OncoTargets and Therapy

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ORIGINAL RESEARCH

# RETRACTED ARTICLE: Long noncoding RNA NEAT1 promotes nasopharyngeal carcinoma progression through regulation of miR-124/NF-κB pathway

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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**Abstract:** Nasopharyngeal carcinoma (NPC) is one of the most common malignancies and seriously endangers people's health. Recently, long noncoding RNA (lncRNA) *NEAT1* has been determined as an oncogenic gene in a variety of cancers. However, the effect of NEAT1 in NPC and its underlying mechanism have not been well elaborated. In this study, the data showed that NEAT1 was upregulated and miR-124 was downregulated in NPC tissues and cells. Loss-of-function revealed that NEAT1 knockdown inhibited proliferation and promoted apoptosis of NPC cells while gain-of-function revealed that upregulated NEAT1 showed an opposite effect. Moreover, NEAT1 was demonstrated to suppress miR-124 expression by direct interaction in NPC cells. Additionally, miR-124 reversed NEAT1-mediated pro-proliferation and anti-apoptosis effect. Furthermore, miR-124 regulated NPC cell proliferation and apoptosis via NF-κB signaling pathway. Mouse models of NPC confirmed that NEAT1 overexpression facilitated tumor growth by modulating miR-124 in vivo. Taken together, this study indicated that upregulated NEAT1 promoted the tumorigenesis and progression of NPC through regulating miR-124/NF-κB signaling pathway, suggesting an attractive therapy target for NPC patients.

**Keywords:** nasopharyngeal carcinoma, lncRNA, NEAT1, miR-124, NF-κB pathway

## Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common nasopharyngeal malignancies in China and Southeast Asia, especially in Southern China.[1] The incidence of NPC is associated with multiple factors, including viral infection, genetics, and environment.[2] Although the developments of radiotherapy and chemotherapy have improved the treatment effect of NPC, the survival rate of NPC patients remained at 50%–70% at 5 years.[3] Thus, it is of great importance to search novel therapy target for NPC.

Long noncoding ribonucleic acids (lncRNAs), a type of transcript with length over 200 nucleotides (nt), play significant roles in multiple fundamental biological processes implicated in cancer progression.[4] A number of documents have elucidated the involvement of lncRNAs in NPC.[5] For instance, Yang et al[6] found that lncRNA LINC01420 downregulation inhibited cell migration and invasion in NPC. Liu et al[7] also detected that lncRNA PCAT7 contributed to the development and progression of NPC via regulating miR-134-5p/ELF2 signal pathway. NEAT1 has been found as an oncogene in a series of cancers,[8] such as endometrial cancer[9] and pancreatic cancer.[10] Additionally, NEAT1 was reported to promote the progression of NPC by regulating epithelial to mesenchymal transition by modulating ZEB1 mediated by miR-204.[11]

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http://dx.doi.org/10.2147/OTT.S151800

Nevertheless, the underlying molecular mechanism of NEAT1 in NPC has not been well understood.

MicroRNAs (miRNAs), a group of small noncoding RNAs of 19–22 nt in length, have important effects in the tumorigenesis and development of many malignances.[12] Increasing evidence reveals that miRNAs display important potential values in cancer diagnosis, treatment, and prognosis.[13] Recent studies have confirmed that miR-124 might act as a tumor suppressor in many cancers, including breast cancer,[14] non-small-cell lung cancer,[15] and bladder cancer.[16] Interestingly, miR-124 was previously confirmed to inhibit cell proliferation and invasion in NPC by targeting Capn4.[17] Recently, the competing endogenous RNA (ceRNA) hypothesis proposes that lots of lncRNAs might act as molecular sponges for miRNAs to influence target mRNA expression, highlighting the importance of such interactions during the tumorigenic process.[18,19] It was previously reported that NEAT1, whose expression was collaboratively controlled by HuR and miR-124-3p, facilitated proliferation and invasion of ovarian cancer cells.[20] However, the effect of interplay between NEAT1 and miR-124 in NPC remains undefined. In this study, it is found that NEAT1 was upregulated and miR-124 was downregulated in NPC. Furthermore, this study suggested that NEAT1 promoted tumorigenesis and development of NPC by regulating miR-124/NF-κB pathway.

## Materials and methods

### Tissue samples and cell culture

NPC tissues and normal nasopharyngeal tissues were obtained from patients who had undergone surgery at the Huaihe Hospital of Henan University (Kaifeng, Henan, China). Written informed consent was obtained from the patients, and this study was approved by the Ethical and Scientific Committees of Huaihe Hospital of Henan University.

Five NPC cell lines (CNE1, CNE2, SUNE1, 6-10B, and SUNE2), normal nasopharyngeal epithelial cells (N69), and 293T cells were purchased from American Tissue Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 100 μg/mL streptomycin (Thermo Fisher Scientific), and 100 U/mL penicillin (Thermo Fisher Scientific), and then were maintained at 37°C in a humidified atmosphere with 5% $CO_2$.

### Cell transfection

The full length sequences of NEAT1 were amplified by PCR and cloned into pcDNA3.1 vector (Thermo Fisher Scientific) to construct pcDNA-NEAT1 overexpression plasmid (NEAT1). All miRNA mimics (miR-negative control [NC], miR-124), miRNA inhibitors (anti-miR-NC, anti-miR-124), and siRNAs (si-NC, si-NEAT1) were obtained from Sangon Biotech (Shanghai, China). Plasmids and oligonucleotides were transfected by using the Lipofectamine™ 2000 transfection reagent (Thermo Fisher Scientific) referring to the manufacturer's instructions.

### RNA extraction and reverse transcription–quantitative polymerase chain reaction

Total RNA from NPC cells was isolated with Trizol reagent (Thermo Fisher Scientific), and then equal amount of RNA (500 ng) was converted into cDNA by M-MLV Reverse Transcription Kit (Thermo Fisher Scientific). The cDNA was subjected to real time–polymerase chain reaction using SYBR Premix Ex Taq GC Kit (Thermo Fisher Scientific) on ABI 7900HT fast real-time PCR detection system (Thermo Fisher Scientific) in order to quantify NEAT1 and miR-124 expression. The relative fold change of gene expression was detected by using $2^{-\Delta\Delta Ct}$ method with GAPDH or U6 as an internal control. For reverse transcription–quantitative polymerase chain reaction (RT-qPCR) analysis, the following primers were used: NEAT1: 5′-GTACGCGGGCAGACTAACAC-3′ (forward) and 5′-TGCGTCTAGACACCACAACC-3′ (reverse); miR-124: 5′-AGGCCUCUCUCCGUGUUCAC-3′ (forward) and 5′-CAGCCCCATTCTTGGCATTCAC-3′ (reverse); GAPDH: 5′-TGCACCACCAACTGCTTAGC-3′ (forward) and 5′-GGCATGCACTGTGGTCATGAG-3′ (reverse); U6: 5′-GCTTCGGCAGCACATATACTAAAAT-3′ (forward) and 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (reverse).

### Colony formation assay

Transfected NPC cells were seeded into six-well plates and were cultured in growth medium. After 12 days, cells were fixed with 70% ethanol and subsequently stained with 0.2% crystal violet solution (Sigma-Aldrich Co., St Louis, MO, USA). The number of colonies with more than 50 cells was counted by using a microscope (Leica Microsystems, Wetzlar, Germany).

