrated by immunohistochemical staining (Fig. 6G-6H). These data demonstrate the tumor-suppressive role of LINC00839 knockdown in NPC tumor growth.

Discussion

NPC is one of the most common malignancies of the head and neck, and it has a multifactorial aetiology, presenting a distinct geographical distribution in occurrence, with high incidence rates [14]. Despite recent advancements in molecularly targeted approaches in NPC treatment, the 5-year survival rate of patients with NPC has not met the expectation due to distant metastases [15]. This triggers a major effort to discover actionable molecular targets to treat patients with NPC. Recently, lncRNAs have been demonstrated as playing critical actions in the progression of NPC [7].

LINC00839 acts as an oncogenic gene and is significantly upregulated in human cancers including breast cancer and osteosarcoma [11, 12]. In our study, we noticed that LINC00839 level was widely higher in NPC tissues from GEO dataset and cell lines than the normal tissues and NP-69. Here, we figured out the role of LINC00839 knockdown on the malignancy of NPC in C666-1 and SUNE-1 cells. The results suggested that knockdown of LINC00839 abated the malignancy of NPC cells in vitro through inhibiting cell proliferation, colony formation, invasion and EMT. The tumorigenicity of NPC cells in vivo was also inhibited by LINC00839 knockdown. Taken all results together, LINC00839 is upregulated in human NPC and its dysregulation expression modulates the initiation and development of NPC.

Specific endogenous lincRNAs that contain miRNA binding sites can function as “sponge” for specific miRNAs and interfere with their functions, thereby regulating gene expression [1, 16]. For example, lncRNA CCDC144NL-AS1 promotes the oncogenicity of osteosarcoma by acting as a molecular sponge for miRNA-490-3p and thereby increasing HMGA2 expression [17]. In this study, we figured out LINC00839 function as “sponge” for miR-454-3p. It is reviewed that miR-454-3p is expressed in various human tissues under normal physiological conditions and pathologic conditions; it is implicated in a series of biological processes, for example proliferation, apoptosis, metastasis, EMT and so on [18]. Although deregulated miRNAs including miR-454-3p is yet fully understood, miRNAs have been recognized as new diagnostic and prognostic biomarkers in NPC, as well as the therapeutic targets. Some researchers indicated that combined miRNA and current chemotherapeutic drugs could induce some promising results in vitro. We observed that overexpression of miR-454-3p could suppress cell proliferation, invasion and EMT in C666-1 and SUNE-1 cells.

In general, as a ceRNA, the function of lincRNAs depends on the miRNA target. Using an online database, we predicted c-Met as a potential target of miR-454-3p, which was confirmed by luciferase reporter and western blot assay. c-Met, a member of the ADAM family, is overexpressed in breast, ovarian, and

prostate cancers [19]. c-Met is a membrane-bound protease that sheds the extracellular domain of various receptors or its ligands from the cell membrane and subsequently activates downstream signaling transduction pathways. A close association of c-Met expression with cervical lymph node metastasis in NPC has been disclosed [20]. Silencing of c-Met suppresses the proliferation and invasion of NPC cells [21]. Additionally, upregulation of LINC00839 significantly reversed miR-454-3p-induced promoting role in NPC cells in vitro, implying a novel LINC00839/miR-454-3p signaling axis in NPC development. Consistent with the observations in vitro, LINC00839 knockdown alleviated the growth of NPC cells and negatively regulated the expression of miR-454-3p in vivo.

To sum up, we showed knockdown of LINC00839 decreased cell proliferation, migration, invasion and EMT in human NPC cell lines C666-1 and SUNE-1 through sponging miR-454-3p and downregulating c-Met, a downstream target of miR-454-3p. Therefore, we conclude that LINC00839/miR-454-3p/c-Met pathway may be a novel mechanism underlying the oncogenic role of LINC00839 in NPC. Our research could be useful for the diagnosis, therapy and prognosis for NPC, and provides new biomarkers for targeted therapy in NPC.

