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MicroRNA-494-3p Promotes Cell Growth, Migration, and Invasion of Nasopharyngeal Carcinoma by Targeting Sox7

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Abstract

Background: There is mounting evidence that microRNAs play an important role in nasopharyngeal carcinoma, which is widely prevalent in South China and is the most prevalent metastatic cancer among head and neck cancers. Recently, it has been shown that miR-494 is involved in the progression and prognosis of nasopharyngeal carcinoma. However, little is known about the function and mechanism of miR-494-3p in nasopharyngeal carcinoma. In the present study, we aimed to investigate the effects of miR-494-3p on the migration and invasion of nasopharyngeal carcinoma and to further explore the underlying mechanisms of these processes. **Methods:** The expression levels of miR-494-3p and Sox7 in nasopharyngeal carcinoma specimens and nasopharyngeal carcinoma cell lines were measured using quantitative reverse transcription polymerase chain reaction. Luciferase reporter assay, quantitative reverse transcription polymerase chain reaction, and Western blotting were used to confirm whether Sox7 was a direct target of miR-494-3p. Additionally, the roles of miR-494-3p and Sox7 on cell proliferation, migration, and invasion of nasopharyngeal carcinoma were analyzed by Cell Counting Kit-8 (CCK-8) assay, wound healing assay, and Boyden chamber assay, respectively. **Results:** Our study demonstrated that miR-494-3p was commonly upregulated in nasopharyngeal carcinoma specimens and nasopharyngeal carcinoma cell lines compared with nontumor nasopharyngeal epithelial tissue or nasopharyngeal cells (NP69). Moreover, miR-494-3p negatively regulated Sox7 at the posttranscriptional level by binding to a specific site in the Sox7 3′-untranslated region. In addition, synthetic miR-494-3p mimics significantly promoted proliferation, migration, and invasion of S18 and S26 nasopharyngeal carcinoma cells, while a synthetic miR-494-3p inhibitor resulted in suppressed nasopharyngeal carcinoma cell migration and invasion. **Conclusion:** miR-494-3p promotes nasopharyngeal carcinoma cell growth, migration, and invasion by directly targeting Sox7. Our results suggest that miR-494-3p might be a potential therapeutic target for nasopharyngeal carcinoma.

Keywords

nasopharyngeal carcinoma, miR-494-3p, Sox7, migration, invasion

Abbreviations

Ct, cycle threshold; FBS, fetal bovine serum; miRNAs, microRNAs; Mt, mutant; NPC, nasopharyngeal carcinoma; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SRY, sex-determining region Y; Wt, wild type; 3′-UTR, 3′-untranslated region.

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Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common malignancies in South China and Southeast Asia.[1-3] Nasopharyngeal carcinoma is a squamous cell carcinoma derived from epithelial cells that are located in the nasopharynx, which is highly malignant with local invasion and distant metastasis.[4] Thus, the majority of patients tend to present with regional lymph node or even distant metastasis at the first time of diagnosis.[5] Although patients with NPC are sensitive to radiotherapy, the prognosis for patients with NPC still remains poor because of recurrence and distant metastasis.[6,7] However, the molecular mechanisms underlying NPC progression and metastasis are not completely understood to date, and further investigation of this mechanism is imminently needed.

MicroRNAs (miRNAs) are a diverse class of endogenous noncoding small RNAs (19-25 nucleotide), which can regulate their target genes messenger RNA (mRNA) by usually base-pairing to the 3′-untranslated region (3′-UTR) and result in either mRNA degradation or translation inhibition.[8] Recent evidence indicated that miRNAs might function either as tumor suppressors or oncogenes, which serve a crucial role in the proliferation, invasion, apoptosis, differentiation, and metabolism in tumor progression.[9] To date, several miRNAs are related to NPC development and progression by regulating cell growth, metastasis, and apoptosis, including let-7a,[10] miR-34,[11] and miR-93,[12] indicating that miRNAs play significant roles in NPC tumorigenesis. Additionally, the role of miR-494 in tumorigenesis has generally been reported as controversial. Some investigations have described it as an onco-miRNA in several carcinomas by enhancing the ability of cancer cells to grow and metastasize *in vivo*, such as lung cancer,[13] hepatocellular carcinoma,[14] and non-small cell lung cancer.[15,16] In contrast, miR-494 was also reported to be downregulated or correlated with poor prognosis in cancers, and the overexpression of miR-494 could inhibit the proliferation, migration, and invasion in breast cancer,[17] ovarian cancer,[18,19] gastric cancer,[20] and osteosarcoma.[21] However, the role of miR-494-3p in NPC remains unclear, though there were studies reported the functional evidence of miR-494 in the tumorigenesis of NPC.[22,23] To better characterize how the altered expression of miR-494-3p might contribute to NPC development, we examined the expression levels of miR-494-3p in NPC and analyze its impacts on the biological functions of NPC cells.

The sex-determining region Y (SRY)-box 7 (Sox7) protein, along with Sox17 and Sox18, belong to the F subfamily of the SRY-related high mobility group box (Sox) transcription factor, which has been shown to regulate multiple biological processes, such as hematopoiesis, vasculogenesis, cardiogenesis endoderm differentiation, and myogenesis during embryonic development.[24] Previous studies demonstrated that Sox7 exerted a tumor suppressive role in human cancers. Downregulation of Sox7 has been observed in several cancers, including prostate,[25] breast,[26] and lung cancers,[27] which inhibits their proliferation, invasion, and colony formation while promoting apoptosis. In addition, Sox7 was found to be the target gene of several miRNAs, and MiR-9 could induce the invasion and adhesion by direct downregulation of Sox7 in lung cancer.[28] MiR-616 could downregulate Sox7 that promoted the growth and metastasis of non-small cell lung cancer.[29] And yet the function of Sox7 in NPC progression has not been documented. In the present study, we investigated the expression levels and potential roles of miR-494-3p and Sox7 in NPC tissues and cells.