### Cell viability determination

NPC cell viability was detected by using Cell Counting Kit-8 (CCK-8; Sigma-Aldrich Co.). At 0, 24, 48, and 72 h after transfection, cells were incubated in 10 μL of CCK-8 solution for 1 h at 37°C, followed by the measurement of absorbance

at 450 nm with a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA).

## Flow cytometry

At 48 h after transfection, SUNE2 and CNE1 cells were trypsinized and resuspended at $1\times10^6$ cells/mL. Apoptotic rate of NPC cells was detected by Annexin V-FITC Assay Kits (Sigma-Aldrich Co.). The reaction system was analyzed with the CellQuest software by using flow cytometer (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA) to differentiate apoptotic cells (Annexin V-positive and propidium iodide [PI]-negative) from necrotic cells (Annexin V- and PI-positive).

## Luciferase reporter assay

MiRcode website was used to search for the potential target miRNAs of NEAT1. The partial sequences of NEAT1 containing the putative binding sites of miR-124 were amplified by PCR and cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega Corporation, Fitchburg, WI, USA), to construct NEAT1 wild-type (WT) reporter vector. Site-directed mutagenesis of miR-124 complementary bases was carried out using GeneArt™ Site-Directed Mutagenesis System (Thermo Fisher Scientific) to construct NEAT1 mutant-type (MUT) reporter vector with mutant miR-124 binding sites. Then the constructed reporter vector was, respectively, transfected in SUNE2 cells together with miR-124 mimics, anti-miR-124, or their corresponding controls (miR-NC, anti-miR-NC). Luciferase activity was assayed using the Dual-Luciferase Reporter (DLR™) Assay System (Promega Corporation).

## RNA immunoprecipitation assay

RNA immunoprecipitation (RIP) assay was performed with an Imprint RNA Immunoprecipitation kit (Sigma-Aldrich Co.). Briefly, lysates were incubated with anti-Argomaute2 (anti-Ago2) or anti-IgG (negative control) overnight at 4°C, followed by the addition of Protein A magnetic beads to get the immunoprecipitation complex. Then, the complex was washed and purified to obtain RNA without extra protein and DNA. At last, RT-qPCR assay was employed to assess the enrichment of NEAT1 and miR-124 in immunoprecipitated RNA.

## NF-κB activity assay

NPC cells transfected with miR-124 or miR-NC were incubated with or without 10 ng/mL of NF-κB activator TNF-α (Bio-Techne, Minneapolis, MN, USA). DNA-binding activity of p65 was detected with TransAM NF-κB p65 kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's instructions.

## Western blot analysis

Total proteins were extracted from SUNE2 cells with the cell lysis buffer (RIPA; Beyotime, Shanghai, China). Samples were boiled for 10 min with 4× loading buffer (Takara, Shiga, Japan) and separated by 12% SDS-PAGE. Then, the proteins were transferred to polyvinylidine difluoride (PVDF) membranes (Sigma-Aldrich Co.). The membrane was blocked with 5% (wt/vol) skimmed milk powder and washed with Tris-buffered saline containing 0.1% Tween-20 (TBST); the PVDF membranes were then incubated with anti-β-actin (Abcam, Cambridge, UK), anti-p-IκBα (Abcam), anti-IκBα (Abcam), anti-p-p65 (Abcam), and anti-p65 (Abcam) overnight at 4°C, respectively. After three washes with TBST, the PVDF membranes were further probed with horseradish peroxidase conjugated secondary antibodies (Stanta Cruz Biotechnology, Santa Cruz, CA, USA). Lastly, protein expression was quantified using VersaDoc 4000MP imaging system (Bio-Rad Laboratories Inc.).

## Lentivirus production and infection

The full length sequence of NEAT1 was cloned into pLV-H1/α-MCS-IRES-Puro vector (Biosettia, San Diego, CA, USA) to construct NEAT1-overexpression lentivirus vector (lenti-NEAT1). Then lenti-NEAT1 vector or empty vector (lenti-control) was transfected into 293T cells together with psPAX2 and pMD2.G (Addgene, Cambridge, MA, USA). After 72 h post-transfection, lenti-NEAT1 or lenti-control lentivirus was collected to infect SUNE2 cells. Next, the infected cells were screened with puromycin for at least 1 week to obtain stable lentivirus-transfected cells.

## Tumor formation in nude mice

Male BALB/c nude mice (18–22 g, 6–8 weeks) were purchased from Henan Research Center of Laboratory Animal (Zhengzhou, China). Approximately $8\times10^6$ SUNE1 cells stably transfected with lenti-control or lenti-NEAT1 were subcutaneously inoculated into the mice to form xenograft mice. One week later, mice were divided into four groups (n=6 in each group): lenti-control+PBS, lenti-NEAT1+PBS, lenti-NEAT1+miR-NC, and lenti-NEAT1+miR-124. Intratumor injection of PBS, miR-NC, or miR-124 mimics was performed once a week for 6 consecutive weeks. Tumor volume was measured with a caliper during the process of experiment. At the end of experiment, mice were euthanized and tumors were excised for weight evaluation and RT-qPCR

analysis. All animal-handling procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and followed the guidelines of the Animal Welfare Act. The study was approved by the Ethics Committee of Henan University.

## Statistical analysis

All data were analyzed with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) using Student's *t*-test and one-way ANOVA. Experimental graphs were constructed using GraphPad Prism version 5 software (GraphPad Software, Inc, La Jolla, CA, USA). All data were displayed as mean ± standard deviation (SD) from at least three independent assays. $P<0.05$ was considered to be statistically significant.

# Results

## NEAT1 expression was upregulated and miR-124 expression was downregulated in NPC tissues and cell lines

To investigate the potential role and molecular mechanism of NEAT1 and miR-124 in NPC, their expression patterns in NPC tissues and cells were first detected using RT-qPCR assays. The results indicated that NEAT1 expression was markedly increased (Figure 1A), while miR-124 level was significantly decreased (Figure 1B) in NPC tissues compared with normal nasopharyngeal tissues. Similarly, compared with epithelial cells N69, NEAT1 expression was significantly upregulated (Figure 1C) and miR-124 level was markedly downregulated (Figure 1D) in NPC cells. These data suggested that NEAT1 and miR-124 might be associated with the development and progression of NPC.

## NEAT1 knockdown inhibited proliferation and promoted apoptosis in NPC cells

To further elucidate the potential function of NEAT1, small interference RNA of NEAT1 (si-NEAT1) was synthesized and then transfected into SUNE2 and CNE1 cells to examine its knockdown efficiency. As shown in Figure 2A, NEAT1 expression level was significantly decreased by si-NEAT1 in SUNE2 and CNE1 cells. Hence, si-NEAT1 was employed to further investigate the effect of NEAT1 depletion on



