Silencing of IncRNA MIR31HG promotes nasopharyngeal carcinoma cell proliferation and inhibits apoptosis through suppressing the PI3K/AKT signaling pathway

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Abstract

Background: MIR31HG has been affirmed to regulate the tumorigenesis of head–neck squamous cell carcinoma (HNSC). This study aims to reveal the function of MIR31HG in nasopharyngeal carcinoma (NPC), which falls into the category of HNSC.

Methods: MIR31HG expression pattern in HNSC tissues was predicted by starBase. FISH and qRT-PCR were employed to detect MIR31HG expression in NPC tissues and to analyze the association between MIR31HG and clinicopathological features. NPC cell viability, colony formation, and apoptosis were measured by MTT assay, colony formation assay, and flow cytometry. The expressions of protein kinase B (AKT), phosphorylated (p)-AKT, phosphoinositide 3-kinases (PI3K) and p-PI3K in NPC cells were analyzed by Western blot. The correlation between MIR31HG expression and AKT1 mRNA expression was analyzed by The Cancer Genome Atlas and starBase.

Results: MIR31HG was highly expressed in HNSC tissues and NPC tissues. Meanwhile, the association between high MIR31HG expression and aggressive clinicopathological traits was significant in NPC patients at tumor stage III–IV (T3–T4) and in those with lymph node metastasis 1–2 (N1–N2). Silencing of MIR31HG suppressed NPC cell viability and colony formation, promoted apoptosis, and decreased the expressions of p-PI3K, and p-AKT. 740Y-P reversed the above effects of si-MIR31HG on NPC cells. Besides, MIR31HG expression was positively correlated with AKT1 mRNA expression in HNSC patients.

Conclusion: MIR31HG silencing promotes NPC cell proliferation and inhibits apoptosis through suppressing the PI3K/AKT signaling pathway.

KEYWORDS
apoptosis, MIR31HG, nasopharyngeal carcinoma, PI3K/AKT signaling pathway, proliferation

1 | INTRODUCTION

Head–neck squamous cell carcinoma (HNSC) becomes the sixth most common cancer around the world, and nasopharyngeal carcinoma (NPC) belongs to the category of HNSC. The pathogenesis of NPC is associated with multiple risk factors including Epstein–Barr virus infection, genetic susceptibility, and environmental stimulation. Recently, great improvement has been made in the prognosis of NPC patients, which is mainly attributed to the abundant treatment approaches. Locally recurrent NPC and some NPC with...
more advanced recurrences including rT3 and rT4 diseases can be treated by nasopharyngectomy, which delivers improved outcome with the advancements in endoscopy technologies. A single-center study involving 28 patients with NPC at rT3 or rT4 stage reported that combined craniofacial resection raises the 5-year overall survival to 52% with almost half of the patients exhibit clear resection margin. Nevertheless, declines in physical functions after reaction significantly reduce the life quality of patients. Additionally, induction chemotherapy improves the progression-free survival and overall survival of patients with locally advanced NPC, and immunotherapy produces favorable outcomes to the 1-year overall survival of patients with advanced recurrent or metastatic NPC.

However, approximately 30% NPC patients have poor prognosis due to distant metastasis. Therefore, physical function decline after resection, along with poor prognosis of metastatic NPC, prompts the need of seeking certain therapeutic approaches that can strangle the development of NPC at an early stage.

Long noncoding RNAs (lncRNAs) are nonprotein coding transcripts consisting of more than 200 nucleotides, residing in the fractions of nuclei or cytoplasm. Findings from cutting edge research confirmed that lncRNAs have significant associations with the occurrence and development of cancers. Several lncRNAs such as HOTAIR, AFAP1-AS1, and LOC401317 are discovered to mediate NPC tumor growth and angiogenesis, affect the prognosis of NPC patients or regulate NPC cell proliferation and apoptosis. Recently, MIR31HG, an lncRNA with the length of 2166 nucleotides, has attracted increasing interest of many scholars, given the fact that it can function as an oncogenic factor in various cancers including osteosarcoma, nonsmall cell lung cancer (NSCLC), hepatocellular carcinoma, pancreatic ductal adenocarcinoma and breast cancer. Of note, MIR31HG is also found to promote HNSC cell proliferation and tumorigenesis through regulating cell cycle progression. Moreover, MIR31HG overexpression is related to shorter overall survival in HNSC. However, the detailed role of MIR31HG in NPC remains undefined.

