

LncRNA SNHG16 Facilitates Nasopharyngeal Carcinoma Progression by Acting as ceRNA to Sponge miR-520a-3p and Upregulate MAPK1 Expression

Qingwei Wu
Yingying Zhao
Runjie Shi
Tao Wang

Department of Otorhinolaryngology
Head and Neck Surgery, Shanghai Ninth
People's Hospital, Shanghai Jiao Tong
University School of Medicine, Shanghai,
People's Republic of China

Background: Accumulating evidence shows that lncRNAs are widely involved cellular processes of various tumors. The aim of this study was to explore the potential role and molecular mechanism of lncRNA SNHG16 in nasopharyngeal carcinoma (NPC).

Methods: SNHG16, miR-520a-3p, and MAPK1 levels were measured by RT-qPCR assay. CCK-8, colony formation, transwell, and flow cytometry assays were adopted to analyze the proliferation, migration, invasion, and apoptosis of NPC cell lines (SUNE1 and 5-8F). Murine xenograft model was used to investigate tumor growth and metastasis in vivo. Immunohistochemical staining was employed to evaluate the levels of Bcl-2, cleaved caspase-3, Bax, and Ki-67. Dual-luciferase reporter assays were conducted to analyze the binding ability between miR-520a-3p and SNHG16 or MAPK1.

Results: SNHG16 was overexpressed in NPC tissues and cells. High SNHG16 expression indicated a poor prognosis. SNHG16 knockdown could cause significant inhibition on cell proliferation and metastasis, induce cell apoptosis in NPC cells, and repressed tumor growth and metastasis in vivo. Additionally, SNHG16 could directly bind to miR-520a-3p, thus positively regulating MAPK1 expression. Moreover, functional analysis indicated that miR-520a-3p exerted a tumor-suppressing role in NPC progression. Rescue assays demonstrated that MAPK1 upregulation could abrogate the inhibitory effects on NPC cell proliferation and metastasis, as well as the promoting effects on NPC cell apoptosis caused by SNHG16 knockdown. In conclusion, SNHG16 contributed to the proliferation and metastasis of NPC cells by modulating the miR-520a-3p/MAPK1 axis.

Conclusion: These results suggest that SNHG16 acts as an oncogene in the progression of NPC via modulating the miR-520a-3p/MAPK1 axis.

Keywords: lncRNA SNHG16, miR-520a-3p, MAPK1, nasopharyngeal carcinoma, ceRNA

Correspondence: Runjie Shi; Tao Wang
Department of Otorhinolaryngology
Head and Neck Surgery, Shanghai Ninth
People's Hospital, Shanghai Jiao Tong
University School of Medicine, No. 639
Zhi Zao Ju Road, Huangpu District,
Shanghai, 200011, People's Republic of
China
Email shirunjie35@163.com;
TaoWang3775@163.com

Introduction

As a kind of malignant tumor originating from epithelial cells in nasopharynx, nasopharyngeal carcinoma (NPC) is the most common head and neck carcinomas in southern China.¹ There were approximately 13,000 new cases and 73,000 deaths owing to NPC in 2018 around the world.² By now, local invasion and distant metastasis are still major causes for the low survival rate of NPC patients. Hence, it is important to investigate the molecular mechanism of NPC and discover more effective NPC therapies.

Accumulating studies indicated that lncRNAs could act as competing endogenous RNAs (ceRNAs) which competitively bind to miRNAs and restrain the binding between miRNAs and their target genes, thus participating in mRNA regulation. For example, lncRNA KCNQ1OT1 regulated the CCND2 level and promoted osteosarcoma progression by competitively binding with miR-4458.³ Wang et al uncovered that lncRNA HCP5 contributed to the development of breast cancer by modulating BIRC3 via competitive interaction with miR-219a-5p.⁴ LncRNA MT1JP could competitively bind with miR-92a-3p and modulate FBXW7 level in gastric cancer.⁵ A large number of lncRNAs have been identified as oncogene in NPC. For instance, SNHG5 accelerated NPC development via the miR-1179/HMGB3 pathway.⁶ SOX2-induced lncRNA ANRIL upregulation facilitated NPC tumor growth.⁷ SNHG7 promoted cell proliferation of NPC by regulating ELAVL1 via miR-514a-5p.⁸ It has been reported that SNHG16 exerts important functions in a diversity of human cancers,⁹ including cervical cancer,¹⁰ gastric cancer,¹¹ osteosarcoma,¹² pancreatic cancer,¹³ and hepatocellular carcinoma.¹⁴ Additionally, SNHG16 also could function as a ceRNA for miRNAs in cellular processes of various cancers. For instance, SNHG16 facilitated cell growth in colon cancer via regulating the miR-302a-3p/AKT axis.¹⁵ SNHG16 contributed to glioma malignancy by upregulating E2F1 via competitive interaction with miR-20a-5p.¹⁶ SNHG16 accelerated development of colorectal cancer via miR-124-3p/MCP-1 axis.¹⁷ Furthermore, a report from Zou et al also revealed that SNHG16 was overexpressed in high-risk NPC.¹⁸ However, the molecular mechanisms of SNHG16 in NPC need to be further investigated.

MicroRNAs (miRNAs) are defined as a class of single-stranded RNAs (20–23 nucleotides), which can function as either oncogene or tumor suppressor in human cancers.^{19,20} MiR-520a-3p has been identified as a tumor suppressor in multiple cancers. To cite an instance, miR-520a-3p regulated CCND1 and CD44 to suppress breast cancer.²¹ MiR-520a-3p restrained cell malignant properties in papillary thyroid carcinoma through JAK/STAT pathway.²² MiR-520a-3p regulated HOXD8 to inhibit non-small cell lung cancer development.²³ However, its potential regulating mechanism in NPC is still unclear.

In this study, we revealed a new mechanism that SNHG16 enhanced cell proliferation and metastasis, and inhibited cell apoptosis in NPC through modulating MAPK1 level via competitive interaction with miR-520a-3p. The findings of the present study might provide a novel insight into NPC tumorigenesis.

Materials and Methods

Data Mining and Analysis

The expression data and clinical data of NPC tissues and normal tissues were obtained from the Gene Expression Omnibus (GEO) profile database (<http://www.ncbi.nlm.nih.gov/geo/>), accession number GSE12452 and GSE70970.