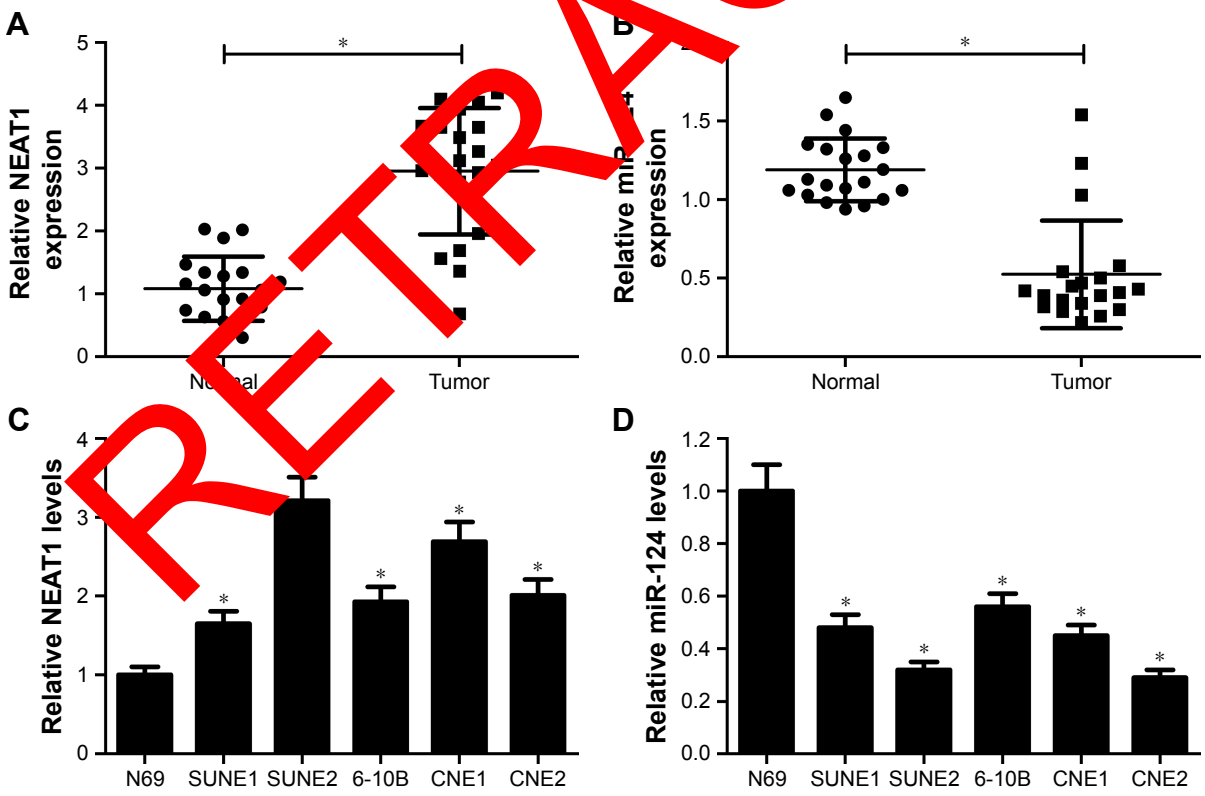


**Figure 1** NEAT1 was upregulated and miR-124 was downregulated in NPC tissues and cells. RT-qPCR assay was performed to assess the expression patterns of NEAT1 (**A**) and miR-124 (**B**) in NPC tissues (n=20) and normal nasopharyngeal tissues (n=20).The expression patterns of NEAT1 (**C**) and miR-124 (**D**) in NPC cell lines (SUNE1, SUNE2, 6-10B, CNE1, and CNE2) and normal nasopharyngeal epithelial cells (N69) were examined; *$P<0.05$ vs respective control.
**Abbreviations:** NPC, nasopharyngeal carcinoma; RT-qPCR, reverse transcription–quantitative polymerase chain reaction.

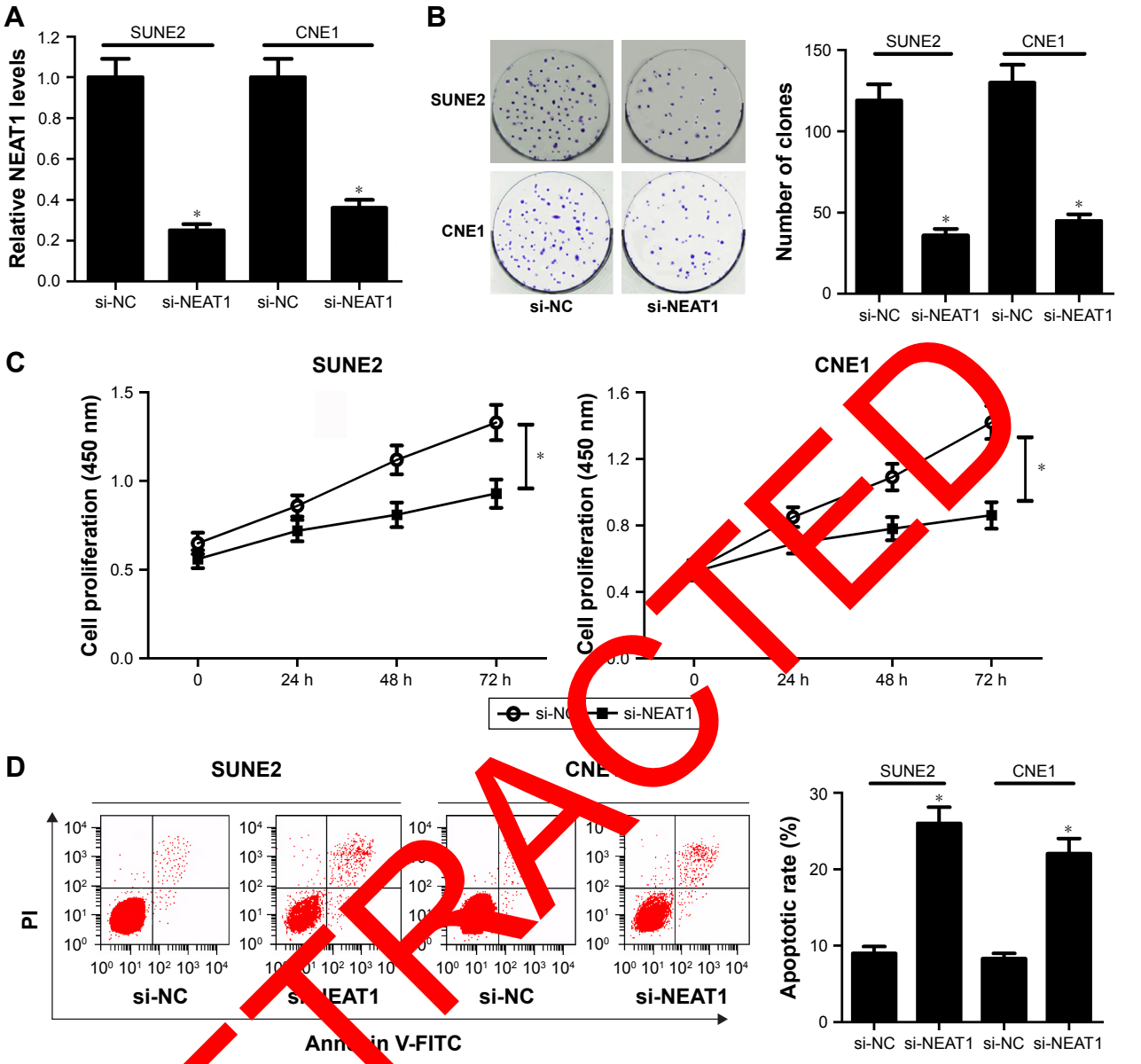




**Figure 2** NEAT1 knockdown inhibited proliferation and enhanced apoptosis in NPC cells. (**A**) The expression level of NEAT1 was detected by RT-qPCR in si-NC- or si-NEAT1-transfected NPC cells. (**B**) The effect of NEAT1 knockdown on the colony-forming capacity of SUNE2 and CNE1 cells was assessed by colony formation assay. (**C**) The viability of NPC cells with si-NEAT1 was introduced was determined by Cell Counting Kit-8 at OD 450 nm. (**D**) Flow cytometry was employed to evaluate the effect of NEAT1 knockdown on apoptosis in NPC cells following treatment with Annexin V-FITC Apoptosis Assay kit; *$P<0.05$ vs si-NC.
**Abbreviations:** NPC, nasopharyngeal carcinoma; RT-qPCR, reverse transcription–quantitative polymerase chain reaction; NC, negative control; PI, propidium iodide.

