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LncRNA SNHG1 promotes nasopharyngeal carcinoma development via targeting miR-424-5p

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Summary. Objective. Nasopharyngeal carcinoma (NPC) is a malignant tumor of the head and neck. Distant metastasis and drug resistance are the main causes of cancer-related death. A better understanding of the molecular mechanisms that affect the progression of NPC would contribute to clinical treatment. This paper aims to investigate the effects of the long noncoding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) on biological phenotypes of NPC cells and its related mechanisms.

Methods. The expression of SNHG1 and miR-424-5p in non-cancerous nasopharyngeal mucosa tissues and NPC tissues, as well as in normal nasopharyngeal epithelial cells and NPC cells was detected by qRT-PCR. HK1 and C666-1 cells were transfected with SNHG1 overexpression vector (OE-SNHG1), miR-424-5p mimic, SNHG1 knockdown vector (sh-SNHG1), or miR-424-5p inhibitor, followed by detection of transfection efficiency by qRT-PCR, cell viability by MTT, and invasive and migratory abilities by transwell invasion assay and cell scratch test. Moreover, the relationship between SNHG1 and miR-424-5p was detected by dual-luciferase reporter and RIP assays.

Results. In NPC tissues and cells, SNHG1 was upregulated but miR-424-5p was downregulated. Transfection with OE-SNHG1 or miR-424-5p inhibitor promoted proliferative, invasive, and migratory phenotypes of HK1 and C666-1 cells; transfection of sh-SNHG1 or mi-miR-424-5p induced reverse trends. Mechanistically, SNHG1 negatively regulated miR-424-5p expression, and transfection of miR-424-5p inhibitor counteracted the inhibitory effects of sh-SNHG1 on the proliferative, invasive, and migratory phenotypes of HK1 and C666-1 cells.

Conclusion. LncRNA SNHG1 promoted proliferation, invasion and migration of NPC cells by repressing miR-424-5p expression.

Key words: Nasopharyngeal carcinoma, Long non-coding RNA SNHG1, MicroRNA-424-5p, Proliferation, Invasion, Migration

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Introduction

Nasopharyngeal carcinoma (NPC) incidence demonstrates significant geographic variation and people in Southeast Asia and south China have high age standardized rate (Tan et al., 2020). There are approximately 80,000 new cases of NPC and 50,000 deaths from the disease every year globally, and the incidence in Southeast Asia is 80 times higher than in the West (Yin and Zhu, 2019). Unfortunately, since early NPC is relatively asymptomatic, many patients with NPC present with locally advanced disease or distant metastasis at initial diagnosis (Sun et al., 2019). Generally, patients with early NPC can be cured by radiotherapy alone, while patients with middle and advanced NPC need the comprehensive treatment mode of radiotherapy combined with chemotherapy for better efficacy (Liao et al., 2019). However, side effects such as xerostomia, nausea, and vomiting often occur after radiotherapy, chemotherapy, and targeted therapy. Moreover, about 20% of NPC patients are at risk of recurrence or metastasis after therapy (Liu et al., 2019; Li et al., 2020c). Therefore, deeper insights into the mechanism of initiation and development of NPC are necessary to improve clinical outcome.

Long noncoding RNAs (lncRNAs) are non-coding RNAs that participate in the regulation of gene expression at transcriptional and posttranscriptional levels (Li et al., 2021). LncRNAs have been shown to play important roles in epigenetic regulation, cell cycle, cell differentiation, and other biological processes (Pan et al., 2019; Xiong et al., 2020). In recent years, the lncRNA small nucleolar RNA host gene 1 (SNHG1) was found to be involved in the development of various cancers, such as esophageal squamous cell cancer, hepatocellular carcinoma, breast

cancer, and gastric cancer by regulating downstream genes (Li et al., 2020a; Qu and Yang, 2020; Zhang and Wang, 2020). SNHG1 is considered a promising biomarker in various malignancies including NPC (Huang et al., 2018). It is reported to be expressed at a high level in NPC and induce epithelial-mesenchymal transition (EMT) through the microRNA (miR)-145-5p/NAUK1 axis (Lan and Liu, 2019). In addition, SNHG1 was demonstrated to promote the proliferation and migration of cancer cells by sponging miRs (Zheng et al., 2019). However, more efforts are required to deepen the understanding about the mechanisms behind the tumor-promoting effects of the lncRNA SNHG1 in NPC.

MiRs are single-stranded small RNAs produced by the processing of Dicer protein from single-stranded RNA precursors with hairpin structure of ~70-90 bases (Koufaris, 2016). MiRs can regulate various biological processes such as proliferation and apoptosis (Witwer and Halushka, 2016). For example, miR-424-5p enhanced the proliferative capacity of laryngeal squamous cell carcinoma as well as invasive and migratory capacities (Li et al., 2019). The implication of miR-424-5p in cancer development has also been found in pancreatic cancer and breast cancer (Lu et al., 2019; Dastmalchi et al., 2020). Interestingly, miR-424-5p acted as a tumor suppressor in NPC (Zhao et al., 2020). Moreover, miR-424-5p is documented to impede lncRNA CDKN2B-AS1-mediated liver cancer cell progression (Shen et al., 2020). Considering the different roles of miR-424-5p in cancers, its functions in NPC still need further identification. In addition, online software starBase predicted that there was a binding site between SNHG1 and miR-424-5p. Hence, we postulated that SNHG1 may affect NPC development by regulating miR-424-5p. Consequently, this study was designed to verify this hypothesis, in an attempt to provide a new candidate for the clinical treatment of NPC.