Materials and Methods

Cell Culture

Nasopharyngeal epithelial cell (NP69) and nasopharyngeal carcinoma cell lines S18, S26 were gifted by professor Chao-Nan Qian (Cancer Center of Sun Yat-sen University). An immortalized nasopharyngeal epithelial cell NP69 was cultured in keratinocyte/serum-free medium (Invitrogen, Massachusetts, USA) supplemented with bovine pituitary extract (BD Biosciences, New Jersey, USA). The human NPC cell lines (S18, S26, CNE-1, CNE-2, HONE-1, and 5-8F) were maintained in RPMI-1640 (Invitrogen, Massachusetts, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) in a humidified atmosphere containing 5% CO_2 at 37°C.

Clinical Specimens

Thirty freshly frozen NPC samples and 12 noncancerous nasopharynx tissues were collected from the Affiliated Hospital of Guangdong Medical University (Zhanjiang, Guangdong Province, China) between March 2013 and November 2014. No patients had received any forms of tumor-specific therapy before diagnosis. All samples were analyzed by pathologists to confirm the diagnosis. Informed consent was obtained from each patient, and the research protocols were approved by ethics committee of the Affiliated Hospital of Guangdong Medical College (HM-81201672).

Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction

To quantitate mRNA expression, total RNA was extracted from clinical tissue samples and NPC cell lines with RNAiso Plus (Takara, Tokyo, Japan). The isolated total RNA was reverse transcribed using the One Step PrimeScript miRNA cDNA Synthesis Kit (Takara) for miR-494-3p and the PrimeScript RT Master Mix (Perfect Real Time; Takara) for Sox7, according to the manufacturer's instructions. Relative expression was calculated via the comparative cycle threshold (Ct) method and was normalized to the expression of U6 small RNA or β-actin. The sequence-specific forward primers for the mature miR-494-3p and U6 internal control were 5′-GTATCT-TAGGTTGTCCGTGTTGTC-3′ and 5′-CTCGCTTCGGCAG-CACA-3′, respectively. The Uni-miR quantitative polymerase chain reaction primer was included in the kit. Forward and reverse primers sequences for Sox7 mRNA were 5′-GGCC

TAAGCTGGACTCTCCT-3′ and 5′-TTCGATGATGGCTA CCAAGA-3′, respectively. Forward and reverse primers sequences for β-actin mRNA were 5′-CTGGCACCA-CACCTTCTACAATG-3′ and 5′-CCTCGTAGATGGGCA-CAGTGTG-3′, respectively. The quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using SYBR Premix ExTaq II (Takara) on a Light Cycler (Roche Diagnostics, Basel, Switzerland). Relative quantification of miRNA expression was calculated by using the $2^{-\Delta\Delta Ct}$ method.[30] The raw data were presented as the relative quantity of target miRNA or mRNA, normalized with respect to U6 or β-actin and relative to a calibrator sample. All qRT-PCR reactions were performed in triplicates.

MicroRNA Transfection

MiR-494-3p mimic, inhibitor, and the negative control were obtained from RiboBio (Guangzhou, China). MicroRNA transfection was performed using Lipofectamine 3000 (Invitrogen), according to the manufacturer's instruction. The relative level of miR-494-3p in transfected cells was examined by qRT-PCR. Total RNAs and protein were prepared 48 hours after transfection for qRT-PCR and Western blotting analysis.

Luciferase Reporter Assays

The Sox7 wild-type (Wt) and mutant (Mt) 3′-UTRs were created and cloned to the firefly luciferase-expressing vector pLUC. For the luciferase assay, HEK293T was seeded in triplicates in 12-well plates the day before transfection, and cotransfected with the Sox7 Wt or Mt 3′-UTR reporter vector, and miR-494-3p mimics or miR-494-3p mimics-Negative control (NC), using Lipofectamine 3000 (Invitrogen). After 48 hours of transfection, the cells were harvested and lysed, and the luciferase activities were assayed using the Dual-Luciferase Reporter System (Promega, Madison, Wisconsin, USA). Three independent experiments were performed.

Cell Proliferation Assay

Cell proliferation was analyzed using Cell Counting Kit-8 (CCK-8) assay. Briefly, 3×10^3 cells were seeded into a 96-well plate with quadruplicate repeat for each condition. Subsequently, cells were incubated in low-serum culture media in 5% FBS (Gibco) to be kept quiescent for 24 hours and to ensure that the cells reached 50% to 60% confluence. Then miR-494-3p mimics, inhibitor, and NC were transfected for another 24, 48, 72 hours, respectively. Then the medium was removed and replaced with fresh medium (90 μL) with 10 μL CCK-8 (Dojindo Molecular Technologies, Tokyo, Japan) added to each well, incubated for 2 hours at 37°C. Optical density was measured on a microplate reader (Thermo Electron Corp, Massachusetts, USA) using an absorbance wavelength at 450 nm. The percentage of surviving cells from each group relative to the control group was defined as the proliferation rate. For these studies, all experiments were repeated at least 3 times.

Wound Healing

For the scratch wound healing assay, S18 and S26 cells (5×10^5) were seeded into 6-well plates and cultured in 10% FBS (Gibco) medium for 24 hours at 37°C. When the cells reached 50% to 60% confluence, they were transfected with miR-494-3p mimics, inhibitor, and NC, respectively, for another 24 hours, then scraping the cell monolayer with a 200-μL pipette tip. Cell migration was determined by measuring the movement distance of cells into the scraped area. Representative images (10×) of wound closure were captured between 0 and 24 hours using an inverted microscope.