There are some pathways have been demonstrated to be involved in the progression of NPC, such as Ras/Raf/MEK/ERK and Wnt/β-catenin, especially phosphoinositide 3-kinases (PI3K)/protein kinase B (AKT). YBX3 regulates the metastasis of NPC via PI3K/AKT pathway. ADORA1 promotes NPC progression through regulation of PI3K/AKT/GSK-3β/β-catenin pathway. MicroRNAs, including MicroRNA-29 and MicroRNA-124-3p, inhibit the progression of NPC via PI3K/AKT pathway. Moreover, a previous study has demonstrated that turning off MIR31HG on the EGFR/PI3K/AKT signaling pathway by silencing MIR31HG shows enhancement in NSCLC cell apoptosis, and inhibition in proliferation and cell cycle, indicating that the regulation on the PI3K/AKT signaling pathway acts as a mechanism by which MIR31HG modulates the proliferation and apoptosis of some cancer cells.

This study is herein committed to defining the role of MIR31HG in NPC, and investigating how the EGFR/PI3K/AKT signaling pathway is involved in MIR31HG-regulated phenotype changes of NPC cells.

## 2 MATERIALS AND METHODS

### 2.1 Ethics statement

The study obtained the approval of the Ethics Committee of The Affiliated Hospital of Medical School of Ningbo University in 2019. Histological confirmation on NPC at different stages (T1-T2, T3-T4, N0 and N1-N2) was conducted according to the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) cancer staging system (8th edition). Prior to the sample collection, no any treatment was reported to be applied on these patients. The harvested tissues were instantly frozen in liquid nitrogen at −80°C before being subjected to qRT-PCR analysis. The correlation between MIR31HG expression and clinicopathological features of the NPC patients was presented in Table 1.

### 2.2 Clinical sample

Nasopharyngeal carcinoma tumor tissues and the corresponding normal tissues in pairs were harvested from 50 NPC patients diagnosed at The Affiliated Hospital of Medical School of Ningbo University in 2019. Histological confirmation on NPC at different stages (T1-T2, T3-T4, N0 and N1-N2) was conducted according to the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) cancer staging system (8th edition). Prior to the sample collection, no any treatment was reported to be applied on these patients. The harvested tissues were instantly frozen in liquid nitrogen at −80°C before being subjected to qRT-PCR analysis. The correlation between MIR31HG expression and clinicopathological features of the NPC patients was presented in Table 1.

| Characteristics | IncRNA MIR31HG expression | p Value |
|-----------------|---------------------------|--------|
| Age(years)      |                           |        |
| <60             | 12                        | 9      | 0.536 |
| ≥60             | 14                        | 15     |       |
| Drinking        |                           |        |
| Yes             | 10                        | 6      | 0.308 |
| No              | 16                        | 18     |       |
| Smoking(y×p)    |                           |        |
| <400            | 14                        | 8      | 0.144 |
| ≥400            | 12                        | 16     |       |
| T-category      |                           |        |
| T1-T2           | 12                        | 4      | 0.026 |
| T3-T4           | 14                        | 20     |       |
| Lymph node metastasis |                  |        |
| N0              | 22                        | 10     | 0.002 |
| N1-N2           | 4                         | 14     |       |
| Differentiation |                           |        |
| Well            | 9                         | 8      | 0.924 |
| Moderate-to-Poor| 17                        | 16     |       |
2.3 | Bioinformatics analyses

MIR31HG expression pattern in HNSC was analyzed by starBase (http://starbase.sysu.edu.cn/index.php). The correlation between MIR31HG expression and AKT mRNA expression was analyzed by The Cancer Genome Atlas database (TCGA). The Kaplan–Meier survival curve analysis of MIR31HG in HNSC was performed using UALCAN database (http://ualcan.path.uab.edu/index.html).