Materials and methods

Clinical tissue sample

Thirty seven patients with NPC (NPC group) who were hospitalized in the Otolaryngology Department of Third Xiangya Hospital, Central South University from August 2017 to November 2019 were recruited. None of the patients received preoperative radiotherapy or chemotherapy, and matched non-cancerous nasopharyngeal mucosa tissues (normal group) were resected from the patients as the control. Notably, all patients signed informed consent, and all experiments were conducted with the approval of the Ethics Committee of Third Xiangya Hospital, Central South University (approval No. 22142) and in conformity with the Declaration of Helsinki.

Cell culture

Normal nasopharyngeal epithelial cells NP69 as well as HK1 and TW03 NPC cell lines were purchased from YaJi Biotechnology Co., Ltd. (Shanghai, China), and C666-1, S18, and SUNE-2 NPC cell lines were purchased from TongPai Biotechnology Co., Ltd. (Shanghai, China). NP69 cells were cultured in keratinocyte serum-free medium (KSFM; Gibco, Carlsbad, California, USA), and NPC cell lines were all cultured in Roswell Park Memorial Institute 1640 (RPMI1640; Gibco) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. These cells were cultured in an incubator with 5% CO_2 at 37°C.

Cell transfection and grouping

miR-424-5p mimic (mi-miR-424-5p) (100 nM), pcDNA3.1-SNHG1 overexpression vector (OE-SNHG1) (2 μg), miR-424-5p inhibitor (in-miR-424-5p) (100 nM), SNHG1 knockdown vector [short hairpin RNA for SNHG1 (sh-SNHG1)] (2 μg), and their negative controls (NCs) [mimic NC, pcDNA3.1a (OE-NC), inhibitor NC, and sh-NC] were purchased from RiboBio (Guangzhou, China). HK1 and C666-1 cells in logarithmic phases were selected for transfection and a Lipofectamine 2000 transfection kit (Thermo Fisher Scientific, MA, USA) was used for transfection in accordance with the instructions. Subsequently, transfected cells were added to serum-free RPMI1640 and cultured in a constant temperature incubator with 5% CO_2 at 37°C.

MTT assay

HK1 and C666-1 cells were counted after being respectively transfected for 24, 48, 72, and 96h. Then, 96-well plates were used to culture 100 μL of cell suspension (10^4-10^5 cells) and there were three replicate wells for each group. The cells were incubated at 37°C with 5% CO_2. Next, each well was added with 20 μL of MTT solution (5 mg/mL, Sigma, USA). After the cells were incubated continuously for 4h at 37°C with 5% CO_2, the cell culture was terminated and the culture medium was discarded. Instantly, 150 μL of DMSO was added to each well and oscillated gently for 10 min to promote crystal dissolution. The absorbance value (OD_{490}) of each well was measured at a wavelength of 490 nm. Then, the MTT curve was plotted with the absorbance value as the ordinate and the time as the abscissa. Moreover, the absorbance value of each group was measured three times to obtain the average.

Transwell invasion assay

At first, transwell chambers coated with matrigel were taken out from a refrigerator (-20°C) and equilibrated to room temperature. Then, the transwell chambers and 24-well plates (Corning, New York, USA) were added with 0.5 mL of serum-free culture medium for 2h of cell culture (37°C, 5% CO_2), and then all the culture medium was removed. Next, the transwell

chambers were inoculated with 1×10^5 cells, and culture medium containing 1% FBS was added to make the final volume 500 μL. The basolateral chamber was added with 0.75 mL of culture medium containing 15% FBS. After those cells were cultured with 5% CO_2 under constant temperature (37°C) and humidity for 20h, the transwell chamber was taken out and the culture medium was discarded. After twice calcium-free PBS washing, the invaded cells were immobilized with 4% paraformaldehyde for 10 min, washed twice with PBS, and stained with crystal violet for 10 min. Afterwards, the matrigel and non-invaded cells were gently rubbed off with cotton swabs before thrice PBS washing. Under a microscope (20×), cells in five random fields were observed and counted.

Cell scratch test

As described previously (Nasser et al., 2011), the cells of the control and experimental groups were pipetted into six-well plates. Once cell confluence reached 90%, three scratches were vertically made in the plate with a 100 μL pipette tip. Next, floating cells were removed with PBS and the cells were cultured in serum-free medium. A low-power phase contrast microscope (Olympus MK, Tokyo, Japan) was used to observe the scratch gap. After 24h of continuous culture, the changes of the scratch gap were observed.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Based on the specifications of kits, total RNA was extracted from tissues or cells using TRIZOL (Thermo Fisher Scientific, MA, USA), and the extracted RNA was converted into cDNA using a reverse transcription kit (Thermo Fisher Scientific) after quantification by Nanodrop 2000 (Waltham, MA, USA). Next, the PCR system was configured according to the fluorescence qPCR kit (Takara, Dalian, China). The PCR reaction was performed using the ABI7500 qPCR instrument (Applied Biosystems, USA) as follows: 95°C for 10 min, 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 34 s. All primer sequences are shown in Table 1 and synthesized by GENEWIZ (Beijing, China). GAPDH was the internal reference of lncRNA, and miRNA expression was normalized to U6. Data analysis was performed using the $2^{-\Delta\Delta Ct}$ method (Burja et al., 2019), and the formula was as follows: $\Delta\Delta Ct = [Ct_{(objective\ gene)} - Ct_{(internal\ genes)}]_{experimental\ group} - [Ct_{(objective\ gene)} - Ct_{(internal\ genes)}]_{control\ group}$.

Dual-luciferase reporter assay

The mutant and wild sequences of the target site between SNHG1 and miR-424-5p were synthesized according to the prediction of online software starBase. Then mutant and wild sequences were cloned into PGL3-Basic vector (Promega, Madison, USA), and respectively named as MT-SNHG1 and WT-SNHG1. After the HEK-293T cells were co-transfected with MT-SNHG1 or WT-SNHG1 plus mi-miR-424-5p or in-miR-424-5p for 48h, the luciferase activity of each group was detected by the dual luciferase reporter kit (Promega).