Migration and Invasion Assays

The migration and invasion assays were performed using cell culture inserts with 8 μM microporous filters (Corning Costar, New York, USA) coated with (invasion) or without (migration) Matrigel. For S18 cells, 3×10^4 cells were plated into each insert. For S26 cells, 5×10^4 cells were plated. Cells suspended in 200 μL serum-free medium (Life Technology) were added to the insert, 600 μL RPMI 1640 containing 20% FBS was added to the bottom chamber, incubated for 24 hours at 37°C. Cells in the upper filters (inside the inserts) were removed, the insert was washed with PBS 2 times, and the migrated or invaded cells in the lower filters (outside the inserts) were fixed in methanol, stained with crystal violet for 15 minutes, respectively, and then counted under a microscope. The number of cells in the membrane counted from 5 randomly selected visual fields (×100 magnification) of each filter from triplicate inserts was averaged.

Western Blotting

Cells were cultured for 72 hours and then harvested in Radio-Immunoprecipitation Assay (RIPA) buffer containing a protease inhibitor mixture (Roche) and incubated on a rocker at 4°C for 15 minutes. The protein concentration of the lysates was measured using the BCA protein assay kit (Pierce, Massachusetts, USA) and was normalized to equal amounts of protein, separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE), transferred to PVDF, and probed with the indicated primary antibodies. After probed with the indicated antibodies, the blot was incubated with species-specific horse radish peroxidase (HRP)-conjugated secondary antibodies, and the immunoreactive bands were visualized by enhanced chemiluminescence (Pierce). The same membranes were then stripped and reprobed with mouse monoclonal antibodies against β-actin to confirm equal loading of the samples.

Statistical Analysis

Data were presented as mean (standard deviation, SD) for at least 3 independent experiments. The Student *t* test was used for comparisons of 2 independent groups. For more than 2 groups' comparison, 1-way analysis of variance was used. The