2.4 | Fluorescence in situ hybridization (FISH)

An RNA FISH kit (Gene Pharma) was used for analyzing MIR31HG expression in NPC based on the manufacturer's instructions. Shortly, tissue sections were dewaxed, treated with protease K and preheated, followed by being hybridized with MIR31HG FISH probes at 37°C for 12h. Thereafter, the tissue sections were washed for 15 minutes (min) and incubated with 4,6-diamidino-2-phenylindole (DAPI) for staining of nuclei. The images were captured using a TCS SP8 laser scanning confocal microscope (Leica).

2.5 | Cell culture and treatment

Human NPC cell lines, C666-1 cells and HK1 cells were ordered from Cell Line Resource Center of Central South University. Both of the cells were cultured in RPMI 1640 Media (A4192301, ThermoFisher) supplemented with 10% fetal bovine serum (FBS, F2442, Sigma-Aldrich), 100 U/ml penicillin (P3032, Sigma-Aldrich) and 100 μg/ml streptomycin (S9137, Sigma-Aldrich) at 37°C with 5% CO₂. After cell transfection, the cells were incubated with 740Y-P (a PI3K agonist, 10 μM, HY-P0175, MedChemExpress) at 37°C with 5% CO₂ for 24h. 2.6 | Cell transfection

Small interfering RNA (siRNA) targeting MIR31HG (si-MIR31HG, siB171206021220-1-5) and its negative control (si-NC, siN0000002-1-5) was purchased from RiboBio. Both C666-1 cells and HK1 cells were transfected with si-MIR31HG and si-NC by Lipofectamine 3000 transfection reagent (L3000015, ThermoFisher). Briefly, cells (1 × 10⁴ cells/well) were seeded in 96-well plates to reach 80% confluence. Lipofectamine 3000 transfection reagent, si-MIR31HG (0.2 μg) and si-NC (0.2 μg) were diluted with Opti-MEM medium (31,985,062, ThermoFisher), followed by incubation together at 37°C for 10 min. Then, the cells were added with P3000 reagent and gene-lipid complex, and incubated with the diluted 3000 transfection reagent and siRNA at 37°C for 48h.

2.7 | Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNAs from NPC tumor tissues as well as C666-1 cells and HK1 cells were isolated by Trizol reagent (15596026, ThermoFisher). The total RNAs were stratified by chloroform (151858, Sigma-Aldrich), precipitated by isopropanol (I9516, Sigma-Aldrich), washed by 75% ethanol (E7023, Sigma-Aldrich) and resuspended in RNase-free water (10977023, ThermoFisher). The cDNAs were synthesized from the total RNAs by SuperScript IV reverse transcriptase (18090010, ThermoFisher), followed by determination adopting a PCR detection system (CFX Connect, Bio-Rad) with PowerUp SYBR Green Master Mix (A25742, ThermoFisher). The primers used were as follows: MIR31HG forward primer: 5′-AGTTTGTGGCCCAGTTTCCA-3′ and reverse primer: 5′-CACCCTCAGTATGATCC-3′. The reaction was initiated at 95°C for 10 min, and 40 circles of 95°C for 15 s and 60°C for 60s. The relative gene expressions were estimated by 2^ΔΔCt method and normalized to GAPDH.

2.8 | MTT assay

C666-1 cells and HK1 cells were washed by phosphate buffer solution (PBS, P3619, Sigma-Aldrich) and detached by Trypsin (T1426, Sigma-Aldrich). After being transfected with si-MIR31HG or si-NC and with or without being incubated with 740Y-P, the cells were inoculated in 96-well plates at a density of 1 × 10⁴ cells/well, and then incubated with MTT solution (V900888, Sigma-Aldrich) (20 μl) at 37°C for 4 h at day 1, day 2 and day 3. Later, dimethyl sulfoxide (100 μl) (D2650, Sigma-Aldrich) was added to the cells. The absorbance at 570nm was measured by a microplate reader (ELx808, BioTek).