NPC cell proliferation and apoptosis. Colony formation assay revealed that the number of clones was obviously reduced following NEAT1 downregulation in NPC cells (Figure 2B). Consistently, NEAT1 knockdown led to a suppression of viability in NPC cells (Figure 2C). Moreover, flow cytometry analysis showed that NEAT1 depletion strikingly promoted apoptosis of SUNE2 and CNE1 cells (Figure 2D). Taken together, these results demonstrated that NEAT1 knockdown inhibited proliferation and facilitated apoptosis in NPC cells.

## NEAT1 suppressed miR-124 expression by direct interaction

To further explore the underlying mechanism of NEAT1 in NPC progression, the online software MiRcode was used to search for miRNAs associated with NEAT1. Intriguingly, the data revealed that there existed some complementary sites between NEAT1 and miR-124 (Figure 3A), indicating miR-124 might interact with NEAT1. To validate this assumption, dual-luciferase reporter assay was performed by transfecting constructed WT and MUT NEAT1 luciferase

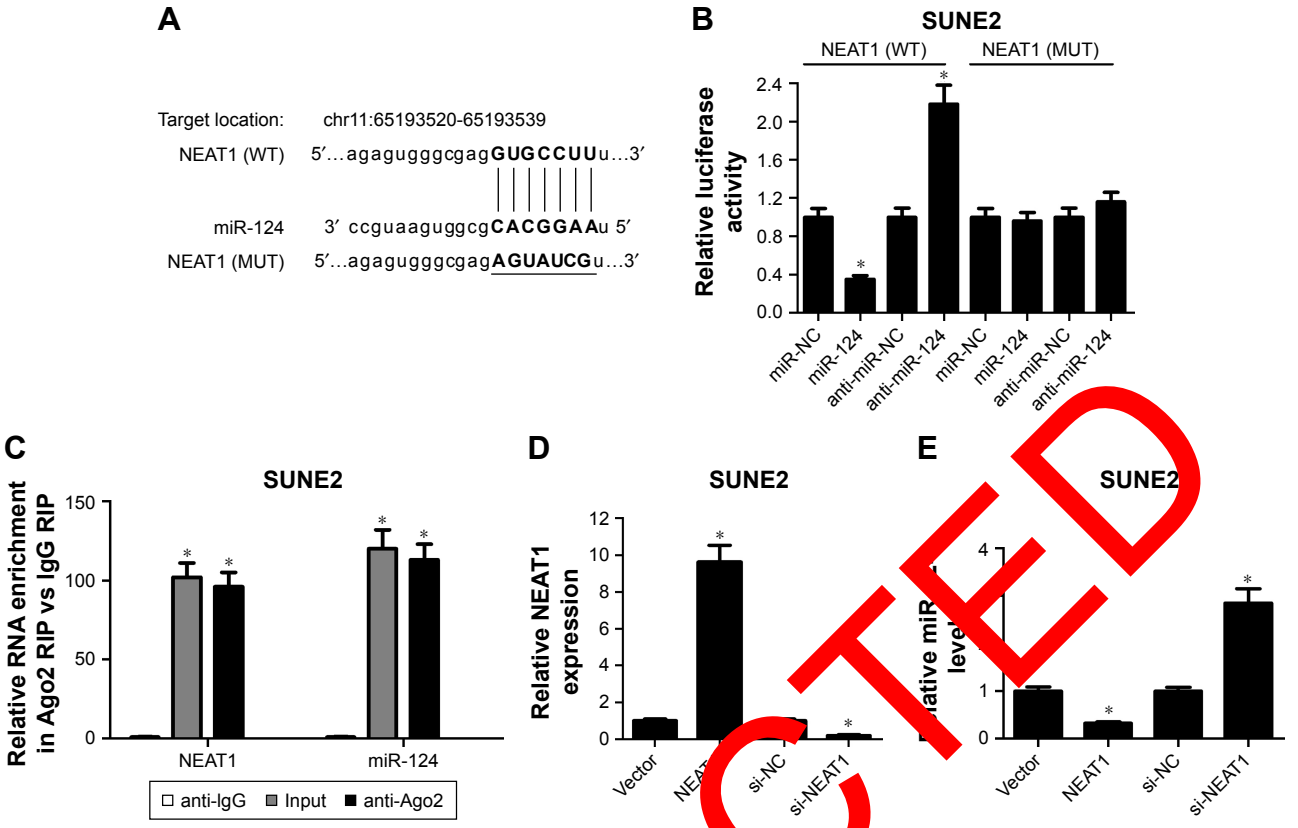


**Figure 3** NEAT1 suppressed miR-124 expression by direct interaction. (**A**) Sequence alignment of miR-124 with the putative binding sites within NEAT1 and mutant miR-124 binding sites. (**B**) Dual-luciferase reporter assays were used to investigate whether NEAT1 could directly interact with miR-124 by the putative binding sites in SUNE2 cells cotransfected with wild-type (WT) or mutant-type (MUT) NEAT1 luciferase vectors and miR-NC, miR-124, anti-miR-NC, or anti-miR-124. (**C**) SUNE2 cells lysate was treated with anti-Ago2 or anti-IgG (negative control) for RNA immunoprecipitation (RIP) assay. NEAT1 expression (**D**) and miR-124 expression (**E**) in NEAT1- or si-NEAT1-transfected SUNE2 cells are presented; *$P<0.05$ vs respective control.
**Abbreviation:** NC, negative control.



vectors into SUNE2 cells together with miR-NC, miR-124 mimics, anti-miR-NC, or anti-miR-124. The experimental data presented that miR-124 upregulation significantly decreased the luciferase activities of NEAT1 (WT), while miR-124 downregulation exhibited opposite effect (Figure 3B). However, mutation of predicted matching sites in the NEAT1 reporter system displayed little change in the luciferase activity following introduction with miR-124 mimics or anti-miR-124 (Figure 3B). Ago2, a component of RNA-induced silencing complex, plays a vital role in the maturation process of miRNAs. Hence, RIP assay was performed using Ago2 antibody to examine the potentially endogenous interaction between NEAT1 and miR-124. The results showed that NEAT1 and miR-124 were substantially enriched by Ago2 antibody compared with control IgG antibody in SUNE2 cells (Figure 3C). To further explore the effect of NEAT1 on miR-124, NEAT1-overexpression plasmid (pcDNA3.1-NEAT1) and siRNA targeting NEAT1 (si-NEAT1) were constructed and synthesized. As displayed in Figure 3D, NEAT1 expression was evidently enhanced in NEAT1-transfected SUNE2 cells and repressed in si-NEAT1-introduced SUNE2 cells. Conversely, the upregulation of NEAT1 significantly decreased miR-124 expression in SUNE2 cells, while NEAT1 knockdown promoted miR-124 expression (Figure 3E). Taken together, these results suggested that NEAT1 might act as a sponge of miR-124 in NPC cells.