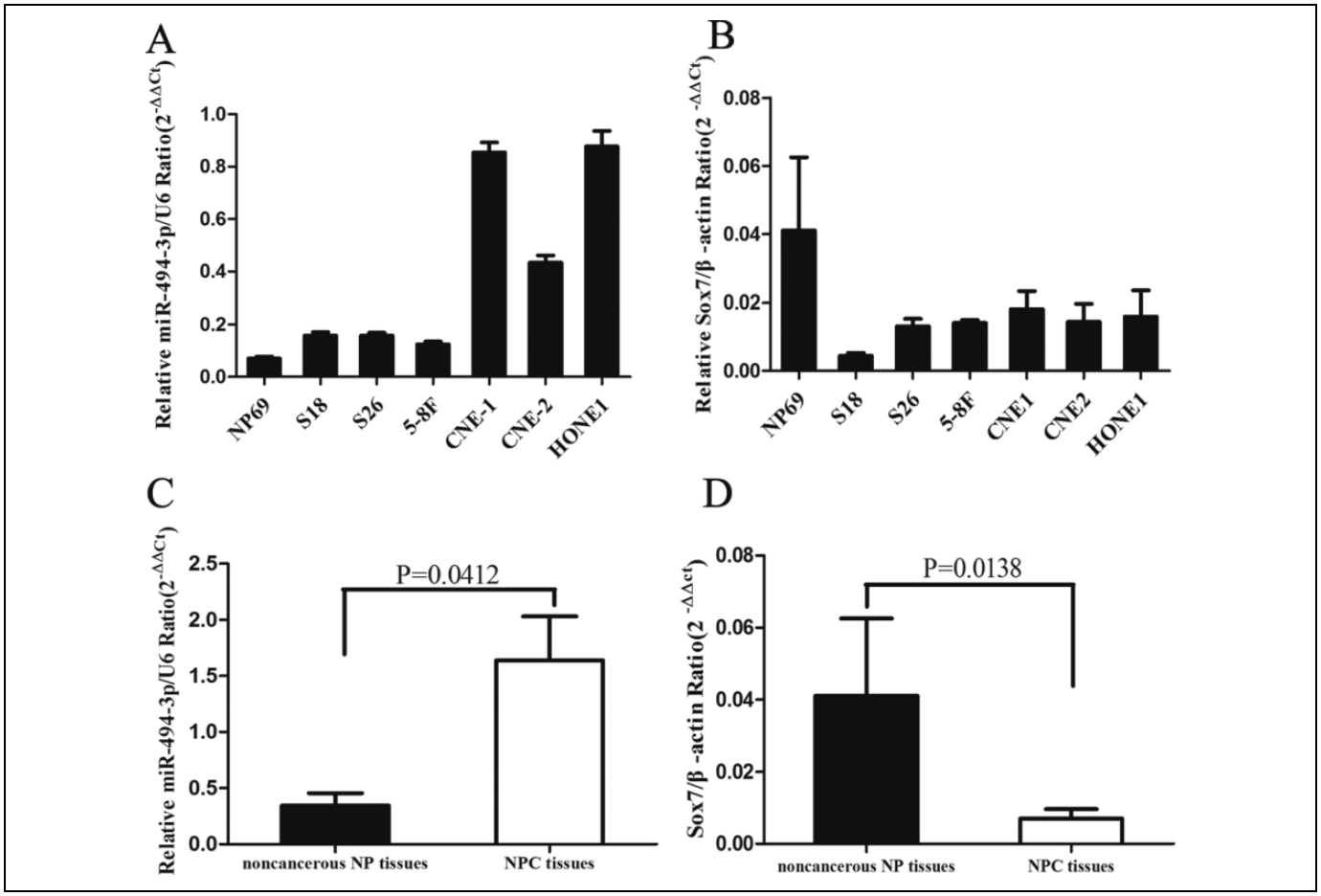

Figure 1. Expression of miR-494-3p and Sox7 in nasopharyngeal carcinoma (NPC) cell lines and tissues. A, Real-time polymerase chain reaction (PCR) analysis of miR-494-3p expression in normal nasopharyngeal epithelial cell (NP69) and NPC cell lines (S18, S26, CNE-1, CNE-2, HONE-1, 5-8F). B, The relative levels of Sox7 in normal nasopharyngeal epithelial cell (NP69) and NPC cell lines (S18, S26, CNE-1, CNE-2, HONE-1, 5-8F) by real-time PCR. C, Real-time PCR analysis of miR-494-3p expression in 30 NPC versus 12 noncancerous nasopharyngeal tissue samples. D, Sox7 expression levels in 30 NPC versus 12 noncancerous nasopharyngeal tissue samples by real-time PCR. Data are presented as the mean (standard deviation, SD; from triplicate replications).

relationship between Sox7 and miR-494-3p expression was explored by Spearman correlation. All statistical analysis was performed with SPSS 17.0 software, and $P < .05$ was considered statistically significant.

Results

MiR-494-3p Was Upregulated and Sox7 Was Downregulated in Human NPC Clinical Specimens and Cell Lines

The miR-494-3p and Sox7 expression levels were tested in a panel of human NPC cell lines (S18, S26, CNE-1, CNE-2, HONE-1, and 5-8F) and immortalized nontumorigenic cell line (NP69). Compared with NP69, NPC cell lines showed higher expression levels of miR-494-3p and lower expression levels of Sox7 mRNA (Figure 1A and B). The miR-494-3p expression level was tested in 30 freshly frozen NPC specimens and 12 noncancerous nasopharynx tissues. The results showed that miR-494-3p was significantly increased in NPC specimens than in noncancerous nasopharynx tissues (Figure 1C; $P < .05$), and Sox7 was decreased in NPC specimens than in noncancerous nasopharynx tissues (Figure 1D; $P < .05$).

MiR-494-3p Promoted Cell Growth, Migration, and Invasion in NPC Cells

To explore the effect of miR-494-3p on cell growth, S18 and S26 cells were transiently transfected with miR-494-3p mimic, miR-494-3p inhibitor, or miR-NC, respectively. The results of CCK-8 assay showed that overexpressed miR-494-3p (miR-494-3p mimics) dramatically promoted cell growth in S18 cell by 78.74% and in S26 cell by 72.37% ($P < .01$), whereas the suppression of miR-494-3p (miR-494-3p inhibitor) significantly reduced cell growth in S18 cell by 71.02% and in S26 cell by 74.65% ($P < .01$), compared with negative control of miR-494-3p (Figure 2A and B). In wound healing method, we found that the overexpression or inhibition of miR-494-3p

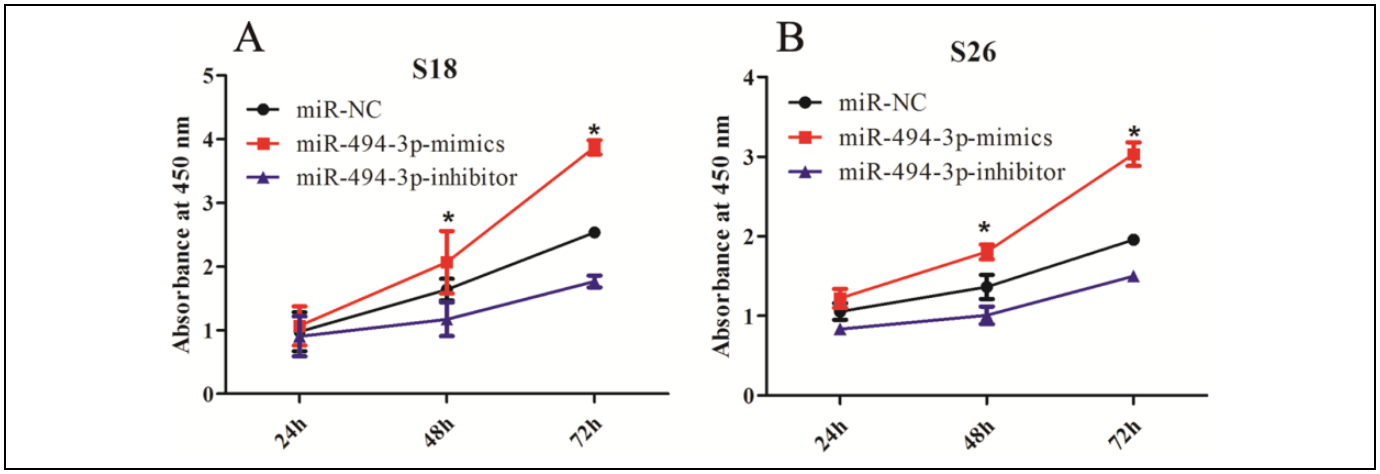

Figure 2. Upregulated miR-494-3p promotes proliferation in nasopharyngeal carcinoma (NPC) cells. A, The growth curves determined by Cell Counting Kit-8 (CCK-8) assay showed that miR-494-3p mimics promoted NPC cell growth, and miR-494-3p inhibitor inhibited NPC cell growth compared with negative control in S18 cell (*$P < .05$). B, The miR-494-3p mimics promoted NPC cell growth, and miR-494-3p inhibitor inhibited NPC cell growth compared with negative control in S26 cell line by CCK-8 assay (*$P < .05$).