RNA immunoprecipitation (RIP) assay

After twice pre-cooled PBS washing, cells were centrifuged at 1500 rpm for 5 min and completely mixed with RIP lysis buffer. After magnetic beads were resuspended in 100 μL of RIP wash buffer, 5 μg of Ago2 antibody (ab186733, 1:100, Abcam, Cambridge, UK) was added for 30-min incubation with the beads, with IgG antibody (ab172730, 1:100, Abcam) serving as the NC. The centrifuge tube was placed on a magnetic rack and the supernatant was discarded. Next, 500 μL of RIP wash buffer was added to rinse the bead-antibody complex twice. After the supernatant was removed, the bead-antibody complex was mixed with RIP wash buffer (500 μL), and the tube was preserved on ice after vibration. Then the tube was placed on the magnetic rack and the supernatant was abandoned, after which RIP buffer (900 μL) was appended. Subsequently, the prepared cell lysate was thawed quickly and centrifuged at 14000 rpm for 10 min (4°C), after which 100 μL of the supernatant was collected for incubation (4°C) with the bead-antibody complex overnight. After transient centrifugation and the removal of the supernatant, the complex was rinsed with RIP wash buffer (500 μL) six times. After that, Proteinase K buffer (150 μL) was used to purify the RNA by incubating with the bead-antibody-RNA complex for 30 min (55°C). After RNA extraction, the expression of related genes was assessed by qRT-PCR. Data were acquired from three independent experiments.

Statistical analysis

SPSS 18.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6.0 (GraphPad Software Inc.) were used for statistical analysis. Data were represented in the form of mean ± standard deviation. *t*-test was used for comparisons between the two groups, and comparisons

Table 1. Primer sequences used for quantitative reverse transcription polymerase chain reaction analysis.

Name of primer	Sequences (5'-3')
LncRNA SNHG1-F	TAACCTGCTTGGCTCAAAGGG
LncRNA SNHG1-R	CAGCCTGGAGTGAACACAGA
miR-424-5p-F	CAGCAGCAATTCATGT
miR-424-5p-R	TGGTGTCGTGGAGTCG
GAPDH-F	ACCACAGTCCATGCCATCAC
GAPDH-R	TCCACCACCCTGTTGCTGTA
U6-F	TCGCTTCGGCAGCACATATAC
U6-R	GCGTGTCATCCTTGCGCAG

LncRNA, long noncoding RNA; F, forward; R, reverse

among multiple groups were gauged through one-way analysis of variance. The expression level of SNHG1 and miR-424-5P in seven NPC cell lines and NP69 cells were compared using homogeneity of variance test (P=0.573>0.05), followed by LSD multi-comparison. P<0.05 was considered statistically significant.

Results

SNHG1 is upregulated and miR-424-5p is downregulated in NPC tissues and cells

To study molecular mechanisms of NPC development, we used qRT-PCR to detect the expression of SNHG1 and miR-424-5p in NPC tissues and cell lines (HK1, S18, SUNE-2, C666-1, and TW03). Compared with the normal group, the NPC group had increased SNHG1 expression but decreased miR-424-5p expression (Fig. 1A,B). Consistently, in comparison with the NP69 cell line, SNHG1 RNA levels were signally elevated and miR-424-5p RNA levels were obviously reduced in the NPC cell lines (Fig. 1C,D). These results suggested that SNHG1 and miR-424-5p were closely related to the occurrence and development of NPC. Notably, the upregulation of SNHG1 and down-regulation of miR-424-5p in HK1 and C666-1 cells were the most significant, and thus these two cell lines were selected for subsequent experiments.

SNHG1 exerts pro-proliferative, pro-invasive, and pro-migratory effects in NPC cells

Next, we transfected HK1 and C666-1 cells with OE-SNHG1, sh-SNHG1, or their NCs (OE-NC and sh-NC) and then did a series of experiments to explore the effects of SNHG1 in biological phenotypes of NPC cells. qRT-PCR detection showed that the level of SNHG1 was remarkably increased in the OE-SNHG1 group, while SNHG1 levels were suppressed in the sh-SNHG1 group (Fig. 2A). Results from MTT assay manifested that the HK1 and C666-1 cell proliferation was enhanced in the OE-SNHG1 group (vs. OE-NC group) but inhibited in the sh-SNHG1 group (vs. sh-NC group) (Fig. 2B,C). In addition, HK1 and C666-1 cell invasiveness and migration in the OE-SNHG1 group were significantly increased versus the OE-NC group; and these properties in the sh-SNHG1 group were significantly decreased versus the sh-NC group (Fig. 2D-G). Taken together, upregulating SNHG1 promoted proliferation, invasion, and migration of NPC cells and downregulating SNHG1 blocked these phenotypes.

MiR-424-5p retards malignant phenotypes of NPC cells

To clarify the effects of miR-424-5p on NPC cell malignancy, we transfected HK1 and C666-1 cells with mi-miR-424-5p, in-miR-424-5p, mimic NC, or inhibitor