Declarations

Conflict of interest statement

We declare that we have no conflict of interest.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Figures

Figure 1

The expression and effect of LINC00839 on cell growth in NPC. (A-B) The differential gene expression in GSE53819 and GSE64634 data subsets was visualized using volcano plots (C) Venn diagrams show the intersection of GSE53819 and GSE64634 data subsets. (D) qPCR examined LINC00839 expression in NPC cell lines (SUNE-1, CNE-1, C666-1 and CNE-2) compared with that in normal nasopharyngeal epidermal cell line NP-69. ** $P < 0.01$ compared with NP-69. (E) qPCR analysis testified LINC00839 expression in C666-1 and SUNE-1 cells after transfected with shRNA against LINC00839 (sh-LINC00839 # or sh-LINC00839 #2) or scrambled shRNA (sh-Ctrl). (F-G) After transfection, cell proliferation was determined by MTT assay. (H) The growth of NPC cells was determined by colony formation assay. ** $P < 0.01$ compared with sh-Ctrl group.

Figure 2

The role of LINC00839 in NPC cell migration and invasion. (A-B) After cell transfected with sh-LINC00839, cell migration was determined by wound healing assay. (C-D) Cell invasion was determined by Transwell assay. (E) The expressions of E-cadherin and N-cadherin were determined by western blot. (F) The mRNA levels of Vimentin and ZO-1 were measured by qPCR assay. ** $P < 0.01$ compared with sh-Ctrl group.

Figure 3

The relationship between LINC00839 and miR-454-3p in SUNE-1 and C666-1 cells. (A) Online software starBase showed the sequence alignment of miR-454-3p with the putative binding sites within LINC00839. (B) The luciferase reporter assay demonstrated the influence of miR-454-3p on the luciferase activity in C666-1 and SUNE-1 cells transfected with LINC00839-wt or LINC00839-mut vector. (C) RIP assay was performed to further identify the potential binding of LINC00839 and miR-454-3p. (D) Levels of miR-454-3p were detected by qPCR after cells transfected with sh-LINC00839. (E) qPCR examined miR-454-3p level in NPC cell lines (SUNE-1, CNE-1, C666-1 and CNE-2) compared with that in NP-69. **P<0.01 compared with NP-69. (F) qPCR analysis testified miR-454-3p expression in C666-1 and SUNE-1 cells after transfected with miR-NC or miR-454-3p mimic. (G) After transfection, the growth of NPC cells was

determined by colony formation assay. (H) The invasion of NPC cells was analyzed using Transwell assay. **P<0.01 compared with miR-NC group.

Figure 4

c-Met was a target gene for miR-454-3p. (A) The predicted miR-454-3p binding sites in the 3'-UTR of c-Met gene according to Targetscan. (B) Luciferase activity of wild-type (wt) or mutant (mut) c-Met 3'-UTR in SUNE-1 and C666-1 cells transfected with miR-454-3p mimic or miR-NC. **P<0.01 compared with miR-NC

group. (C) Western blot detected the effect of miR-454-3p and sh-LINC00839 on c-Met protein expression in SUNE-1 and C666-1 cells. (D) SUNE-1 and C666-1 cells were transfected with miR-454-3p or co-transfected with miR-454-3p and pc-LINC00839. The expression of c-Met was determined by western blot.

Figure 5

LINC00839 reverses the effects of miR-454-3p in SUNE-1 and C666-1 cells. (A) SUNE-1 and C666-1 cells were transfected with miR-454-3p or co-transfected with miR-454-3p and pc-LINC00839. The growth of

NPC cells was measured using colony formation assay. (B) The invasion of NPC cells was assessed using Transwell assay. ** $P < 0.01$ compared with miR-NC group, ## $P < 0.01$ compared with miR-454-3p group.

Figure 6

LINC00839 knockdown suppresses tumor growth in vivo. (A) SUNE-1 cells were silenced LINC00839 expression by lentivirus infection, the level of LINC00839 was assessed by qPCR. (B) SNUE-1 cells were xenograft transplanted into nude mice ($n = 3$). Tumor volumes were measured every week and the growth curve of xenograft tumors was drawn. (C) Representative images of xenograft tumors. (D) Tumor weight. (E) Expression levels of miR-454-3p in xenograft tumors were determined by qPCR. (F) Expression level of c-Met in xenograft tumors was determined by western blot. (G-H) Protein levels of E-cadherin and N-cadherin were examined by immunohistochemical staining. ** $P < 0.01$ compared with sh-Ctrl group.