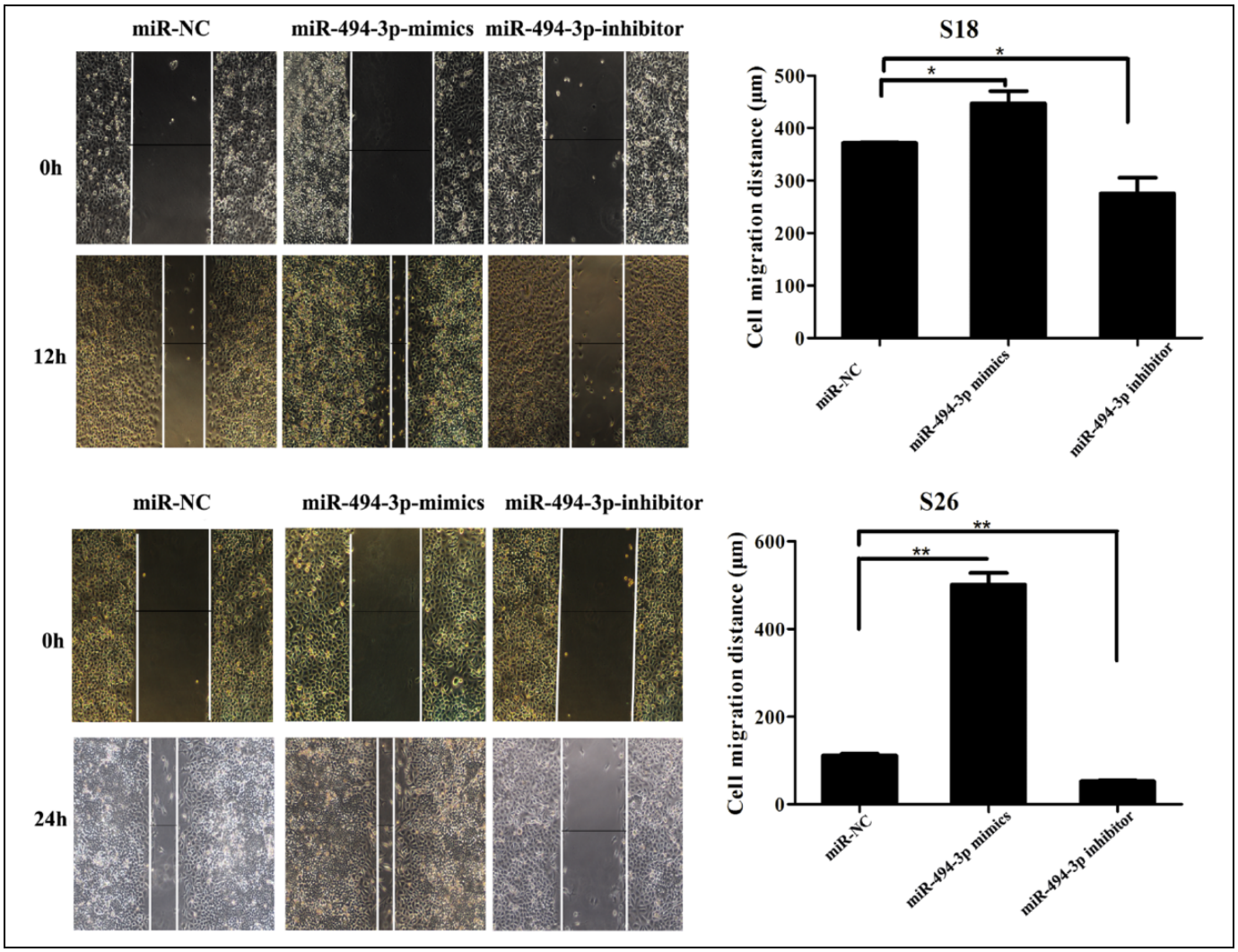

Figure 3. Upregulated miR-494-3p promotes migration in nasopharyngeal carcinoma (NPC) cells. Upregulated miR-494-3p expression dramatically increased the ability of S18 and S26 cell migration *in vitro* by wound healing assay. *$P < .05$, **$P < .01$. The results were expressed as cell number per field to the miR-Negative control (NC). The data are presented as the mean (SD) of triplicate replications.