Sample Tissues

Twenty-six NPC samples and adjacent normal tissues were harvested from patients admitted to Shanghai Ninth people's Hospital from July 2006 to July 2007, and the samples were stored in liquid nitrogen immediately. No NPC patients had ever undergone any radiotherapy or chemotherapy. All the NPC patients enrolled signed the informed consent. Our study was approved by the Ethics Committee of Shanghai Ninth people's Hospital and conducted in accordance with the Declaration of Helsinki. Patient follow-ups were conducted during the 14 years follow-up period.

Cell Culture and Transfection

The NPC cell lines (SUNE1, 5–8F, and C666-1) and normal nasopharyngeal epithelial cell line (NP69) were purchased from BeNa (Beijing, China). The cells were incubated in DMEM with 10% FBS and kept in an incubator with 5% CO₂ at 37°C.

The siRNA targeting SNHG16 (si-SNHG16: 5'-ACAAAGUAUGACAGUUCGGCC-3'), siRNA negative control (si-NC: 5'-CUCUGCGUCAUCUAGAUGUGA-3'), miR-520a-3p mimics (5'-AAAGUGCUUCCCUUUGGACUG-3'), miR-520a-3p inhibitor (5'-UUUCACGAAGGAAACCUGAC-3'), and their corresponding negative controls (NC mimics: 5'-ACGUAGUGAUCAGACCUGAAC-3' and NC inhibitor: 5'-UUCACUUGGCCAUUUAGGGC-3') were acquired from GenePharma (Shanghai, China). The full length of MAPK1 was inserted into pcDNA3.1 to establish MAPK1 overexpression plasmid (MAPK1). With Lipofectamine 2000 (Invitrogen), si-NC, si-SNHG16, MAPK1, pcDNA3.1, miR-520a-3p, miR-520a-3p inhibitor, NC mimics, and NC inhibitor were transfected into SUNE1 and 5–8F cells.

RT-qPCR Analysis

TRIzol reagent (Invitrogen) was employed to isolate RNA from tissues and cells. Reverse transcription (RT) was conducted by using the Takara PrimeScript Kit (Takara). PCR was carried with FastFire qPCR PreMix (SYBR Green, China) on the PCR system platform (Bio-Rad,

USA). By $2^{-\Delta\Delta Ct}$ method, relative gene expression was quantified with GAPDH or U6 as the internal control. The employed primers are as follows: SNHG16 forward (F): 5'-CAGTCAGCCTCAGTTTCCAA-3' and reverse (R): 5'-AGGCAGGGCTGTGCTGAT-3'; MAPK1 F: 5'-TGGAT TCCCTGGTTCTCTCTAAAG-3' and R: 5'-GGGTCT GTTTTCCGAGGATGA-3'; GAPDH F: 5'-CCACTCCTC CACCTTTGAC-3' and R: 5'-ACCCTGTTGCTGTAGC CA-3'; miR-520a-3p F: 5'-CTCAACTGGTGTCGTGG AGTCGGCAATTCAGTTGAGACAGTCCAAA-3' and R: 5'-ACACTCCAGCTGGGAAAGTGCTTCCC-3'; U6 F: 5'-ATGGGTCGAAGT CGTAGCC-3' and R: 5'- AAA ATATGGAACGCTTCACGA-3'.

CCK-8 Assay

After transfection for 24 hours, the cells at a concentration of 5×10^3 per well were seeded on a 96-well plate and cultured for 0, 24, 48, and 72 hours. Then, 10 μ L CCK-8 reagent was added to each well. Subsequently, the cells were cultivated at 37°C for 1.5 hours. The OD value was analyzed under a microplate reader at 450 nm.

Colony Formation Assay

SUNE1 and 5–8F cells (800 cells/well) were seeded into 6-well plate at a concentration of 100 cells/well. Then the cells were cultured in the medium for 2 weeks. After the colonies were formed, the cells were fixed and stained via 0.1% crystal violet for 25 minutes. The number of the formed colonies was counted with a microscope.

Transwell Assay

Transwell assay was employed to analyze cell migrative and invasive capabilities in 24-well transwell chambers with 8- μ m membrane (Corning, New York). For migration assay, 5×10^4 SUNE1 and 5–8F cells were seeded to the upper chamber. The upper chamber contained 200 μ L serum-free medium. The lower chamber contained 600 μ L medium with 20% fetal bovine serum. After 48h incubation, cells on the outer surface of the membrane were fixed and stained with 0.1% crystal violet. For invasion assay, the Matrigel-precoated upper chamber was adopted, and the subsequent steps are the same as those of migration assay. The number of migrated and invaded NPC cells were measured via a microscope at 450 nm.

Flow Cytometry Assay

Briefly, the transfected cells were washed three times with ice-cold PBS, and the cells were suspended again in Annexin-V

binding buffer (5×10^5 /mL). Thereafter, 5 μ L Annexin-V/FITC solution and 10 μ L Propidium Iodide (PI) reagent were supplemented into the suspension. After incubation at 37°C for 15 minutes in darkness, the condition of cell apoptosis was analyzed by flow cytometry (FACScan, China).

Dual-Luciferase Reporter Assay

Partial SNHG16 sequences and 3'-UTR of MAPK1 with or without binding site to miR-520a-3p were integrated into pmirGLO plasmids (Promega, Wisconsin) to generate luciferase reporter vectors (SNHG16-WT, SNHG16-MUT, MAPK1-WT or MAPK1-MUT), respectively. SUNE1 and 5–8F cells were transfected with corresponding reporter vectors and miR-520a-3p-5p mimics or NC-mimics. Dual-luciferase reporter assay system (Promega) was adopted to detect the relative luciferase activity.

Western Blot

RIPA buffer (Beyotime) was used for protein extraction from the cells. Then, equal amounts of proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA) and then blocked with 5% non-fat milk for 2 h. Subsequently, the membranes were incubated with primary antibodies against MAPK1 (sc-136,288, 1:1000 dilution, Santa Cruz Biotechnology) and GAPDH (ab37168, 1:2000 dilution, Abcam) at 4°C overnight. Following washing, the membranes were incubated with secondary antibody at room temperature for 2 h. The chemiluminescence detection system (Beyotime) was applied to visualize the protein blots.