## MiR-124 reversed NEAT1-mediated pro-proliteration and anti-apoptosis effect in NPC cells

PcDNA-NEAT1 overxpression plasmid (NEAT1) was constructed and transfected into SUNE2 and CNE1 cells to further elucidate the effect of NEAT1 on NPC progression. The data revealed that NEAT1 overexpression evidently enhanced colony forming ability (Figure 4A) and the viability (Figure 4B) of SUNE2 and CNE1 cells. Moreover, apoptotic rate of SUNE2 and CNE1 cells was markdely suppressed by the introduction of NEAT1 (Figure 4C). These results proposed that NEAT1 might act as an oncogene in NPC.

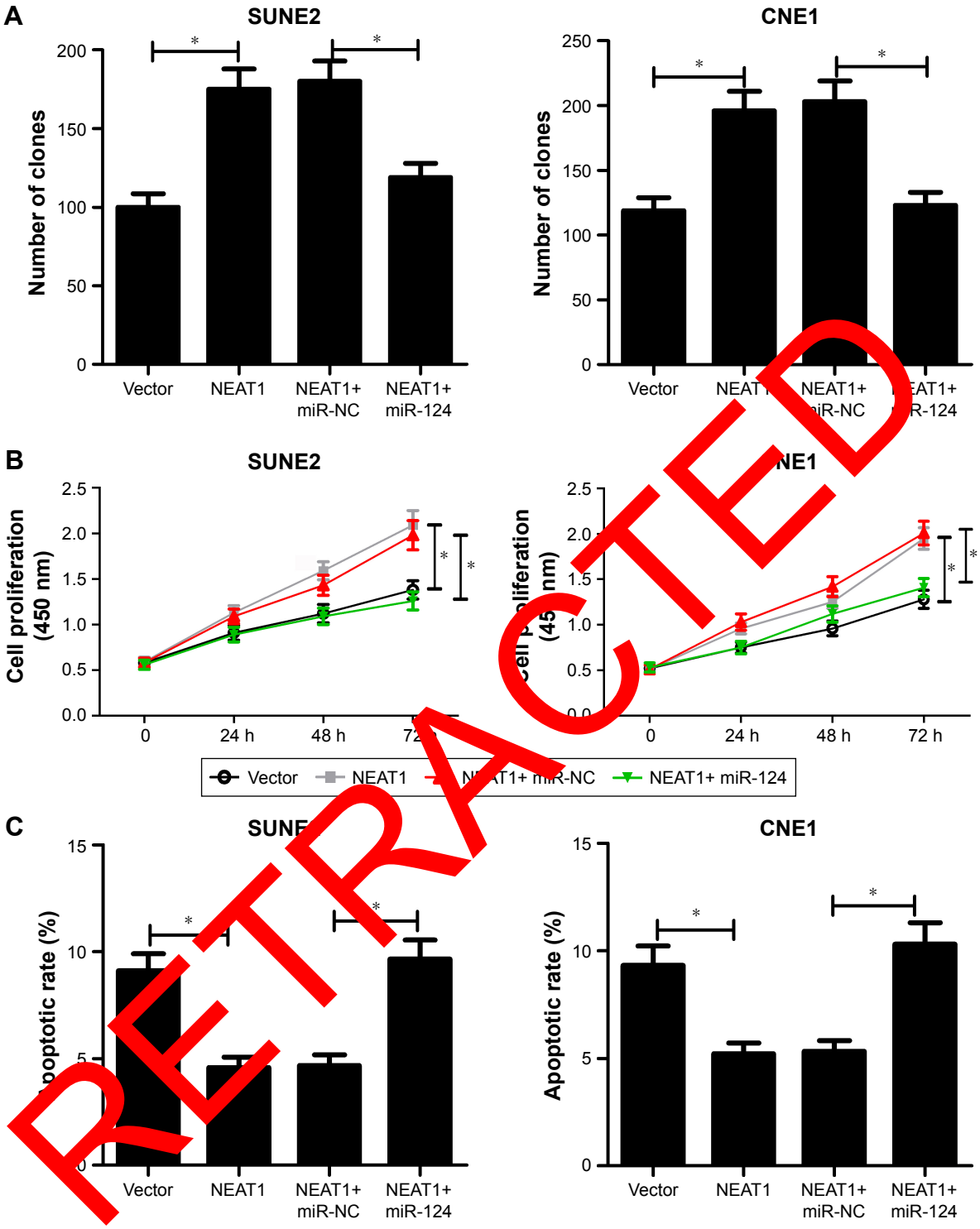




**Figure 4** MiR-124 reversed NEAT1-mediated pro-proliferation and anti-apoptosis effect in NPC cells. SUNE2 and CNE1 cells were introduced with either NEAT1 alone or together with miR-124 mimics. (**A**) Colony-forming assay of cell proliferation in transfected NPC cells. (**B**) CCK-8 analysis of cell viability in transfected NPC cells. (**C**) Flow cytometry assay of apoptosis in transfected NPC cells; *$P<0.05$ vs corresponding control.
**Abbreviations:** NPC, nasopharyngeal carcinoma; CCK-8, Cell Counting Kit-8; NC, negative control.

To further investigate whether the pro-tumor effect of NEAT1 was mediated by miR-124, NPC cells were transfected with NEAT1 alone or in combination with miR-124 mimics. The results showed that the tumor promotion effect of NEAT1 was greatly attenuated after upregulating miR-124, presented as less clones (Figure 4A), lower viability (Figure 4B), and higher apoptosis (Figure 4C) of SUNE2 and CNE1 cells in NEAT1 + miR-124 group than NEAT1 + Vector group. All these data indicated that miR-124 could abrogate the effect of NEAT1 on proliferation and apoptosis in NPC cells.