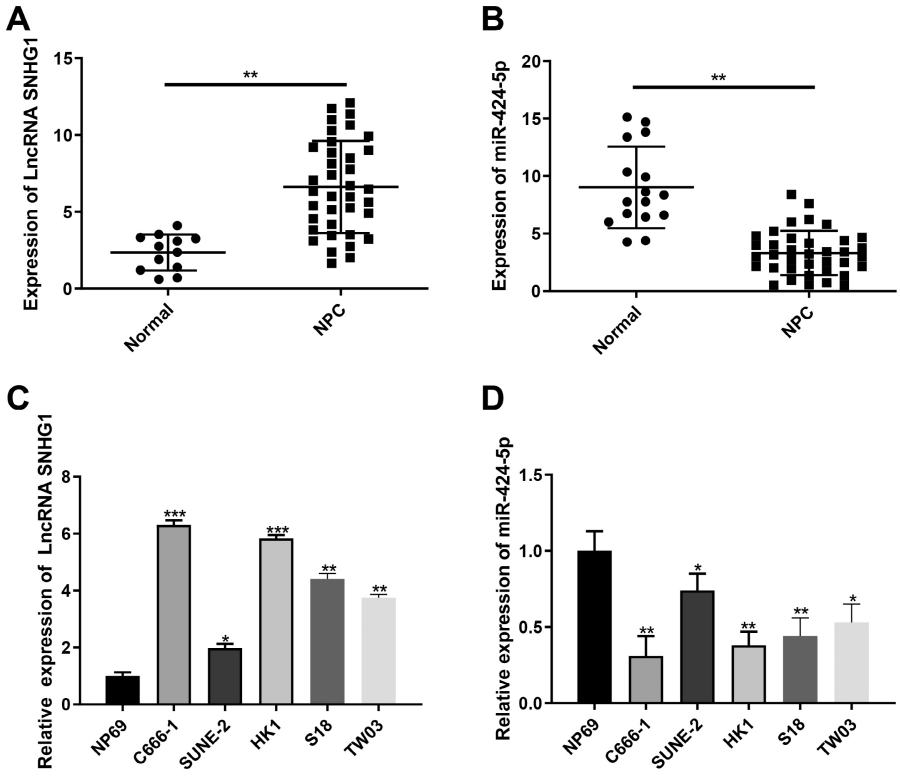

Fig. 1. High expression of SNHG1 and low expression of miR-424-5p in NPC cells or tissues. Expression of SNHG1 **(A)** and miR-424-5p **(B)** was detected by qRT-PCR in non-cancerous nasopharyngeal mucosal tissues (N=12) and NPC tissues (N=37); qRT-PCR examined the expression of SNHG1 **(C)** and miR-424-5p **(D)** in normal nasopharyngeal epithelial cells NP69 and NPC cell lines (HK1, S18, SUNE-2, C666-1, and TW03). Data were expressed as mean ± standard deviation, and cellular experiments were independently repeated three times. *P<0.05, **P<0.01, ***P<0.001 vs. normal or NP69 group. qRT-PCR, quantitative reverse transcription polymerase chain reaction; NPC, nasopharyngeal carcinoma; SNHG1, small nucleolar RNA host gene 1.