2.9 | Colony formation assay

With or without incubation with 740Y-P, C666-1 cells and HK1 cells transfected with si-MIR31HG or si-NC were detached by Trypsin and then seeded in six-well plates at a density of 1 × 10⁵ cells per well. The cells were allowed to clone at 37°C for 2 weeks. After PBS washing, the cells were fixed in 4% paraformaldehyde (P6148, Sigma-Aldrich) for 15 min and stained with 0.1% crystal violet (C0775, Sigma-Aldrich) for 10 min. The number of the colonies in four randomly selected six fields was counted by a microscope (IX71; Olympus) under 100× magnification.

2.10 | Annexin V/propidium iodide (PI) staining assay

C666-1 cells and HK1 cells with or without incubation with 740Y-P and the transfection with si-MIR31HG or si-NC were digested by Trypsin, centrifugated at 1000g for 5 min, and washed by precooled phosphate buffer saline (PBS, P5493, Sigma-Aldrich). Then, the cells were centrifuged at 2000g for 5 min twice with PBS washing at each end of the centrifugation. Cell apoptosis was measured by the Annexin V-FITC/PI apoptosis detection kit (40302ES20, Yeasen, http://www.yeasen.com/products/detail/944). Briefly, the cells were adjusted to a concentration of 1 × 10⁶/ml with 1× Binding Buffer and double stained with Annexin V-FITC solution (5 μl) and PI
solution (10 μl) at room temperature for 10 min in the dark. Later, cell apoptosis was determined by a flow cytometer (Cytoflex, Beckman Coulter) and analyzed by CytExpert software (Version 2.2.0.97, Beckman Coulter).

2.11 Western blot

Processed C666-1 cells and HK1 cells were lysed by RIPA Buffer (89,900, ThermoFisher) blended with a Protease-Phosphatase inhibitor (A32959, ThermoFisher). The concentration of total protein lysate was determined using the BCA kit (A53227, ThermoFisher). The total protein (45 μg) and marker (5 μl) (PR1910, Solarbio) were separately loaded, separated by 10% SDS-PAGE gel (P0670, Beyotime), and transferred onto PVDF membranes (P2438, Sigma-Aldrich). Then, the membranes were blocked by 5% skim milk in Tris Buffered Saline with 1% Tween 20 (TBST, TA-125-TT, ThermoFisher) at room temperature for 1 h. Afterwards, the membranes were incubated at 4°C overnight with primary antibodies against AKT (rabbit, ab8805, 55kDa, 1:500, Abcam), phosphorylated (p)-AKT (rabbit, ab38449, 56kDa, 1:500, Abcam), PI3K (rabbit, #4292, 85kDa, 1:1000, Cell signaling technology), p-PI3K (rabbit, #4228, 85kDa, 1:1000, Abcam), and GAPDH (mouse, ab8245, 36kDa, 1:10000, Abcam). After TBST washing, the membranes were incubated with secondary antibody Goat antiRabbit IgG (A32731, 1:10000, ThermoFisher) or Goat antiMouse IgG (A32733, 1:1000, ThermoFisher). The protein bands were visualized using the enhanced chemiluminescence reagent (WP20005, ThermoFisher) and analyzed using ImageJ software (1.52s version, National Institutes of Health). All protein expressions were normalized to GAPDH.

2.12 Statistical analysis

Measurement data from triplicate experiments were expressed as mean± standard deviation. Statistical analysis was realized using GraphPad prism (version, 8.0, GraphPad Software Inc.). One-way analysis of variance was used to compare the differences among multiple groups, followed by Tukey’s post hoc test. Student-t test was used for comparison between two groups. p <0.05 was considered to be statistically significant.