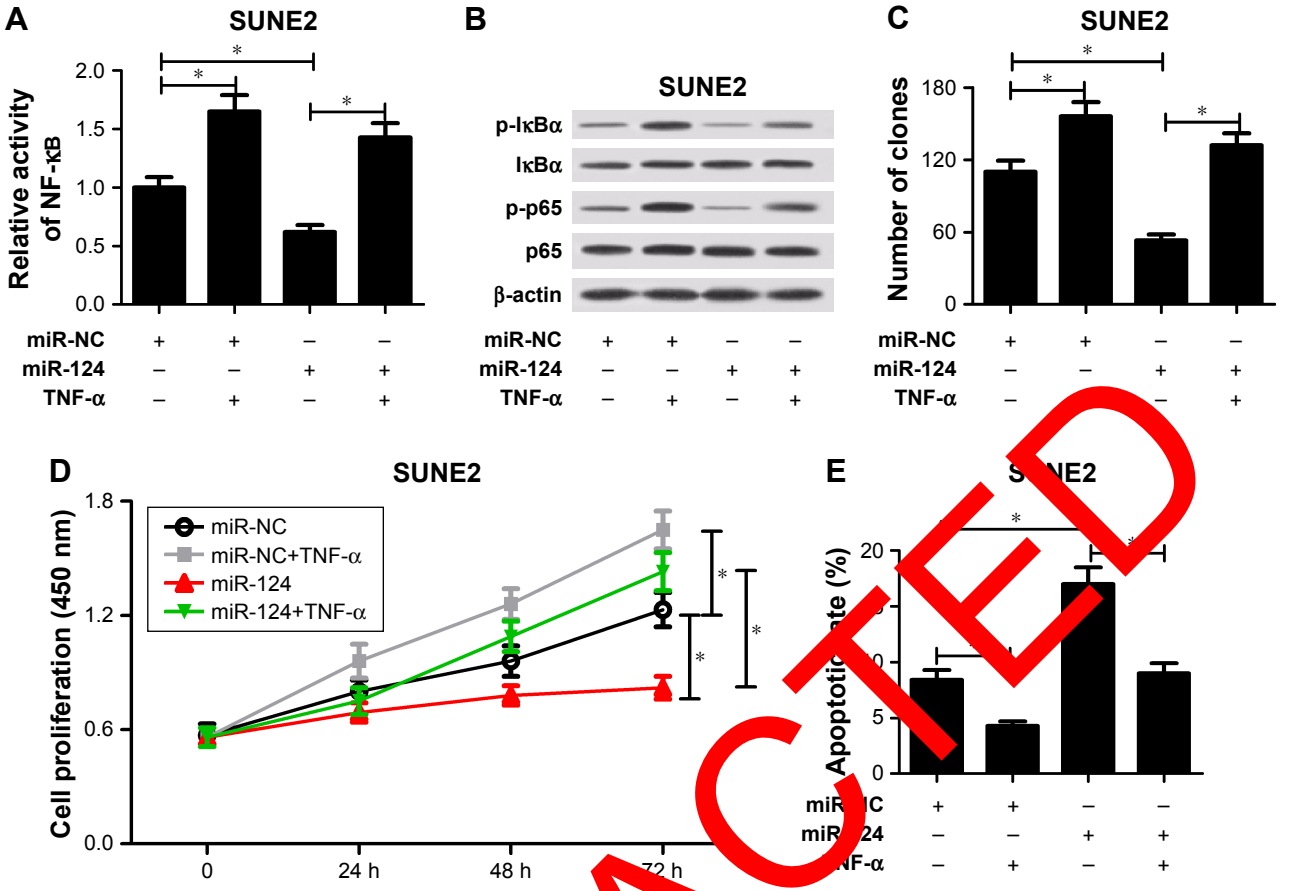


**Figure 5** MiR-124 inhibited cell proliferation and induced apoptosis by regulating NF-κB signal. MiR-NC- or miR-124-transfected SUNE2 cells were treated with or without 10 ng/mL TNF-α. (**A**) Relative activity of NF-κB was analyzed by TransAM NF-κB p65 kit in treated cells. (**B**) Western blot analysis was performed to measure phosphorylation of IκBα and p65 in treated cells. β-actin was used as internal reference. Colony-forming ability (**C**), cell viability (**D**), and apoptosis (**E**) were analyzed in treated cells; *$P<0.05$ vs respective control.

**Abbreviations:** NC, negative control; TNF-α, tumor necrosis factor-α.



## MiR-124 inhibited cell proliferation and induced apoptosis by regulating NF-κB signal

NF-κB had been validated to be involved in miR-124-mediated antitumor effect in some cancers and miR-124 directly targeted the NF-κB/p65.[21] As a result, the effect of miR-124 on NF-κB signaling pathway in SUNE2 cells was further investigated. The data revealed that TNF-α treatment evidently increased the activity of NF-κB (Figure 5A), promoted phosphorylation of IκBα and p65 (Figure 5B), facilitated cell proliferation (Figure 5C and D), and suppressed apoptosis (Figure 5E), whereas miR-124 overexpression decreased the activity of NF-κB (Figure 5A), repressed phosphorylation of IκBα and p65 (Figure 5B), hindered cell proliferation (Figure 5C and D), and induced apoptosis (Figure 5E). These data suggested that miR-124 might play a role in the regulation of NF-κB signaling pathway in NPC cells. Therefore, the effect of NF-κB activation on miR-124-mediated proliferation and apoptosis was further detected in NPC cells. The data showed that NF-κB activation induced by TNF-α notably abated miR-124-triggered anti-proliferation and pro-apoptosis effect in NPC cells (Figure 5C–E). All these results implied that miR-124 regulated NPC cell proliferation and apoptosis by inhibiting NF-κB signal.

## NEAT1 promoted tumor growth by inhibiting miR-124 expression in vivo

As mentioned earlier, in vitro assays indicated that NEAT1 contributed to NPC progression via modulating miR-124 in NPC. Hence, the effect of NEAT1 and miR-124 on tumor growth in xenograft mice was investigated. The results showed that NEAT1 overexpression markedly facilitated tumor growth, revealed by the increase of tumor volume (Figure 6A) and tumor weight (Figure 6B and C). Moreover, the introduction of miR-124 mimics strikingly weakened the pro-tumor effect of NEAT1 in vivo (Figure 6A–C). Additionally, as presented in Figure 6D and E, NEAT1 expression was upregulated and miR-124 expression was downregulated in