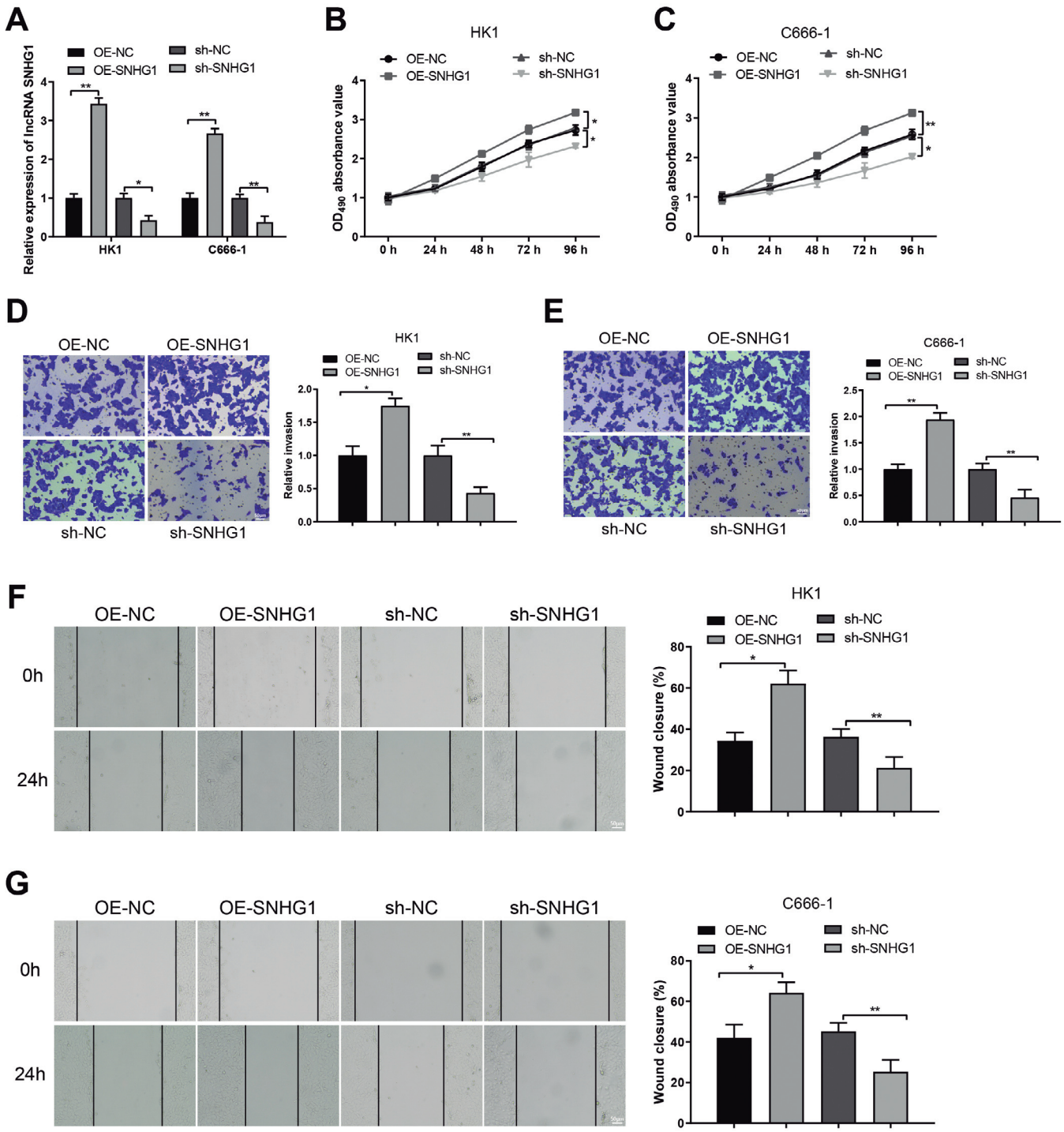

Fig. 2. SNHG1 overexpression promotes proliferation, invasion, and migration of NPC cells. HK1 and C666-1 cells were respectively transfected with OE-SNHG1, sh-SNHG1, OE-NC, or sh-NC. **A.** The expression level of SNHG1 was detected by qRT-PCR. **B, C.** the proliferation of HK1 or C666-1 cells was assessed by MTT assay. **D, E.** invasion ability of HK1 or C666-1 cells was detected by transwell invasion assay. **F, G.** the migration ability of HK1 or C666-1 cells was tested by cell scratch test. Data were expressed as mean ± standard deviation, and cellular experiments were independently repeated three times. **P*<0.05, ***P*<0.01 vs. OE-NC or sh-NC group. qRT-PCR, quantitative reverse transcription polymerase chain reaction; NPC, nasopharyngeal carcinoma; SNHG1, small nucleolar RNA host gene 1.

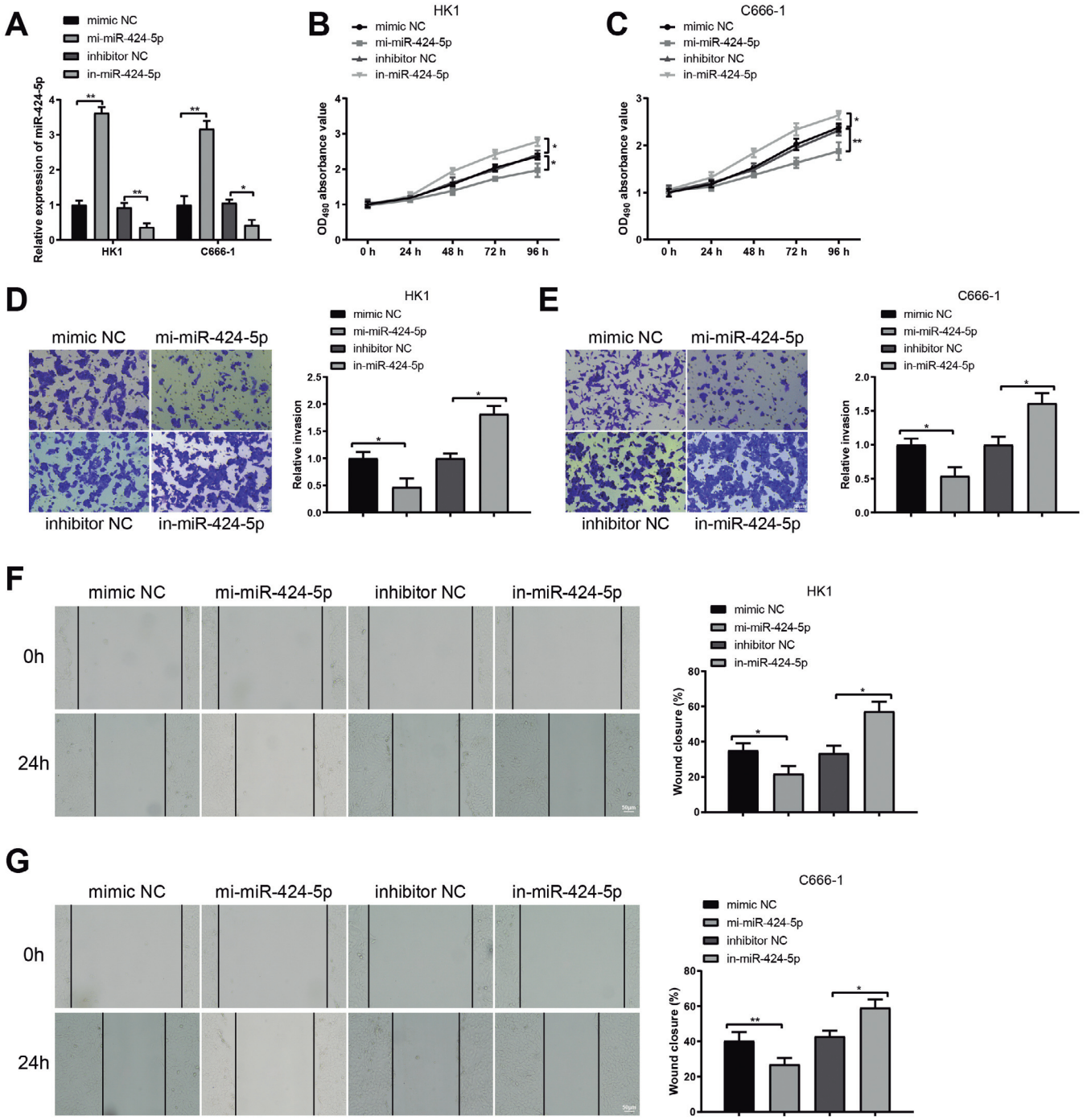

Fig. 3. Overexpressed miR-424-5p inhibits proliferation, invasion, and migration of NPC cells. HK1 and C666-1 cells were respectively transfected with mimic NC, mi-miR-424-5p, inhibitor NC, or in-miR-424-5p. **A.** The expression of miR-424-5p was detected by qRT-PCR. **B, C.** the proliferation ability of HK1 or C666-1 cells was measured by MTT assay. **D, E.** the invasion ability of HK1 or C666-1 cells was detected by transwell invasion assay. **F, G.** the migration ability of HK1 or C666-1 cells was tested by cell scratch test. Data were expressed as mean ± standard deviation, and cellular experiments were independently repeated three times. **P*<0.05, ***P*<0.01 vs. mimic NC or inhibitor NC group. qRT-PCR, quantitative reverse transcription polymerase chain reaction; NPC, nasopharyngeal carcinoma.