Murine Xenograft Model

Six male BALB/C nude mice (4 weeks) acquired from Slac Laboratory Animal Center (Shanghai, China) were randomly put into two groups. Then, SUNE1 cells (5×10^6 cells in 100 μ L) stably expressing si-NC or si-SNHG16 were injected into the right flank of each mouse subcutaneously, respectively. The tumor size was detected every 5 days. Finally, all the mice were sacrificed 30 days after injection, and the xenografted tumors were harvested and weighed. For in vivo metastasis, transfected cells (5×10^6) were injected into nude mice through the tail vein. Six weeks later, the lungs were isolated and metastasis was analyzed using H&E staining. The animal experiments gained approval from the Ethics Committee of Shanghai Ninth people's Hospital and were carried out according to the National Standard of the Care and Use of Laboratory Animals.

Immunohistochemical Staining (IHC)

After resection from nude mice, the tumor tissues were fixed, paraffin-embedded, and sectioned. The sections were dewaxed, rehydrated, and then incubated in citrate buffer for 20 minutes to obtain antigen retrieval. Subsequently, the tissues were cultured with 3% H₂O₂ for 15 minutes, thus blocking endogenous peroxidase activity. Thereafter, the tissue sections were cultivated with antibodies (Bax, cleaved caspase-3, Bcl-2 or Ki-67) at 4°C overnight. Subsequently, the sections were washed with PBS, incubated with HRP-conjugated secondary antibody, and photographed using a light microscope.

Statistical Analysis

All the statistical data collected were represented as mean \pm SD. GraphPad Prism 6 software was employed for statistical analysis. The Student's *t*-test and ANOVA were adopted to assess differences between two groups or multiple groups, respectively. The overall survival of NPC patients was analyzed via Kaplan-Meier analysis and Log rank test. Pearson correlation analysis was employed to assess the correlation between SNHG16 and miR-520a-3p. $P < 0.05$ was regarded as significant statistically.

Results

SNHG16 is Highly Expressed and Indicates Poor Prognosis in NPC

To explore the clinical relevance of SNHG16, the expression of SNHG16 was evaluated in NPC public dataset (GSE12452). The results indicated that SNHG16 was significantly upregulated in NPC tissues (Figure 1A). Furthermore, RT-qPCR analysis demonstrated that SNHG16 expression was upregulated in NPC tissues and NPC cell lines (Figure 1B and C). Additionally, high

SNHG16 expression was positively associated with clinical stage and lymph node metastasis (Table 1). Kaplan-Meier analysis indicated that the high expression of SNHG16 was associated with a low survival time of NPC patients (Figure 1D). To sum up, SNHG16 exhibited a high expression in NPC, and the high expression of SNHG16 predicted poor prognosis of NPC patients.

SNHG16 Depletion Inhibits Cell Proliferation and Metastasis in NPC Cells

SUNE1 and 5-8F cells were used for subsequent experiments due to the high expression of SNHG16. Functional assays were performed to further investigate the effect of SNHG16 on NPC. We firstly decreased the expression of SNHG16 in SUNE1 and 5-8F cells through siRNA interference (Figure 2A). It was found that SNHG16 knockdown obviously restrains the proliferation of SUNE1 and 5-8F cell lines (Figure 2B and C). Moreover, results of flow cytometry assay exhibited SNHG16 silencing expedited apoptosis of SUNE1 and 5-8F cells (Figure 2D). Transwell assays indicated that SNHG16 silencing visibly decreased the migration and invasion capabilities of SUNE1 and 5-8F cells (Figure 2E and F). The above results showed that SNHG16 contributed to NPC proliferation and metastasis.

Knockdown of SNHG16 Restrains NPC Tumor Growth and Metastasis in vivo

In light of the repressive effect of SNHG16 knockdown on NPC cell proliferation and metastasis, xenografted models were used to further investigate the impact of SNHG16 silencing on NPC tumor growth in nude mice. As demonstrated in Figure 3A, tumors from the mice in si-SNHG16 group were smaller than those from the mice in si-NC group. Moreover, the tumor volume and weight were

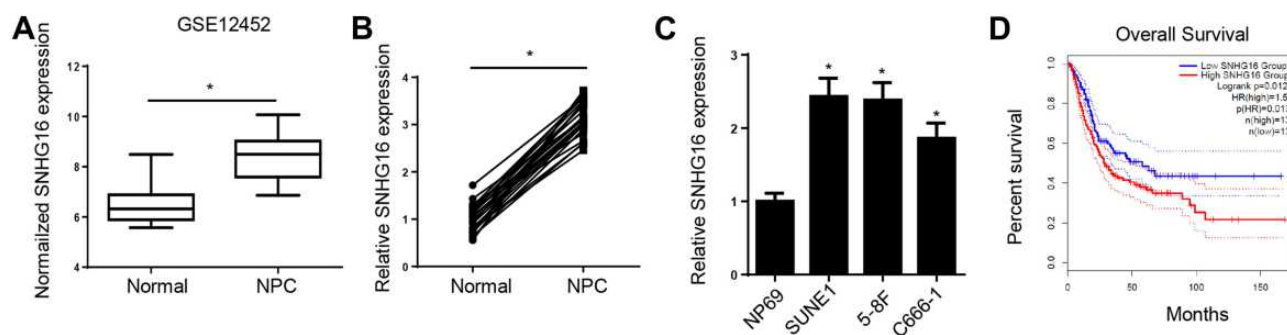


Figure 1 SNHG16 is highly expressed and indicates poor prognosis in NPC. (A) The SNHG16 expression data in NPC tissues were obtained from the GEO datasets in accession GSE12452. (B) SNHG16 expression in 26 NPC tissues and adjacent normal tissues was measured. (C) SNHG16 level was upregulated in NPC cell lines via RT-qPCR. (D) Kaplan-Meier analysis was conducted to illustrate the correlation between SNHG16 expression and survival time of NPC patients. * $p < 0.05$.