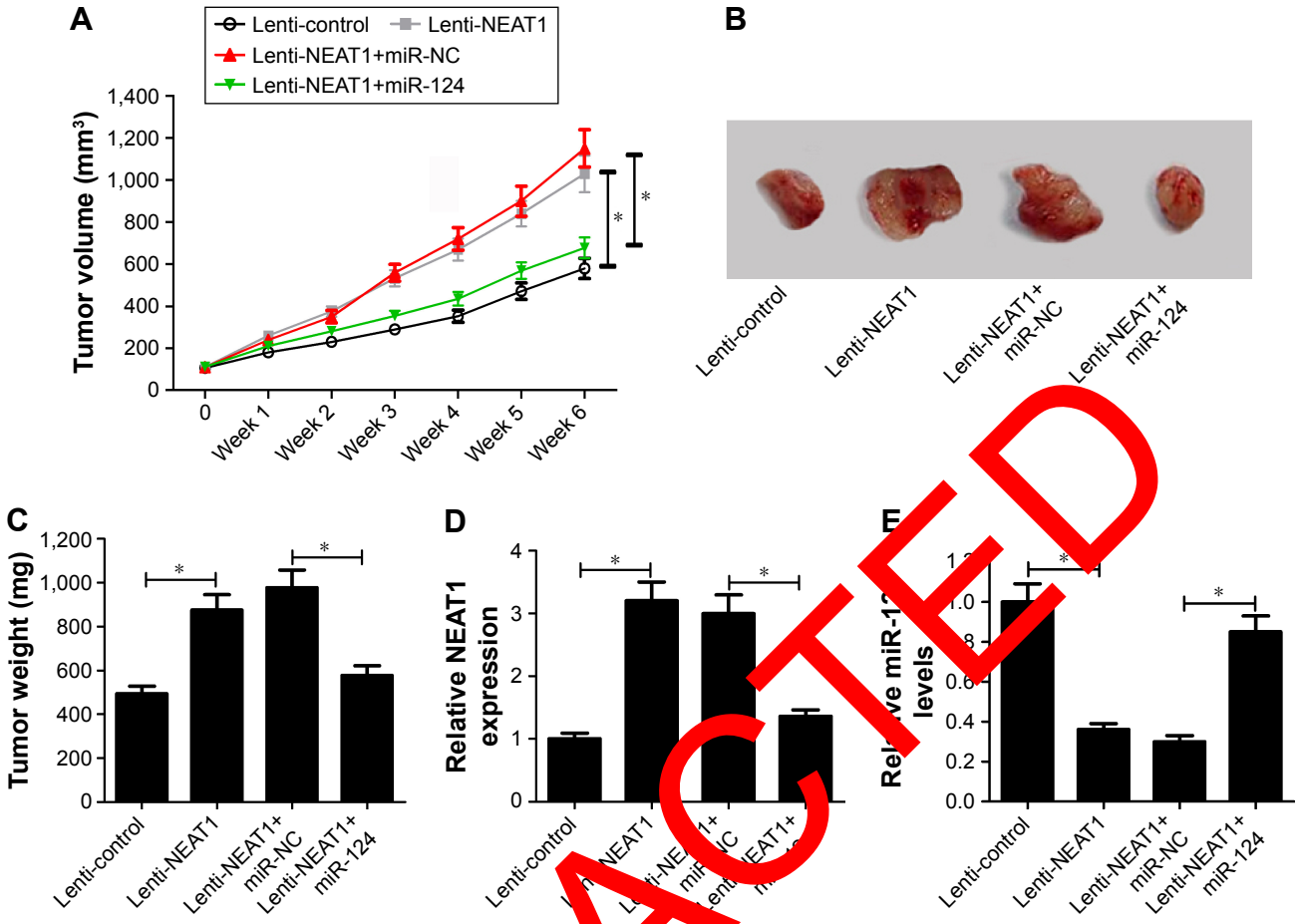


**Figure 6** NEAT1 promoted tumor growth through inhibiting ...24 expre... in vivo. About 8×10⁶ SUNE2 cells stably transfected with lenti-control or lenti-NEAT1 were subcutaneously injected into the nude mice. One wee...ter, in...tumor in...tion of PBS, miR-NC, or miR-124 mimics were performed once a week for 6 consecutive weeks according to indicated groups (n=6 in each gro... lenti-con...l+PBS, len...NEAT1+PBS, lenti-NEAT1+miR-NC, lenti-NEAT1+miR-124. Mice were euthanized for removing tumor masses at 7 weeks after inoculation. ... The tum... was m...easured with a caliper once. (**B**) Representative images of the xenograft tumors isolated from different groups. (**C**) The average weight of ...sect... tum... RT-qPCR ...alysis was applied to test the expression of NEAT1 (**D**) and miR-124 (**E**) in excised tumor tissues; *$P<0.05$ vs corresponding control.

**Abbreviations:** NC, negative control; RT... R, reverse trans...tion–quantitative polymerase chain reaction.



tumors derived from lenti...NEAT1-trans...ted cells. However, these effects were pr...minently reversed by the restoration of miR-124 expressi... The... results revealed that NEAT1 promoted tum...igenes...by inhibi...ng miR-124 in vivo.

## Discussion

NEAT1, a...w lncRNA localized specifically to nuclear paraspeckles, ...found in the nucleus' interchromatin space.[22] Recently, researchers have discovered that NEAT1 acts as an oncogene in a series of cancers.[8] Qian et al[23] found that NEAT1 promoted cell proliferation and migration in breast cancer. In prostate cancer, NEAT1 enhanced cell proliferation and inhibited cell apoptosis, which contributed to tumorigenesis and development.[24] Also, NEAT1 was confirmed to promote epithelial to mesenchymal transition by regulating the miR-204/ZEB1 axis in NPC.[11] In this study, the data revealed that the expression of NEAT1 was significantly increased in NPC tissues and cells. Moreover, NEAT1 knockdown suppressed proliferation and induced apoptosis of SUNE2 and CNE1 cells, while NEAT1 overexpression exhibited an opposite effect. Our study also verified that NEAT1 promoted tumor growth in vivo. These results provided further evidence that NEAT1 might be a critical mediator in the development of NPC.

Recently, lots of studies have verified that NEAT1 may act as a ceRNA of miRNAs, which antagonizes miRNA functions and regulates the expression of miRNA endogenous targets to play vital roles in tumorigenesis and development of many cancers.[25–28] For instance, in human breast cancer, NEAT1 facilitated cell growth and invasion via the miR-211/HMGA2 axis.[26] Similarly, NEAT1 accelerated tumor progression by inhibiting miR-377-3p and activation of E2F3 signaling pathway in non-small-cell lung cancer.[27] NEAT1 also promoted epithelial to mesenchymal transition by regulating the

miR-204/ZEB1 axis in NPC.[11] Therefore, MiRcode online website was employed to search for the potential target miRNAs of NEAT1. Intriguingly, the results showed that miR-124 might interact with NEAT1. Further dual luciferase assays and RIP assays verified that miR-124 was a target of NEAT1, and NEAT1 could suppress miR-124 expression. A previous document also elucidated a close connection between NEAT1 and miR-124-3p in ovarian cancer cells.[20]

MiR-124, as tumor suppressors, has been demonstrated in a variety of cancers. For example, in breast cancer, miR-124 inhibited cell migration and invasion by regulating CDK4 expression.[29] Silber et al indicated that miR-124 might be efficacious for the treatment of glioblastoma multiforme.[30] In the present study, it was found that miR-124 was significantly decreased in NPC tissues and cells. These results illustrated that miR-124 might serve as a tumor suppressor in the tumorigenesis and progression of NPC. In line with our results, Xu et al[31] proved that miR-124-3p inhibited NPC cell proliferation and invasion, as well as facilitated NPC cell apoptosis through regulating the expression of STAT3. Peng et al[32] found that miR-124 suppressed tumor growth and metastasis by targeting Foxq1 in NPC. Hence, we investigated whether NEAT1 exerted its oncogenic effect by regulating miR-124. The results showed that exogenous expression of miR-124 reversed NEAT1-mediated pro proliferation and anti-apoptosis effect in NPC cell in vitro. Additionally, the pro-tumor effect of NEAT1 was markedly attenuated following the restoration of miR-124 expression in vivo. Taken together, the data suggested that NEAT1 might facilitate tumor growth through antagonizing miR-124 functions in vitro and in vivo.

The potential mechanism implicated in antitumor effect of miR-124 in NPC was investigated. A previous document found that miR-124 exerted a tumor-suppressive role by decreasing MYC and BCL expression via direct targeting of p65, which is associated with NF-κB pathway in B-cell lymphoma.[33] Moreover, numerous studies revealed that constitutive activation of NF-κB pathway played vital roles in the development and progression of NPC. Sun et al[33] confirmed that NF-κB/p65 enhanced cell migration by secreting matrix metalloproteinase (MMP-9) in NPC. Zhao et al[34] confirmed that knockdown of TIGAR repressed NPC tumor growth via NF-κB pathway. This study verified that the upregulation of miR-124 significantly inhibited phosphorylation of IκBα and NF-κB activity in SUNE2 cells. Furthermore, our data validated that miR-124 regulated NPC cell proliferation and apoptosis by inhibiting NF-κB signal. In agreement with our findings, miR-124 was recently reported to regulate autophagy, inflammation, and cell death via targeting p62 and p65/NF-κB in KRAS mutant mesenchymal NSCLC cells.[35] In addition, Sun et al[15] revealed that miR-124 upregulation attenuated cancer cell migration and NF-κB pathway hindered miR-124 expression in non-small-cell lung cancer. Therefore, more research about the regulatory relationship between miR-124 and NF-κB in NPC are needed in future work.