NC. qRT-PCR analysis for miR-424-5p expression exhibited an increment in the mi-miR-424-5p group compared with the mimic NC group, and a massive decrease in the in-miR-424-5p group compared to the inhibitor NC group (Fig. 3A), which indicated successful transfection of mi-miR-424-5p and in-miR-424-5p vectors. As expected, in HK1 and C666-1 cells, the proliferative, invasive, and migratory abilities were significantly suppressed in the mi-miR-424-5p group (vs. the mimic NC group), and these properties were enhanced in the in-miR-424-5p group (vs. the inhibitor NC group) (Fig. 3B-G). Collectively, overexpression of miR-424-5p suppressed the malignant properties of NPC cells.

SNHG1 negatively regulates miR-424-5p

Next, we found that there was a binding site between SNHG1 and miR-424-5p through the online software starBase. Thus, we hypothesized that SNHG1 may modulate the biological phenotypes of NPC cells by regulating the expression of miR-424-5p. Furthermore, results from qRT-PCR showed that the expression of miR-424-5p in the OE-SNHG1 group was significantly reduced (vs. the OE-NC group), and that in the sh-SNHG1 group it was significantly increased (vs. the sh-NC group) (Fig. 4A).

Subsequently, the potential binding site of SNHG1 and miR-424-5p as well as the corresponding mutation site are shown in Fig. 4B. To verify the targeting relationship, we constructed WT-SNHG1 and MT-SNHG1 to carry out luciferase reporter assay. According to the results, the cells co-transfected with WT-SNHG1 and mi-miR-424-5p had decreased luciferase activity compared with the mimic NC group; and the luciferase activity of the cells co-transfected with WT-SNHG1 and in-miR-424-5p was significantly increased compared with the inhibitor NC group (Fig. 4C). Furthermore, RIP assay displayed that the Ago2 group had higher RNA levels of SNHG1 and miR-424-5p compared with the IgG group (Fig. 4D), suggesting that SNHG1 bound to miR-424-5p. To further explore the relationship between SNHG1 and miR-424-5p, we transfected HK1 and C666-1 cells with OE-SNHG1 and mi-miR-424-5p or sh-SNHG1 and in-miR-424-5p, and then detected the expression of miR-424-5p using qRT-PCR. In comparison with the OE-SNHG1 group, the expression of miR-424-5p was evidently increased in the OE-SNHG1 + mi-miR-424-5p group; compared with the sh-SNHG1 group, the sh-SNHG1 + in-miR-424-5p group had lower miR-424-5p expression (Fig. 4E). These