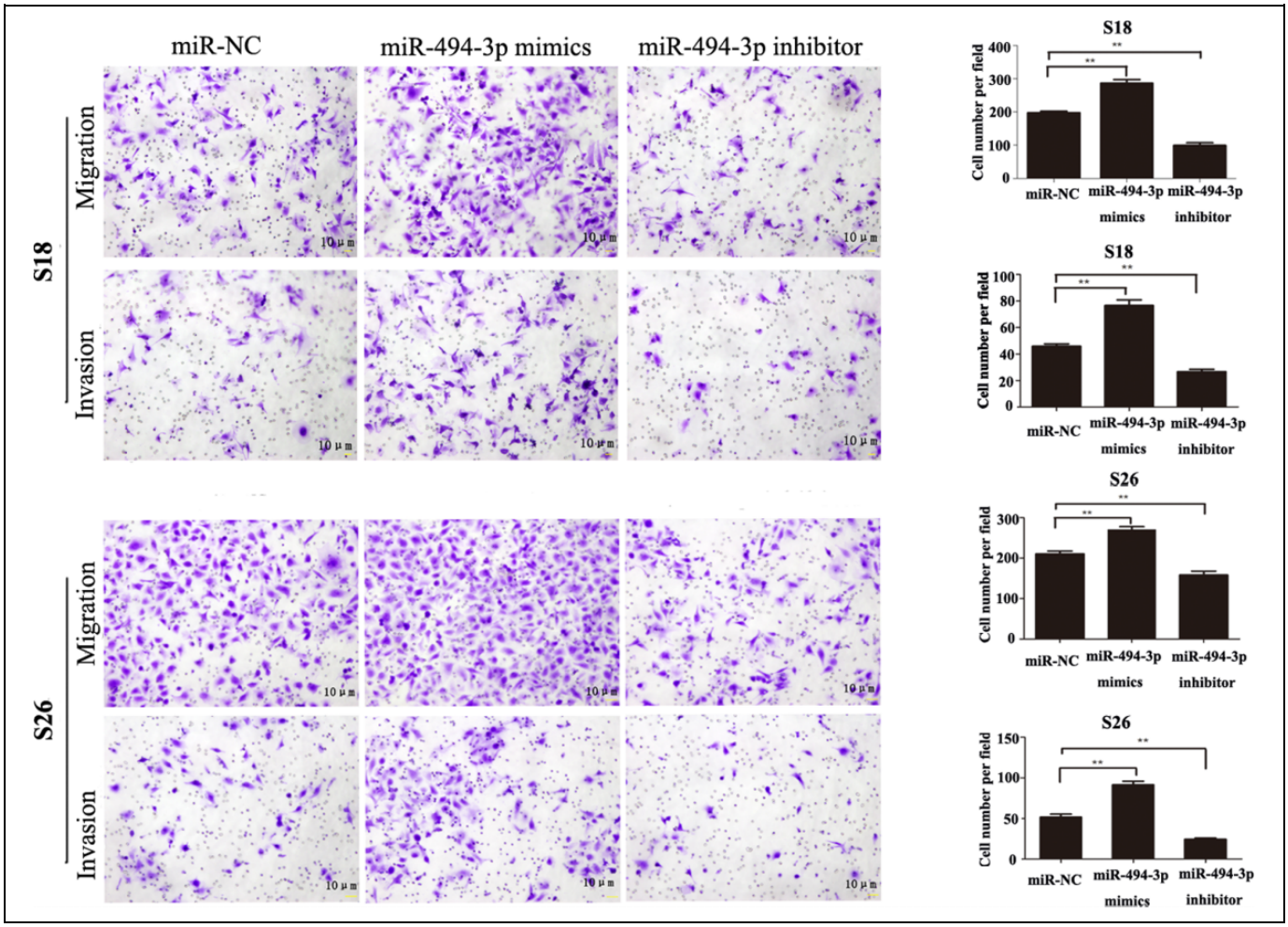

Figure 4. Upregulated miR-494-3p promotes migration and invasion in nasopharyngeal carcinoma (NPC) cells. Elevated miR-494-3p expression promoted S18 and S26 cell migration and invasion as evaluated by the transwell assays. *$P < .05$. Photomicrographs are 100× (right panel), and the results were expressed as cell number per field to the miR-NC. The data are presented as the mean (SD) of triplicate replicates.

increased or reduced S18 and S26 cell migration, respectively, compared with miR-494-3p negative control (Figure 3; $P < .05$ in S18 and $P < .01$ in S26). And overexpression of miR-494-3p that significantly promoted the cell migration and invasion capacity of S18 and S26 cells or miR-494-3p inhibitor has the contrasting results, compared with miR-control via transwell assay (Figure 4; $P < .01$).

Sox7 Was a Direct Target of miR-494-3p in NPC Cells via Binding to Its 3′-UTR

Based on the miRanda and TargetScan software, potential binding sites of miR-494-3p in the 3′-UTR of Sox7 were predicted (Figure 5A). Then we performed luciferase reporter assay to determine whether miR-494-3p could directly target the 3′-UTR of Sox7. We found that cotransfection of miR-494-3p mimics and pLUC-Sox7-wt significantly decreased the luciferase activity in H293T cell as compared with the control. Moreover, the activity of mutant 3′-UTR vector was unaffected by a simultaneous transfection with miR-494-3p mimics when cotransfected with pLUC-Sox7-mut (Figure 5B; $P < .05$). Taken together, these data suggested that Sox7 is one of direct targets of miR-494-3p.

Overexpression of miR-494-3p Led to Downregulation of Sox7 in S18 and S26 Cells

To elucidate whether the growth-promoting effect of miR-494-3p was mediated by repression of Sox7 in NPC cells, we performed gain-of-function and loss-of-function studies. The successful overexpression and suppression of miR-494-3p in the S18 and S26 cells were confirmed by qRT-PCR compared with the control (Figure 6A; $P < .05$). Then we found that transfection of miR-494-3p mimics significantly decreased Sox7 expression in S18 and S26 cells, while the expression of Sox7 was increased by miR-494-3p inhibitor as compared with the control (Figure 6B; $P < .05$). To better understand whether miR-494-3p could regulate Sox7, we further analyzed the Sox7 protein expression by Western blotting and quantitative analysis after transfecting S18 or S26 cells with