3 RESULTS

3.1 High MIR31HG expression in HNSC tissues and NPC tissues indicated aggressive clinicopathological traits

Prediction from starBase indicated that MIR31HG is highly expressed in HNSC relative to that in the normal tissues (Figure 1A, p = 0.012). No relevance was observed between the high expression of MIR31HG and the poor overall survival of NPC patients (Figure 1B). FISH analysis revealed that MIR31HG expression level was higher in NPC tissues than that in the corresponding normal adjacent tissues (Figures 1C), which was further verified by qPCR in fresh NPC samples (n = 50) and normal adjacent tissues (n = 50) (Figure 1D, p < 0.001). Subsequently, further analyses were conducted on the correlation between the clinicopathological features of NPC patients and MIR31HG expression. It turned out that NPC patients at T3-T4 exhibited a higher MIR31HG expression level than patients at T1-T2. Meanwhile, MIR31HG expression, presenting no obvious association with patients’ age, drinking, smoking or tumor subtype, was increased in NPC patients with N1-N2, as compared with that in NPC patients with N0 (no lymph node metastasis) (Table 1 and Figure 1C, p < 0.01). These findings suggested that high MIR31HG expression was associated with enhanced aggressiveness of NPC.

3.2 Si-MIR31HG repressed NPC cell viability and colony formation, but promoted apoptosis

To figure out whether downregulated MIR31HG could lead to the inhibited aggressiveness of NPC, the transfection of si-MIR31HG into C666-1 cells and HK1 cells was carried out. As depicted in Figure 2A, MIR31HG expression was successfully decreased (p < 0.001). Then, MTT assay, colony formation assay and flow cytometry were performed, the results of which uncovered that MIR31HG silencing decreased C666-1 and HK1 cell viability at day 2 and day 3, inhibited colony formation, but promoted apoptosis (Figure 2B–D, p < 0.05, p < 0.001). These observations suggested that decreasing MIR31HG expression suppressed NPC cell viability and proliferation, yet promoted apoptosis.

3.3 740Y-P reversed the inhibitory effect of si-MIR31HG on the phosphorylation of PI3K and AKT

A previous study has disclosed that MIR31HG overexpression can modulate EGFR/PI3K/AKT signaling pathway, thereby affecting NSCLC cell proliferation and apoptosis. Thus, it is reasonable to hypothesize that MIR31HG silencing-caused changes in NPC cell phenotypes may result from the regulation on the PI3K/AKT signaling pathway. For finding evidence to support this hypothesis, 740Y-P, a PI3K agonist, was used to incubate si-MIR31HG-transfected NPC cells. Western blot analyses showed that MIR31HG silencing significantly decreased the protein expression levels of p-AKT and p-PI3K as well as the ratios of p-AKT to AKT and p-PI3K to PI3K in C666-1 cells and HK1 cells, while treatment with 740Y-P explicitly reversed the repressive impacts of MIR31HG silencing upon above aspects (Figure 3A–D, p < 0.001). However, neither si-MIR31HG-transfected NPC cells nor si-MIR31HG-transfected NPC cells undergoing treatment of 740Y-P showed any changes in the protein expression levels of PI3K and AKT.
FIGURE 1  High MIR31HG expression in HNSC tissues and NPC tissues indicated aggressive clinicopathological traits. (A) MIR31HG expression pattern in HNSC tissues and normal tissues was predicted by starBase (http://starbase.sysu.edu.cn/index.php). (B) The effect of MIR31HG expression on the survival curve of HNSC patients. (C) FISH analyses on MIR31HG expression in the NPC pathological tissues and normal adjacent tissues. Scale bar, 100 μm. (C) MIR31HG expression in NPC tumor tissues and the corresponding normal tissues was analyzed by qRT-PCR. (D) MIR31HG expression in NPC tumor tissues at T-category and tissues at lymph node metastatic stage was analyzed by qRT-PCR. (HNSC: Head–neck squamous cell carcinoma; NPC: nasopharyngeal carcinoma; qRT-PCR: quantitative reverse transcription polymerase chain reaction). **p or ***p < 0.01; + versus Normal; * versus T1-T2, # versus N0.
Figure 2  Si-MIR31HG repressed NPC cell viability and colony formation, while promoting apoptosis. (A) MIR31HG expression in si-MIR31HG or si-NC-transfected C666-1 cells and HK1 cells was analyzed by qRT-PCR. (B) The viability of si-MIR31HG- or si-NC-transfected C666-1 cells and HK1 cells was measured by MTT assay at day 1, day 2 and day 3. (C) The colony-forming ability of si-MIR31HG or si-NC-transfected C666-1 cells and HK1 cells was assessed by colony formation assay. (D) The apoptosis of si-MIR31HG or si-NC-transfected C666-1 cells and HK1 cells was measured by flow cytometry. (NPC: nasopharyngeal carcinoma; qRT-PCR: quantitative reverse transcription polymerase chain reaction; si-MIR31HG: small interfering RNA targeting MIR31HG; si-NC: siRNA-negative control) *p < 0.05; **p < 0.001; * versus si-NC.