## Conclusion

This study indicated that upregulated NEAT1 promoted tumorigenesis and progression of NPC through regulating miR-124/NF-κB signaling pathway, indicating that NEAT1 might be a useful marker and potential therapeutic target for NPC.

## Disclosure

The authors report no conflicts of interest in this work.



## References


1. Tang LL, Chen WQ, Xue WQ, et al. Global trends in incidence and mortality of nasopharyngeal carcinoma. *Cancer Lett*. 2016;374(1):22–30.
2. Cai W, Zheng H, Cheung AK, Lung ML. Genetic and epigenetic landscape of nasopharyngeal carcinoma. *Chin Clin Oncol*. 2016;5(2):16.
3. Tang LL, Tan EH, Lim DW, et al. Advances in systemic treatment for nasopharyngeal carcinoma. *Chin Clin Oncol*. 2016;5(2):21.
4. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009;10(3):155–159.
5. He R, Hu Z, Wang Q, et al. The role of long non-coding RNAs in nasopharyngeal carcinoma: as systemic review. *Oncotarget*. 2017;8(9):16075–16083.
6. Yang L, Tang Y, He Y, et al. High expression of LINC01420 indicates an unfavorable prognosis and modulates cell migration and invasion in nasopharyngeal carcinoma. *J Cancer*. 2017;8(1):97–103.
7. Liu Y, Tao Z, Qu J, Zhou X, Zhang C. Long non-coding RNA PCAT7 regulates ELF2 signaling through inhibition of miR-134-5p in nasopharyngeal carcinoma. *Biochem Biophys Res Commun*. 2017;491(2):374–381.
8. Xin Y, Zheng L, Zheng H, Chan MT, Wu WK. NEAT1: a novel cancer-related long non-coding RNA. *Cell Prolif*. 2017;50(2).
9. Li Z, Wei D, Yang C, Sun H, Lu T, Kuang D. Overexpression of long noncoding RNA, NEAT1 promotes cell proliferation, invasion and migration in endometrial endometrioid adenocarcinoma. *Biomed Pharmacother*. 2016;84:244–251.
10. Huang B, Liu C, Wu Q, et al. Long non-coding RNA NEAT1 facilitates pancreatic cancer progression through negative modulation of miR-506-3p. *Biochem Biophys Res Commun*. 2016;482(4):828–834.
11. Lu Y, Li T, Wei G, et al. The long non-coding RNA NEAT1 regulates epithelial to mesenchymal transition and radioresistance in through miR-204/ZEB1 axis in nasopharyngeal carcinoma. *Tumor Biol*. 2016;37(9):11733–11741.
12. Calin GA, Croce CM. Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. *J Clin Invest*. 2007;117(8):2059–2066.
13. Cho WCS. MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. *Int J Biochem Cell Biol*. 2010;42(8):1273–1281.
14. Liang YJ, Wang QY, Zhou CX, et al. MiR-124 targets Slug to regulate epithelial–mesenchymal transition and metastasis of breast cancer. *Carcinogenesis*. 2013;34(3):713–722.

15. Sun Y, Ai X, Shen S, Lu S. NF-κB-mediated miR-124 suppresses metastasis of non-small-cell lung cancer by targeting MYO10. *Oncotarget*. 2015;6(10):8244–8254.
16. Wang X, Wu Q, Xu B, et al. miR-124 exerts tumor suppressive functions on the cell proliferation, motility and angiogenesis of bladder cancer by fine-tuning UHRF1. *FEBS J*. 2015;282(22):4376–4388.
17. Hu H, Wang G, Li C. miR-124 suppresses proliferation and invasion of nasopharyngeal carcinoma cells through the Wnt/β-catenin signaling pathway by targeting Capn4. *Onco Targets Ther*. 2017;10:2711–2720.
18. Liz J, Esteller M. lncRNAs and microRNAs with a role in cancer development. *Biochim Biophys Acta*. 2016;1859(1):169–176.
19. Yoon JH, Abdelmohsen K, Gorospe M. Functional interactions among microRNAs and long noncoding RNAs. *Semin Cell Dev Biol*. 2014;34(4):9–14.
20. Chai Y, Liu J, Zhang Z, Liu L. HuR-regulated lncRNA NEAT1 stability in tumorigenesis and progression of ovarian cancer. *Cancer Med*. 2016;5(7):1588–1598.
21. Jeong D, Kim J, Nam J, et al. MicroRNA-124 links p53 to the NF-κB pathway in B cell lymphomas. *Leukemia*. 2015;29(9):1868–1874.
22. Souquere S, Beauclair G, Harper F, Fox A, Pierron G. Highly ordered spatial organization of the structural long noncoding NEAT1 RNAs within paraspeckle nuclear bodies. *Mol Biol Cell*. 2010;21(22):4020–4027.
23. Qian K, Liu G, Tang Z, et al. The long non-coding RNA NEAT1 interacted with miR-101 modulates breast cancer growth by targeting EZH2. *Arch Biochem Biophys*. 2016;615:1–9.
24. Chakravarty D, Sboner A, Nair SS, et al. The oestrogen receptor alpha-regulated lncRNA NEAT1 is a critical modulator of prostate cancer. *Nat Commun*. 2014;5:5383.
25. Zhang Z, Zhu Z, Watabe K, et al. Negative regulation of lncRNA GAS5 by miR-21. *Cell Death Differ*. 2013;20(11):1558–1568.
26. Li X, Wang S, Li Z, et al. The lncRNA NEAT1 facilitates cell growth and invasion via the miR-211/HMGA2 axis in breast cancer. *Int J Biol Macromol*. 2017;105(Pt 1):346–353.
27. Sun C, Li S, Zhang F, et al. Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-E2F3 pathway. *Oncotarget*. 2016;7(32):51784–51814.
28. Sun C. Long non-coding RNA NEAT1 functions as a ceRNA to regulate E2F3 expression by sponging miR-377-3p in non-small cell lung cancer. *Mol Ther*. 2016;24(1):232.
29. Feng T, Xu D, Tu C, et al. MiR-124 inhibits cell proliferation in breast cancer through downregulation of CDK4. *Tumour Biol*. 2015;36(8):1–11.
30. Silber J, Lim DA, Petritsch C, et al. miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. *BMC Med*. 2008;6(1):14.
31. Xu S, Zhao N, Hui L, et al. MicroRNA-124-3p inhibits the growth and metastasis of nasopharyngeal carcinoma cells by targeting STAT3. *Oncol Rep*. 2016;35(3):1385–13
32. Peng XH, Huang HR, Lu et al. MicroRNA-124 suppresses tumor growth and metastasis by targeting Foxq1 in nasopharyngeal carcinoma. *Mol Cancer*. 2014;13(1):6.
33. Sun W, Guo MM, n P, et al. Id-1 and the p65 subunit of NF-κB promote migration of nasopharyngeal carcinoma cells and are correlated with poor prognosis. *Carcinogenesis*. 2012;33(4):810–817.
34. Zhao M Liu Y, et al. Oncogenic role of the TP53-induced glycolysis and apoptosis regulator in nasopharyngeal carcinoma through NF-κB pathway activation. *Int J Oncol*. 2016;48(2):756–764.
35. AK, Hua K, ipple W, et al. MiR-124 suppresses p62 and p65/NFkB to regulate autophagy, inflammation and cell death in KRAS mutant mesenchymal NSCLC cells. *Mol Cell Biol*. 2017; abstract 252