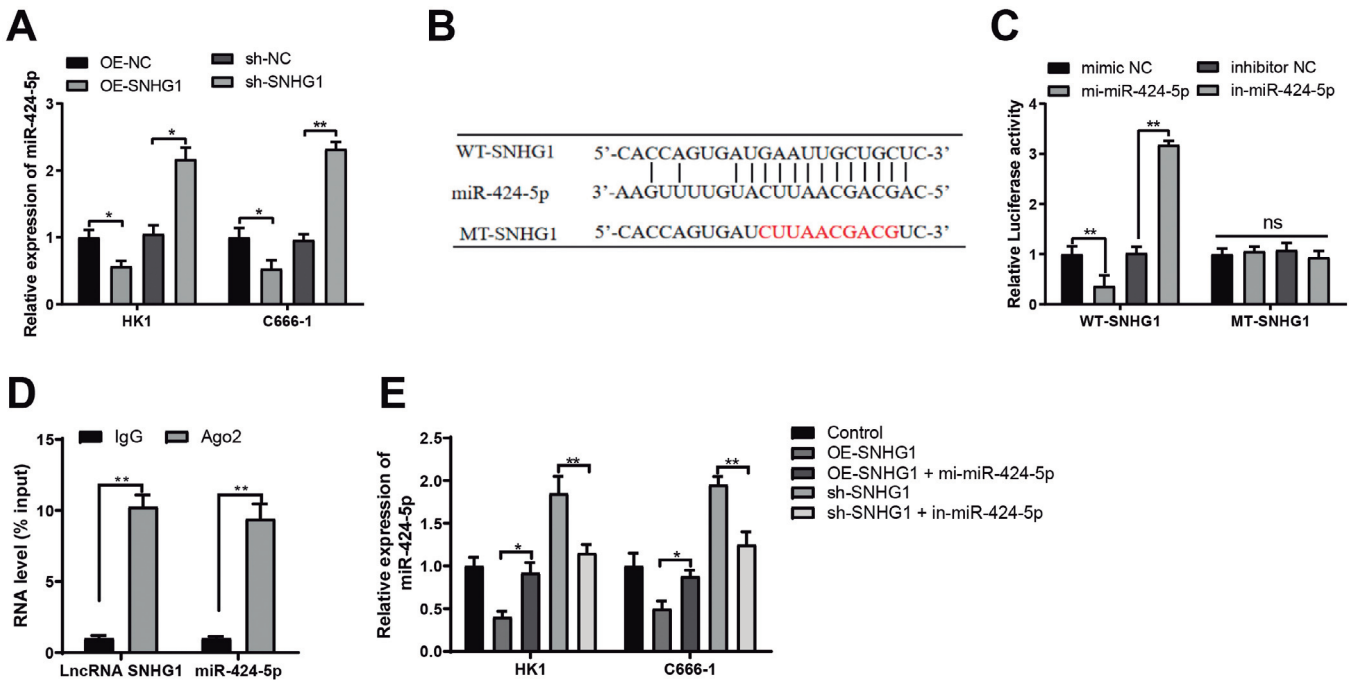

Fig. 4. SNHG1 negatively regulates miR-424-5p. **A.** Expression of miR-424-5p was detected by qRT-PCR after HK1 and C666-1 cells were transfected with OE-SNHG1 or sh-SNHG1. **B.** online software starBase predicted the target site of miR-424-5p and SNHG1 and the SNHG1 mutation site (red) was designed. **C.** HEK-293T cells were co-transfected with mimic NC, mi-miR-424-5p, inhibitor NC, or in-miR-424-5p and WT-SNHG1 or MT-SNHG1 and then dual luciferase reporter assay were used to detect the luciferase activity. **D.** RIP assay was used to detect the interaction between miR-424-5p and SNHG1. **E.** Expression of miR-424-5p was measured by qRT-PCR after HK1 and C666-1 cells were transfected with OE-SNHG1 and mi-miR-424-5p or sh-SNHG1 and in-miR-424-5p. Data were expressed as mean ± standard deviation, and cellular experiments were independently repeated three times. **P*<0.05, ***P*<0.01 vs. mimic NC, inhibitor NC, OE-NC, sh-NC, sh-SNHG1, OE-SNHG1, or IgG group. qRT-PCR, quantitative reverse transcription polymerase chain reaction; RIP, RNA immunoprecipitation; NPC, nasopharyngeal carcinoma; SNHG1, small nucleolar RNA host gene 1.

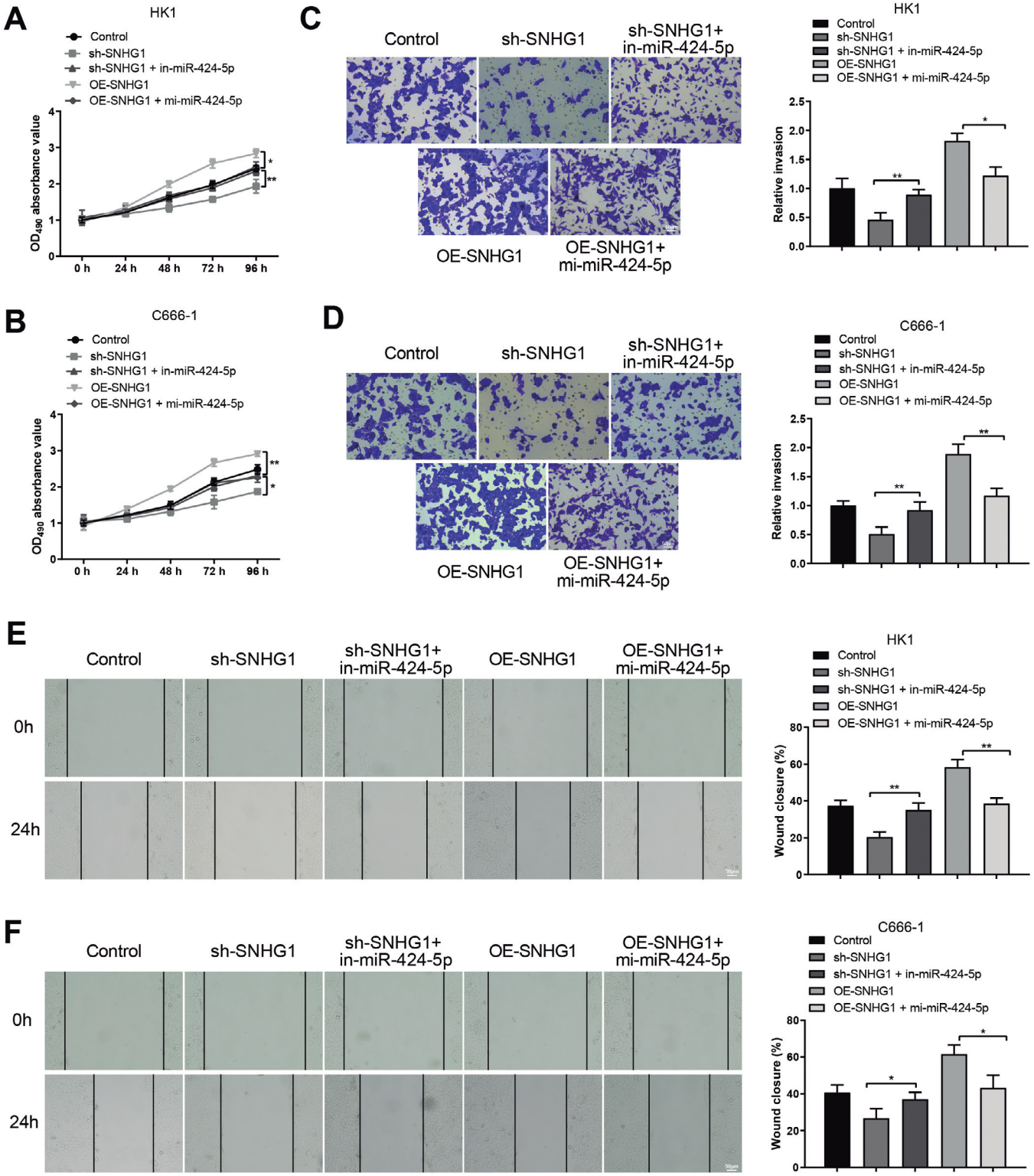

Fig. 5. SNHG1 promotes proliferation, invasion, and migration of NPC cells through miR-424-5p. HK1 and C666-1 were transfected with sh-SNHG1 and in-miR-424-5p or OE-SNHG1 and mi-miR-424-5p. **A, B.** Proliferation ability of HK1 and C666-1 cells was detected by MTT assay. **C, D.** Invasion ability of HK1 and C666-1 cells was measured by transwell invasion assay. **E, F.** Migration ability of HK1 and C666-1 cells was evaluated by cell scratch assay. Data were expressed as mean ± standard deviation, and cellular experiments were independently repeated three times. **P*<0.05, ***P*<0.01 vs. sh-SNHG1 or OE-SNHG1 group. NPC, nasopharyngeal carcinoma; SNHG1, small nucleolar RNA host gene 1.

results demonstrated that SNHG1 directly bound to and downregulated miR-424-5p in NPC cells.