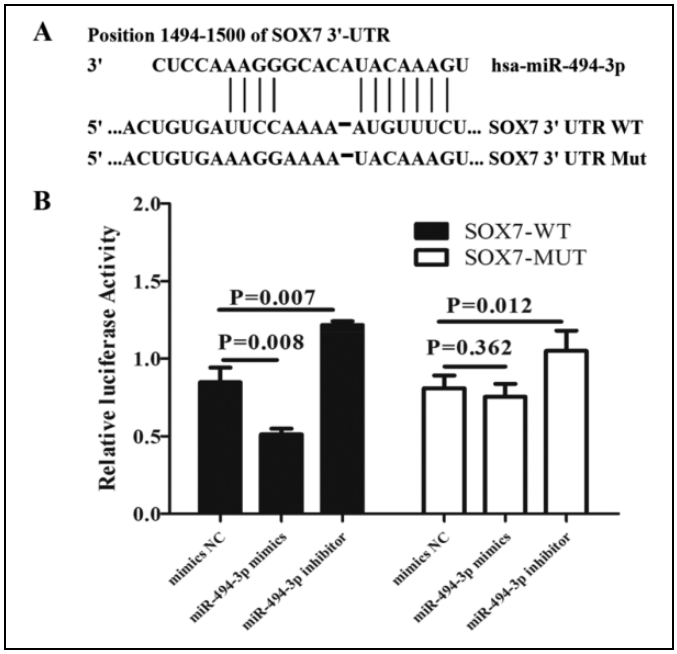

Figure 5. Sox7 is a direct target of miR-494-3p in nasopharyngeal carcinoma (NPC). A, Wild-type (Wt) or mutated (Mut) miR-494-3p target sequences of the Sox7 messenger RNA (mRNA) 3′-untranslated region (3′-UTR) predicted by TargetScan. B, Relative luciferase activity of HEK293T cells after cotransfection with Wt or Mut Sox7 3′-UTR reporter genes (μg) and miR-494-3p mimics (50 nM), miR-494-3p inhibitor (100 nM), or miR-Ctrl (50 nM). The data are presented as the mean (SD) of triplicate replications. $^{**}P < .01$ compared with the miR-Ctrl group, *P* values were calculated using the Student *t* test.

miR-494-3p mimics, inhibitor, and negative control. Sox7 protein in S18 and S26 cells significantly increased after ectopic miR-494-3p expression with miR-494-3p mimics, and miR-494-3p inhibitor upregulated Sox7 protein level (Figure 6C and D; $P < .05$).

Discussion

Recent findings have reported that miRNAs can either serve functionally as oncogenes or tumor suppressor genes and regulate multiple cellular processes and modulate oncogenic or tumor suppressor pathways in tumorigenesis, which could be served as prognostic biomarkers in various cancers.[31,32] The reason may be more than 50% of miRNA genes are located at the chromosomal regions with fragile sites, in which deletion and amplification are frequently presented in human cancers.[33] Identifying cancer-specific miRNAs and their targets genes are critical for understanding their roles in tumor development and progression, and it is important to exploring novel therapeutic targets.[10]

Metastasis is the greatest cause of death in almost all types of malignancies. Distant metastasis is also a botched and complex problem for the treatment of NPC, which can be driven by over 100 genes. Blocking distant metastasis of the first cancer can effectively improve the curative effect of NPC; therefore, more studies are warranted to further unveil the mechanisms underlying NPC metastasis.[34] Several studies indicated that miRNAs were abnormally expressed in NPC, and the dysregulated miRNAs could regulate NPC cell growth and metastasis.[35] Latest research showed that miR-338 could inhibit the migration and proliferation by targeting hypoxia-induced factor 1α in NPC.[36] MiR-223 targeting MAFB suppressed the proliferation and migration of NPC cells.[37] Interestingly, our findings identified that miR-494-3p could increase cell proliferation and migration in NPC cells. Both transwell migration assay and wound healing assay were demonstrated that miR-494-3p could promote the migration of NPC cells.

Previous findings suggested that miR-494-3p might play a diverse role in different tumors. MiR-494-3p was found to be downregulated in prostate cancer. Artificial overexpression of miR-494-3p could inhibit the cell growth, migration, and invasion and promote the apoptosis of prostate cancer cells *in vivo*.[38] Although miR-494-3p upregulated in glioblastoma cells, overexpressed miR-494-3p could promote proliferation, migration, invasion and inhibit apoptosis in gliomas through phosphatase and tensin homolog/serine-threonine protein kinase (PTEN/AKT) pathway.[39] Moreover, miR-494-3p was significantly higher in nasal natural killer (NK) cell lymphoma tissues than those in tumor-adjacent normal tissues, which displays the oncogenic effects through inhibiting PTEN with secondary activation of AKT in NK92 cells.[40]

In the present study, synthetic miR-494-3p inhibitor or mimics were transfected into S18 or S26 cells, respectively, to determine the biological function of miR-494-3p in NPC cells. Overexpressed miR-494-3p could enhance NPC cell growth and migration, yet cell growth was suppressed by miR-494-3p downregulation, suggesting miR-494-3p plays a potential tumor-promoting role in NPC. Similarly, some studies showed miR-155-5p expression was upregulated in most CRC and promoted proliferation, invasion, and metastasis of CRC cells.[41] But miR-155-5p was downregulated in gastric cancer cells.[42] These controversial results suggest that dysregulation of miRNAs can play different roles in diverse cancer cells. Indeed, the tissue and time-dependent expression of miRNAs influenced protein translation during distinct cellular processes, and the aberrant expression of their target genes affected different biological pathways with diverse functions.[43]

Additionally, we firstly detected the antioncogenic effects of Sox7 that predicted as a target gene of miR-494-3p, which was downregulated in NPC cells and NPC tissues. Therefore, we supposed that Sox7 might play a crucial role in NPC tumorigenesis and miR-494-3p regulated NPC cell metastasis through targeting Sox7. Although Sox7 appears to execute a tumor suppressive function in a broad range of cancers, such as hepatocellular carcinoma[44] and gastric cancer,[45] our findings were consistent with earlier studies. There might be 2 main reasons to explain the suppressive role of Sox7 in NPC cells. One is Sox7 that activates its targeted genes, such as SPRY1 and SLIT2 by serving as a transcriptional factor.[46] The other is Sox7 antagonism of Wnt/β-catenin signaling, a commonly upregulated pathway in human cancers involved in the regulation of cell proliferation, survival, and migration.[45,47,48] But the