Table 1 The Correlation Between SNHG16 Expression and Clinicopathological Characteristics in Nasopharyngeal Carcinoma

Variables		Case (n=26)	SNHG16		P value
			Low (n=13)	High (n=13)	
Gender	Male	11	6	5	0.8652
	Female	15	7	8	
Age	<50	16	9	7	0.4520
	≥50	10	4	6	
Smoking history	Yes	13	5	8	0.3360
	No	13	8	5	
Clinical stage	I–II	11	8	3	0.0281
	III–IV	15	5	10	
Lymph node metastasis	Yes	17	6	11	0.0180
	No	9	7	2	

also decreased by knocking down SNHG16 expression (Figure 3B and C). Moreover, IHC showed that the levels of Bax and cleaved caspase-3 were upregulated in si-SNHG16 group compared with si-NC group, while the levels of Bcl-2 and Ki-67 showed an opposite trend (Figure 3D). Furthermore, RT-qPCR assay showed that SNHG16 and MAPK1 levels were clearly reduced and miR-520a-3p expression was obviously increased in si-SNHG16 group, relative to the control group (Figure 3E). In addition, less metastatic nodules were detected in si-SNHG16 group compared with control group, which were confirmed using HE staining (Figure 3F and G). Therefore, our experiments showed that SNHG16 inhibition suppressed NPC tumor growth and metastasis in vivo.

SNHG16 Negatively Regulates miR-520a-3p Expression

To further investigate the downstream mechanism of SNHG16 in NPC, Starbase website was used and predicted a potential binding sequence between miR-520a-3p and SNHG16 (Figure 4A). Besides, we discovered that miR-520a-3p level was apparently downregulated in NPC tissues as well as in NPC public dataset (GSE70970) (Figure 4B and C). Moreover, Kaplan-Meier analysis showed that high miR-520a-3p expression was closely associated with high survival time in NPC patients (Figure 4D). Then, the transfection efficiency of miR-520a-3p mimics and miR-520a-3p inhibitor was confirmed

(Figure 4E). To further investigate whether miR-520a-3p was directly targeted by SNHG16, a dual-luciferase reporter assay was conducted. The data demonstrated that miR-520a-3p mimics largely reduced the luciferase activity of SNHG16-WT, whereas the miR-520a-3p inhibitor remarkably increased the luciferase activity of SNHG16-WT; however, it had no effect on SNHG16-MUT (Figure 4F). Moreover, SNHG16 silencing upregulated miR-520a-3p expression, while SNHG16 overexpression could negatively regulate miR-520a-3p expression (Figure 4G). In addition, it was also verified that miR-520a-3p expression was negatively correlated with SNHG16 (Figure 4H). Hence, we demonstrated that SNHG16 directly targeted miR-520a-3p in NPC cells.

miR-520a-3p Suppressed NPC Cell Proliferation and Metastasis

To further study the role of miR-520a-3p in NPC, a series of functional experiments were conducted. As shown in Figure 5A and B, the upregulation of miR-520a-3p remarkably suppressed cell proliferation in SUNE1 and 5–8F cells. Moreover, flow cytometry assay indicated that miR-520a-3p overexpression accelerated apoptosis in SUNE1 and 5–8F cells (Figure 5C). In addition, miR-520a-3p upregulation markedly decreased the migrative and invasive abilities of SUNE1 and 5–8F cells as indicated by transwell assay (Figure 5D and E). In conclusion, the above results showed that miR-520a-3p restrained NPC progression.

SNHG16 Promoted NPC Progression via miR-520a-3p/MAPK1 Axis

According to the prediction of Starbase, MAPK1 might be a target of miR-520a-3p (Figure 6A). The results from dual-luciferase reporter assay identified that the transfection with miR-520a-3p mimics significantly reduced the luciferase activity of MAPK1-WT, and transfection with miR-520a-3p inhibitor increased the luciferase activity of MAPK1-WT; whereas the luciferase activity of MAPK1-MUT exhibited little change (Figure 6B), suggesting that miR-520a-3p directly targeted MAPK1. Moreover, the mRNA and protein levels of MAPK1 were significantly upregulated in NPC cell lines compared with normal nasopharyngeal epithelial cell line (Figure 6C). According to the RT-qPCR and Western blot assays, miR-520a-3p inhibition obviously increased MAPK1 expression, while miR-520a-3p