Figure 3  740Y-P reversed the inhibitory effect of si-MIR31HG on the phosphorylation of PI3K and AKT. (A, B). The protein expressions of AKT, p-AKT, PI3K and p-PI3K in si-MIR31HG or si-NC-transfected C666-1 cells and HK1 cells incubated with or without 740Y-P were analyzed by Western blot, with GAPDH serving as a reference gene. (AKT: protein kinase B; NPC: nasopharyngeal carcinoma; PI3K: phosphoinositide 3-kinases; si-MIR31HG: small interfering RNA targeting MIR31HG; si-NC: siRNA-negative control; p: phosphorylated). ***p or ###p < 0.001; * versus si-NC, # versus si-MIR31HG.
3.4 | Si-MIR31HG impeded NPC cell viability and colony formation while promoting apoptosis via the PI3K/AKT signaling pathway

Furthermore, the results of MTT assay, colony formation assay and flow cytometry showed that treatment with 740Y-P remarkably reversed the effects of MIR31HG silencing on decreasing C666-1 and HK1 cell viability at day 2 and day 3, inhibiting colony formation, and augmenting apoptosis (Figure 4A–C, p < 0.05, p < 0.01, p < 0.001). To further determine the involvement of AKT activation, the correlation between MIR31HG and AKT in NPC patients was analyzed by TCGA database. The result illustrated that MIR31HG expression was positively correlated with AKT expression in NPC patients (Figure 4D). These results collectively suggested that the effects of MIR31HG silencing on NPC cells may be achieved by the suppression of the PI3K/AKT signaling pathway.

4 | DISCUSSION

Nasopharyngeal carcinoma demonstrates the strongest metastatic potential among all head and neck cancers.36 The incidence of metastasis is around 5% at initial diagnosis, while during the follow-up period, the rate of recurrence and/or metastasis in patients with locally advanced NPC soars to 15% – 30%.37 Moreover, the overall survival of NPC patients with synchronous metastases is hardly longer than 30 months, and the patients with metastatic diseases can only survive for around 20 months.38 Therefore, early diagnosis and containment of NPC tumor growth are critical to improving the prognosis of NPC patients.

Current prediction of NPC prognosis mainly resorts to the clinical TNM staging.39 However, due to the observations of different clinical outcomes in the NPC patients at the same stage, the TNM stage appears to be not precise enough to predict the prognosis of NPC.40 Accumulating evidence has proved the implication of molecular targeted therapies in NPC.40 The surveillance on the level change of molecular biomarkers can help patients be aware of the carcinogenesis of NPC at an earlier time, with which the subgroups of NPC could be classified more precisely.41 Furthermore, targeting these molecular biomarkers whose levels are abnormally changed contributes to combating and eradicating NPC tumor cells.42