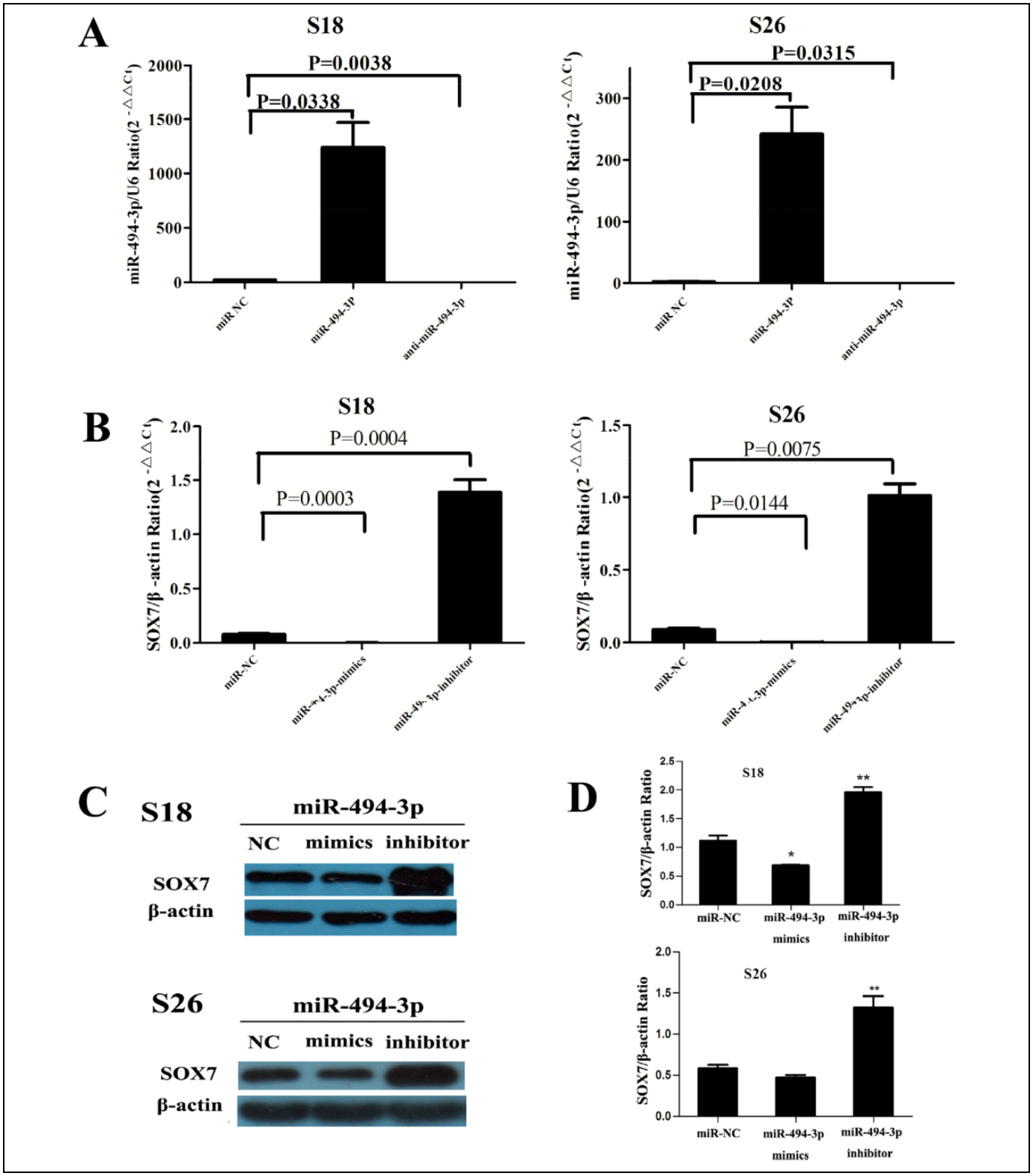

Figure 6. Alteration in miR-494-3p expression changes Sox7 expression. S18, S26 cells were transfected with miR-494-3p mimics and inhibitor. A, MiR-494-3p levels (normalized to U6) determined by quantitative real-time polymerase chain reaction (PCR; $*P < .05$). B, Sox7 messenger RNA (mRNA; normalized to β-actin) expression levels tested after miR-494-3p transfected in S18 and S26 by qRT-PCR ($**P < .001$ compared with miR-NC). C, Sox7 protein levels determined by Western blotting in miR-494-3p knockdown cells were both significantly increased. D, Quantitative analysis showed that Sox7 protein levels decreased in miR-494-3p mimics-transfected S18 and S26 cells relative to those transfected with miR-NC and increased in miR-494-3p inhibitor-transfected S18 and S26 cells relative to those transfected with miR-NC. $*P < .05$. The data are presented as the mean (SD) of triplicate replications. *P* values were calculated using the Student *t* test.

precise molecular mechanism behind these effects remains unclear. In addition, decreased Sox7 expression was reported to be correlated with poor prognosis in patients with both metastatic lung adenocarcinoma and breast cancer,[26,45,49] indicating that it can serve potential role as a valuable prognostic marker. As a transcription factor, Sox7 in the context of cancer prognosis needs to further validated on larger sizes of samples.

In summary, our study demonstrated that miR-494-3p might play an oncogenic role, partly through repression of Sox7 in NPC. MiR-494-3p could promote the proliferation, migration, and invasion of NPC cells by targeting Sox7. MiR-494-3p/Sox7 may play an important role in tumor metastasis and may be a novel diagnostic marker and potential therapeutic target for the treatment of NPC in future.

Authors' Note

H.H., X.L., and Q.Y. contributed equally.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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