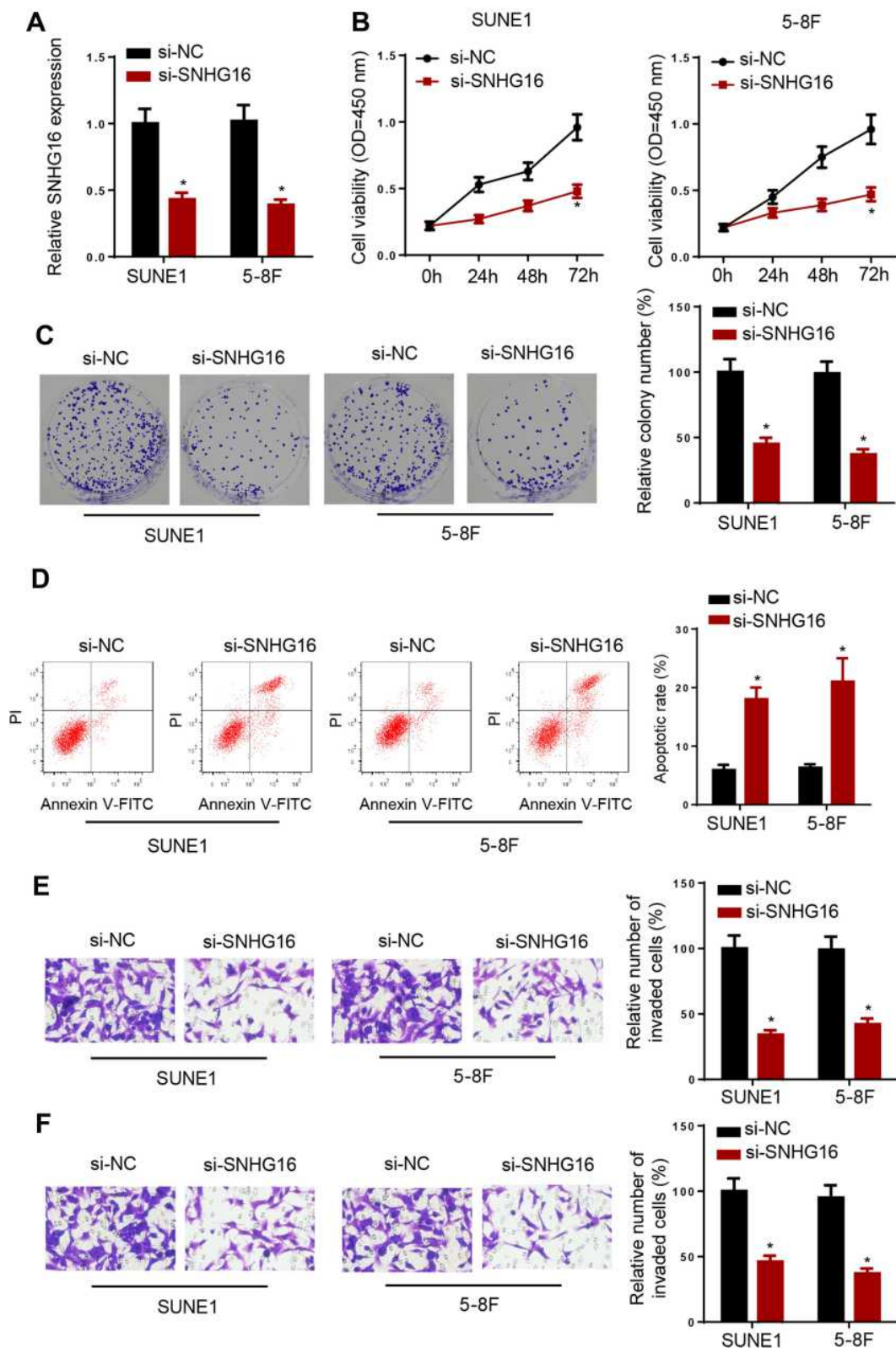


Figure 2 SNHG16 depletion inhibits cell proliferation and metastasis in NPC. **(A)** Expression detection of SNHG16 in SUNE1 and 5-8F cells. **(B and C)** CCK-8 and colony formation assays were carried out to test the effects of SNHG16 knockdown on proliferation. **(D)** Flow cytometry assay was carried out to analyze cell apoptosis of SUNE1 and 5-8F cells. **(E and F)** Transwell assay was conducted to analyze migration and invasion. * $p < 0.05$.

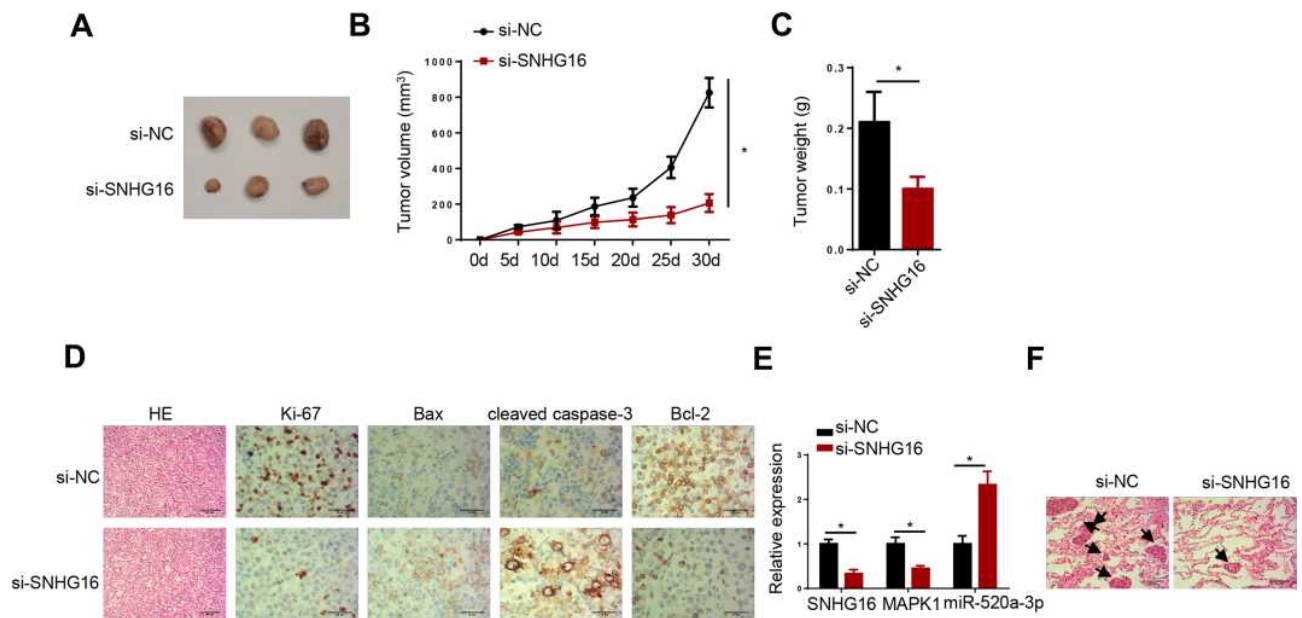


Figure 3 SNHG16 knockdown restrains NPC tumor growth and metastasis in vivo. The tumors excised from mice in si-SNHG16 group or si-NC group. (A and B) The tumor volume and weight were confirmed in SNHG16-knockdown group and control group. (C) Tumor sections under H&E staining and IHC staining to detect Bcl-2, cleaved caspase-3, Bax and Ki-67. (D) RT-qPCR was used to measure the levels of SNHG16, miR-520a-3p and MAPK1. Representative H&E-stained sections (E) of lung tissues and the number of tumor nodules on lung surfaces (F). * $p < 0.05$. The arrows in panel F represent stained metastatic nodules.

overexpression visibly decreased MAPK1 expression in 5–8F and SUNE1 cells (Figure 6D and E). Moreover, SNHG16 knockdown repressed MAPK1 expression, and such suppression could also be abrogated by miR-520a-3p inhibitor (Figure 6F and G). The levels of MAPK1 were significantly upregulated in NPC cells transfected with MAPK1 overexpression plasmid (Figure 6H). Rescue assays further revealed that MAPK1 overexpression reversed the suppression on cell proliferation and metastasis, and promotion on cell apoptosis caused by knocking down SNHG16 expression in 5–8F and SUNE1 cells (Figure 6I–M). To sum up, our data proved that SNHG16 could act its functions via miR-520a-3p/MAPK1 axis.