SNHG1 promotes malignant phenotypes of NPC cells through miR-424-5p

According to the above experimental results, we intended to explicate whether SNHG1 regulates the biological phenotypes of NPC cells by negatively modulating miR-424-5p. After transfection of OE-SNHG1 and mi-miR-424-5p or sh-SNHG1 and in-miR-424-5p in HK1 and C666-1 cells, cell proliferative, invasive, and migratory abilities were markedly enhanced in the sh-SNHG1 + in-miR-424-5p group versus the sh-SNHG1 group, and these properties were restrained in the OE-SNHG1 + mi-miR-424-5p group versus the OE-SNHG1 group (Fig. 5A-F). In short, in-miR-424-5p reversed the inhibitory effects of sh-SNHG1 on the malignant phenotypes of NPC cells, while mi-miR-424-5p reversed the promoting effects of OE-SNHG1 on NPC cells. In summary, SNHG1 promoted NPC cell malignancy via negatively regulating the expression of miR-424-5p.

Discussion

Recurrence and metastasis have long been a concern after NPC treatment (Zhao et al., 2020). Previous studies have shown that abnormal expression of lncRNAs in NPC is involved in cancer progression and can be considered potential targets for the treatment of NPC (Su et al., 2019; Xu et al., 2020). In our study, SNHG1 was found to be upregulated in NPC tissues and cells, and miR-424-5p was downregulated. In addition, we identified that SNHG1 negatively modulated miR-424-5p expression, and SNHG1 promoted proliferation, invasion, and migration of NPC cells by suppressing the expression of miR-424-5p.

A previous study found that the lncRNA NPCCAT1 promoted NPC progression by upregulating YY1 (Su et al., 2019). Lian et al. showed that the lncRNA AFAP1-AS1 facilitated NPC metastasis by regulating the Rho/Rac pathway (Lian et al., 2018). These facts represented the involvement of dysregulated lncRNAs and NPC development. In this study, strong expression of SNHG1 was identified in NPC cells and tissues. The lncRNA SNHG1 has been demonstrated to play an essential role in tumorigenesis of a variety of cancers including NPC (Thin et al., 2019). For instance, high SNHG1 expression has been found in esophageal squamous cell cancer and SNHG1 inhibition led to impaired xenograft tumor growth by sponging miR-204 and increasing HOXC8 (Li et al., 2020a). Researchers also found that SNHG1 upregulation was associated with breast cancer progression through *in vitro* and *in vivo* studies (Li et al., 2020b). Hu et al. indicated that SNHG1 could upregulate DNMT1 expression and thus boost proliferation of gastric cancer cells (Hu et al., 2017). In addition, SNHG1 was demonstrated to enhance the invasiveness and migration as well as tumor growth in non-small cell lung cancer (NSCLC) by decreasing miR-497 expression (Li et al., 2018). Herein, we found SNHG1 presented pro-proliferation, pro-invasive and pro-migratory effects on NPC cells, which was consistent with the previous study (Lan and Liu, 2019).

Given that lncRNAs can regulate biological processes of cancers by sponging miRs, we noticed a potent decrease in miR-424-5p expression in NPC tissues and cells. The inhibitory role of miR-424-5p has been reported in the progression of NPC (Zhao et al., 2020). Moreover, miR-424-5p suppressed SMAD7 pathway-triggered EMT in esophageal squamous cell carcinoma (Wang et al., 2016). Also, this miR has been demonstrated to perturb invasiveness and metastasis in hepatocellular carcinoma (Zhang et al., 2014; Du et al., 2019). Consistently, we found that miR-424-5p overexpression inhibited proliferation, invasion and migration in NPC cells.

In the succeeding experiments, we found that SNHG1 negatively regulated miR-424-5p. Looking back on previous research, studies on the effects of the interaction between lncRNAs and miRs on NPC are not rare. For example, the lncRNA IUR inhibited NPC cell proliferation by downregulating the expression of miR-144 (Liu et al., 2020). The lncRNA SNHG15 deteriorated the development of NPC by downregulating miR-141-3p and upregulating KLF9 (Yi et al., 2020). Besides, miR-34a impedes the development of NPC via targeting the lncRNA MCM3AP-AS1 (Sun et al., 2020). Wang et al. reported that miR-424-5p could be sponged by lncRNA PVT1 and counteract the promotive effects of PVT1 on cancer cell malignancy in NSCLC (Wang and Hu, 2019). All these findings implied that lncRNAs can sponge miRs to affect cancer progression. The present study revealed that miR-424-5p was a tumor suppressor, and it was able to partly reverse the oncogenic effects of SNHG1 on NPC cell aggressiveness and proliferation. Collectively, our experiments confirmed that SNHG1 promotes invasion and migration of NPC cells through downregulating miR-424-5p. However, since lncRNAs exert promiscuous actions, the lncRNA SNHG1 may regulate multiple miRs. Moreover, the present study cannot exclude the possibility that SNHG1 and miR-424-5p regulate other downstream genes in NPC. Future studies should be carried out on the downstream genes of miR-424-5p or miR-424-5p-mediated signaling pathways.

Conclusion

For the first time, we verified that miR-424-5p is a target gene of SNHG1 in NPC, and SNHG1 can facilitate invasion and migration of NPC cells by inhibiting miR-424-5p. In conclusion, we demonstrated the effect of the interaction between SNHG1 and miR-424-5p on the development of NPC, which may provide a theoretical basis for the clinical practice of NPC.

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