Discussion

It is well known that lncRNAs participate in the regulation of various cancers.²⁴ Previously, SNHG16 has been demonstrated as an oncogene in multiple human cancers. For example, Du et al disclosed that SNHG16 exerted its oncogenic role in regulating breast cancer cell proliferation and invasion through regulating RRM2 level.²⁵ Liu et al discovered that SNHG16 promoted pancreatic cancer progression through regulating miR-218-5p expression.²⁶ In our study, it was discovered that SNHG16 was highly expressed in NPC tissues

and cell lines, and high SNHG16 expression was positively related to the poor prognosis of NPC patients. Functional assays proved that SNHG16 knockdown inhibited the proliferation and metastasis and stimulated apoptosis of NPC cells in vitro. Moreover, SNHG16 knockdown suppressed NPC tumor growth and metastasis in vivo. To sum up, the data indicated the oncogenic function of SNHG16 in NPC progression.

A lot of lncRNAs have been demonstrated to serve as ceRNAs of miRNAs in NPC progression. For instance, Lan et al reported that lncRNA SNHG1 contributed NPC progression by regulating NUA1 expression via sponging miR-145-5p.²⁷ Lian et al identified that lncRNA AFAP1-AS1 facilitated NPC metastasis by downregulating miR-423-5p expression.²⁸ A report from Zheng et al discovered that lncRNA FAM225A acted as a ceRNA of miR-590-3p/miR-1275 to facilitate NPC tumorigenesis and metastasis.²⁹ In our study, it was discovered that miR-520a-3p exhibited low expression in NPC tissues and cells, and SNHG16 negatively modulated miR-520a-3p expression in NPC cells. Moreover, we also confirmed that miR-520a-3p exerted tumor-suppressing effects in NPC by inhibiting proliferative and metastatic capacities and promoting apoptosis of NPC cells.

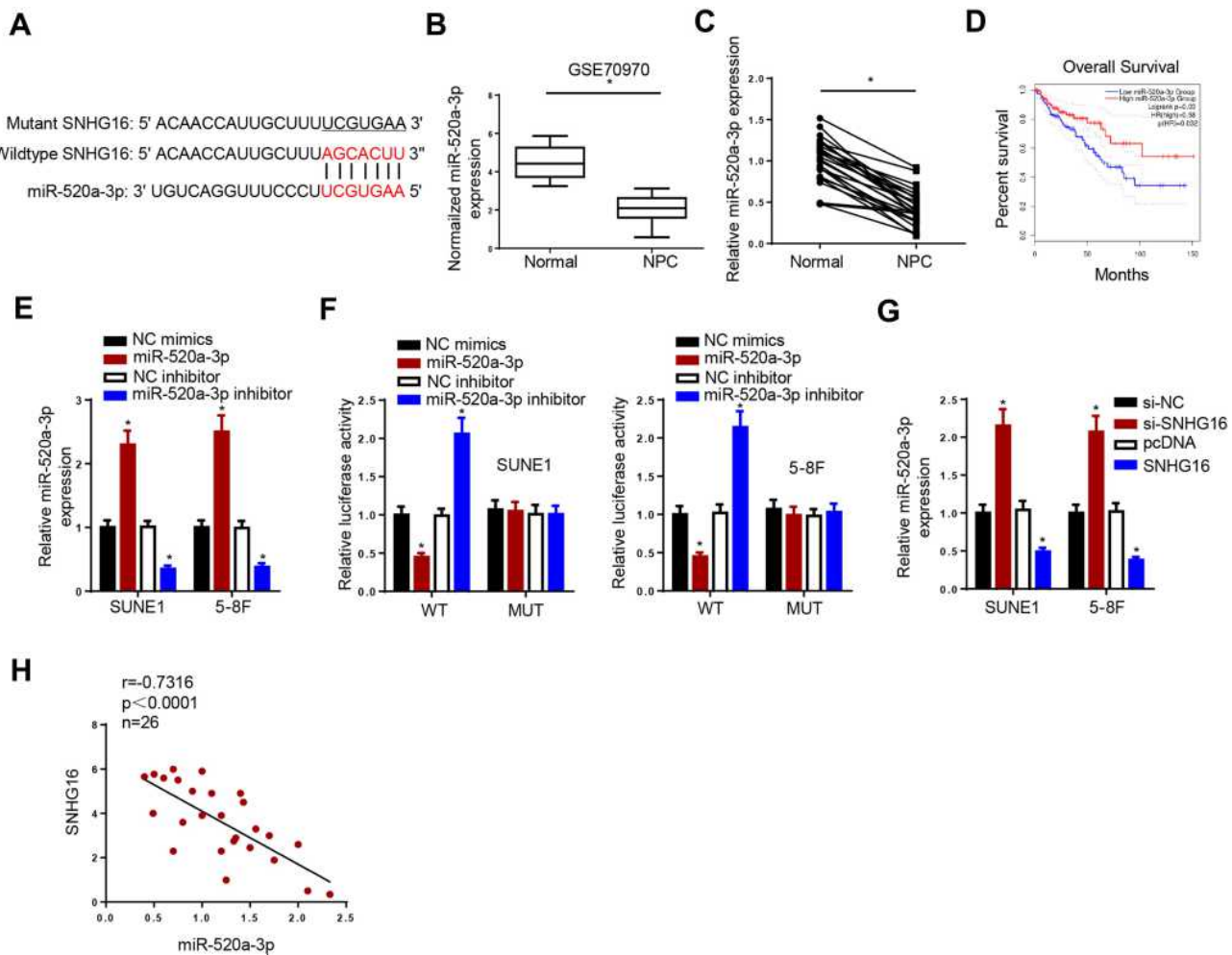


Figure 4 SNHG16 directly sponges miR-520a-3p in NPC cells. **(A)** StarBase website predicted the binding site between SNHG16 and miR-520a-3p. **(B)** miR-520a-3p expression in 26 NPC tissues and adjacent normal tissues was measured via RT-qPCR. **(C)** The miR-520a-3p expression data in NPC tissues were obtained from the GEO datasets in accession GSE70970. **(D)** Kaplan-Meier analysis was conducted to illustrate the correlation between miR-520a-3p expression and survival time of NPC patients. **(E)** Expression analysis of miR-520a-3p after transfection with miR-520a-3p mimics, miR-520a-3p inhibitor or negative controls. **(F)** Luciferase reporter assay was performed to analyze the activity of SNHG16-WT or SNHG16-MUT reporter in SUNE1 and 5-8F cells. **(G)** miR-520a-3p expression was analyzed after transfection with indicated vectors. **(H)** Pearson correlation analysis for correlation between SNHG16 and miR-520a-3p. * $p < 0.05$.

It has been widely recognized that multiple miRNAs exert tumor-suppressing roles in cellular processes of a diversity of cancers by targeting specific mRNAs.^{30,31} MAPK1 (mitogen-activated protein kinase 1) has been discovered upregulated in several cancers, including gastric cancer,³² cervical cancer,³³ and papillary thyroid cancer.³⁴ Herein, we predicted that MAPK1 was a downstream target of miR-520a-3p in NPC cells. Additionally, results of RT-qPCR analysis showed that MAPK1 level was downregulated by miR-520a-3p overexpression and upregulated by miR-520a-3p inhibition. Moreover, SNHG16 knockdown repressed MAPK1 expression; whereas miR-520a-3p silencing partially abrogated the inhibitory effect of SNHG16 blocking on MAPK1 expression. Furthermore, rescue assay indicated

that MAPK1 overexpression could reverse the suppressive impact on proliferation and metastasis, and the promoting effect on the apoptosis of NPC induced by SNHG16 knockdown. These findings suggested that SNHG16 contributed to NPC progression by modulating MAPK1 via sponging miR-520a-3p.

To sum up, this investigation revealed the oncogenic effect of SNHG16 on NPC progression in vitro and in vivo for the first time. Additionally, our data also showed that SNHG16 competitively interacted with miR-520a-3p to positively regulate MAPK1 expression, thereby expediting NPC progression. The newly discovered SNHG16/miR-520a-3p/MAPK1 regulatory network may provide novel insights into NPC tumorigenesis and metastasis and new ideas about gene therapies for NPC treatment.

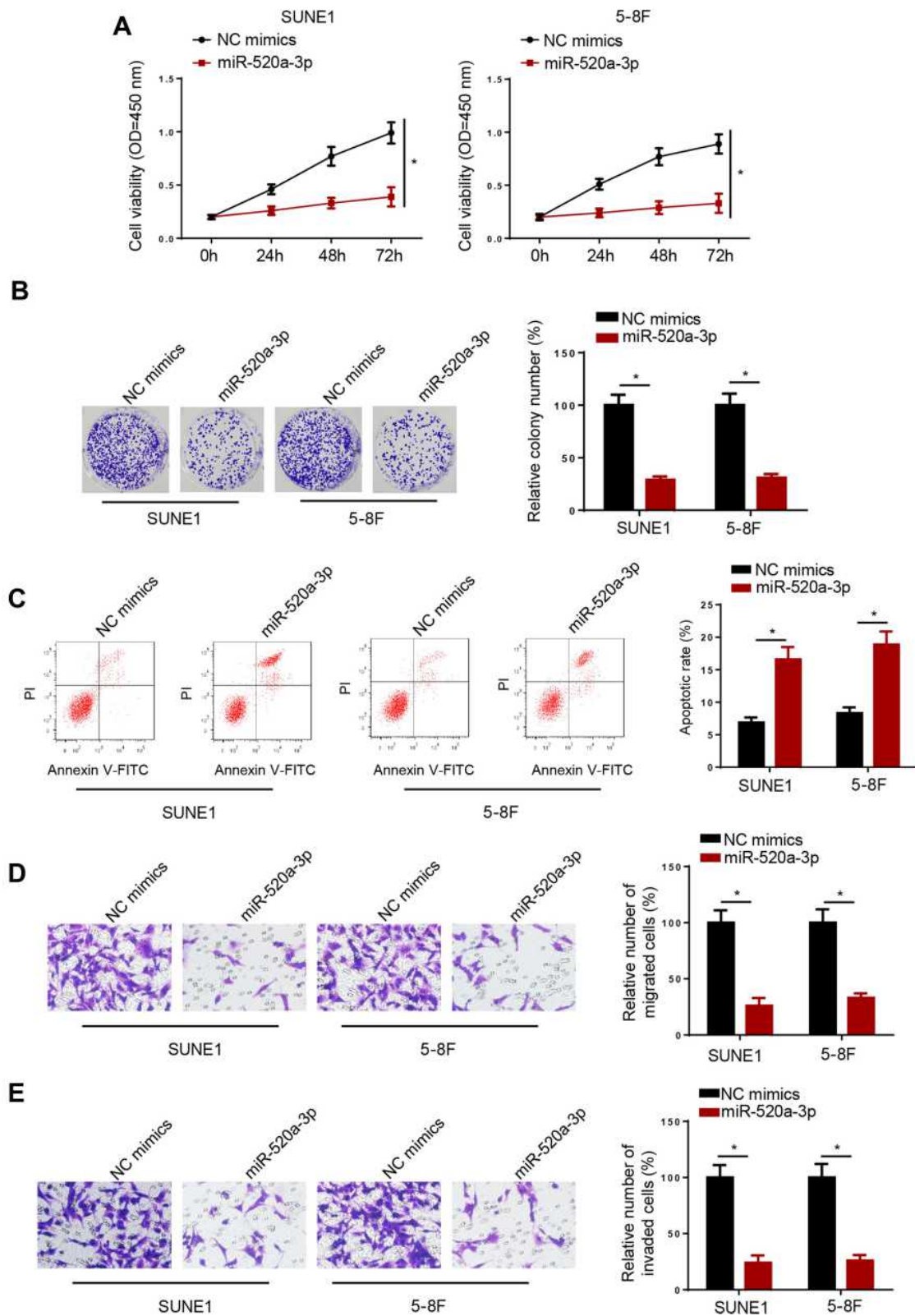


Figure 5 MiR-520a-3p suppressed NPC cell proliferation and metastasis. **(A and B)** CCK-8 and colony formation assays were carried out to test the effects of miR-520a-3p knockdown on proliferation. **(C)** Flow cytometry assay was carried out to analyze cell apoptosis of SUNE1 and 5-8F cells. **(D and E)** Transwell assay was conducted to analyze migration and invasion. * $p < 0.05$.

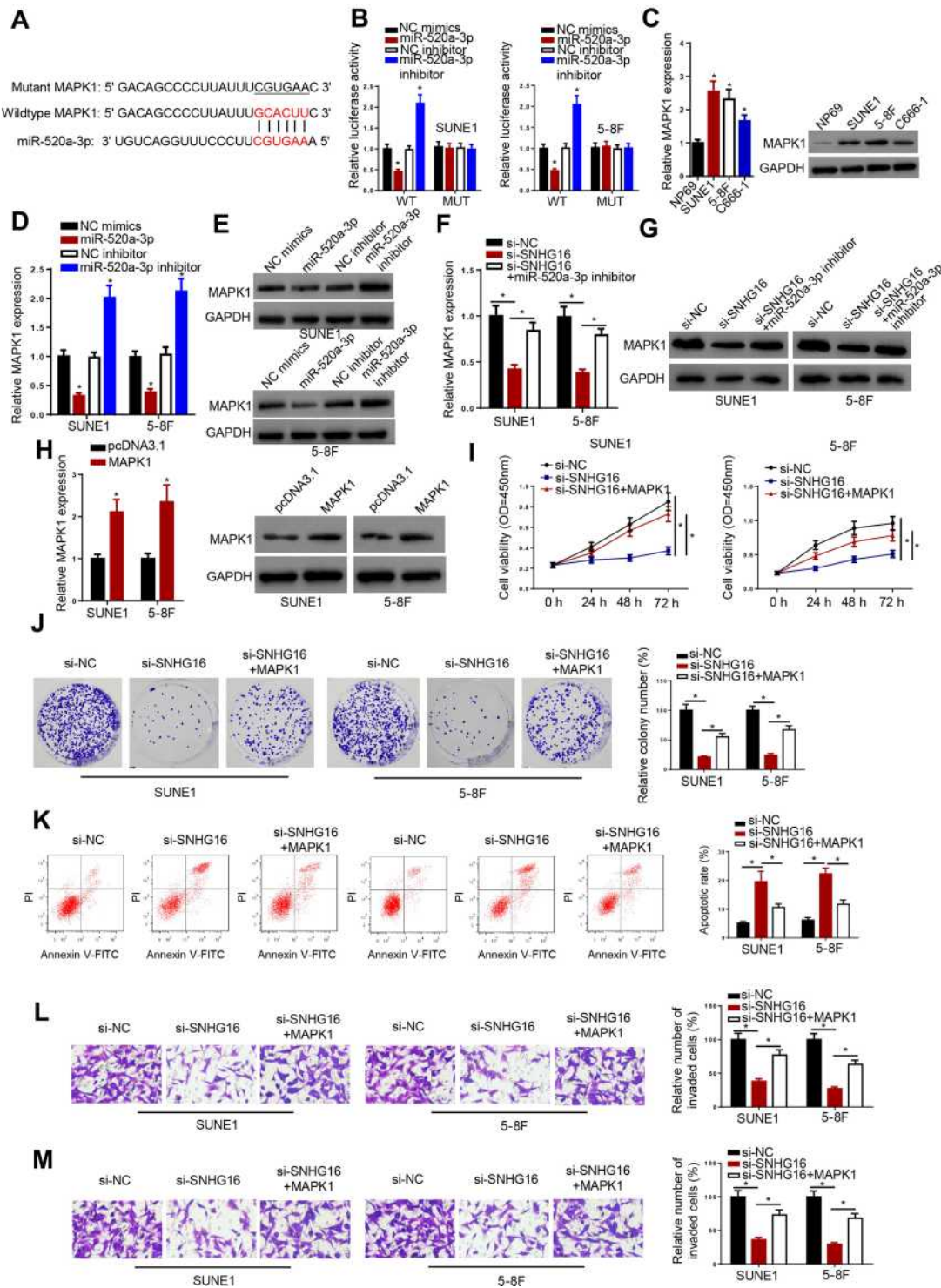


Figure 6 SNHG16 promoted the proliferation and metastatic abilities of NPC cells via miR-520a-3p/MAPK1 axis. **(A)** StarBase website predicted the complementary sequence between MAPK1-3'UTR and miR-520a-3p. **(B)** Luciferase reporter assay was performed in SUNE1 and 5-8F cells co-transfected with MAPK1-WT or MAPK1-MUT, as well as NC mimics, NC inhibitor, miR-520a-3p, and miR-520a-3p inhibitor, respectively. **(C)** The mRNA and protein levels of MAPK1 in NPC cell lines and normal nasopharyngeal epithelial cell line were measured by RT-qPCR and Western blot. **(D and E)** MAPK1 expression was measured in SUNE1 and 5-8F cells transfected with NC mimics, NC inhibitor, miR-520a-3p, and miR-520a-3p inhibitor, respectively. **(F and G)** RT-qPCR and Western blot were performed to measure MAPK1 expression in SUNE1 and 5-8F cells transfected with si-NC, si-SNHG16, and si-SNHG16+miR-520a-3p inhibitor, respectively. **(H)** RT-qPCR and Western blot were used to measure the expression of MAPK1 in cells transfected with pcDNA3.1 and MAPK1 overexpression plasmid. Then, SUNE1 and 5-8F cells were transfected with si-NC, si-SNHG16, and si-SNHG16+MAPK1, respectively. **(I and J)** CCK-8 and colony formation assays were carried out to detect the proliferation of SUNE1 and 5-8F cells. **(K)** Flow cytometry assay was carried out to analyze cell apoptosis of SUNE1 and 5-8F cells. **(L and M)** Transwell assay was performed to examine cell migration and invasion in SUNE1 and 5-8F cells. **p* < 0.05.

Disclosure

The authors report no conflicts of interest in this work.

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