LncRNAs belong to the series of molecular biomarkers that play vital roles in the diagnosis, prognosis and potential treatment of NPC.33 A wide range of lncRNAs has been detected to display abnormal expression patterns in NPC.43 Recent researches have also reported that lncRNA-PVT1, lncRNA-FAM225A and lncRNA-ZFAS1 expressions were all upregulated in NPC and were associated with reduced radio-sensitivity for treatment or poor survival of NPC patients.44–46 The prediction of starBase uncovered that MIR31HG was highly expressed in HNSC, which guided us to make a speculation that MIR31HG may also be highly expressed in NPC and indicated the poor prognosis of NPC patients. Our results showed that NPC tissues presented high MIR31HG expression. More intriguingly, we found high MIR31HG expression in the locally advanced NPC and lymph node metastatic NPC. As these hints signified, high MIR31HG expression is associated with highly malignant local lesion and lymph node metastasis, which is analogous to that in laryngeal squamous cell cancer.42

Since upregulation of MIR31HG indicates poor prognosis in NPC, inhibition of MIR31HG may deliver a tumor-suppressing effect. As reported in Wu’s study, knockdown of lncRNA-ANRIL results in inhibited NPC cell proliferation in vitro and in vivo.47 The study of Shi et al. showed that lncRNA XIST exhibits upregulated expression in NPC, and its inhibition suppresses NPC cell proliferation, migration and invasion, and meanwhile promotes apoptosis, significantly slowing NPC tumor growth.48 It has been elucidated that lncRNAs can participate in the containment of cancer progression by triggering apoptosis, a vital biological process of cancer cells.49 Our studies demonstrated that consistent with the effects delivered by inhibition of the above mentioned lncRNAs, MIR31HG silencing pronouncedly decreased NPC cell viability, inhibited colony formation, and promoted apoptosis, thereby restraining NPC cell progression.

A previous study, set out for investigating the mechanism by which MIR31HG affects gefitinib resistance in NSCLC, has revealed that the suppression of the EGFR/PI3K/AKT signaling pathway explains MIR31HG knockdown-induced cell proliferation inhibition, cell cycle arrest, apoptosis promotion and increased cell sensivity to gefitinib,51 emphasizing the involvement of the PI3K/AKT signaling pathway in MIR31HG-related antitumor activities. Meanwhile, Tang et al. showed that enhanced PI3K/AKT signaling cooccurs with promoted NPC cell proliferation and colony formation, and overexpressing activated AKT can reverse the inhibition on NPC cell growth.50 Indeed, activation of PI3K/AKT signaling pathway has been confirmed to promote cancer progression long before these above studies.51 In line with these findings, our results revealed that levels of p-AKT and p-PI3K, together with ratios of p-AKT to AKT and p-PI3K to PI3K, were diminished in both C666-1 cells and HK1 cells after silencing MIR31HG, indicating that MIR31HG silencing suppressed PI3K/AKT signaling pathway in NPC. Meanwhile, MIR31HG silencing-induced repression of PI3K/AKT signaling pathway was accompanied by NPC cell progression inhibition and apoptosis promotion. Notably, in a previous study, the PI3K/AKT signaling-related mechanism has been proposed to be mediated by MIR31HG knockdown for regulating cancers,52 which is also valid in NPC here. Subsequent analyses using TCGA database showed a positive correlation between MIR31HG expression and AKT1 mRNA expression in HNSC patients, which further supported the validity of this MIR31HG-mediated mechanism in NPC. Additionally, the competitive endogenous RNA (ceRNA) theory between miRNA and lncRNA proposed by Salmena et al.52 is currently widely prevalent. LncRNA can function as a miRNA sponge via ceRNA activity, thereby reducing the inhibitory effects of miRNAs on their targeted mRNAs. In a recent study, lncRNA MIR31HG has been proved to ameliorate rheumatoid arthritis fibroblast-like synoviocyte-mediated inflammation via miR-214-PTEN-AKT signaling pathway.53 We would continue to carry out in-depth work in...
the future, and a ceRNA network (lncRNA-miRNA-mRNA) could be constructed through bioinformatics analysis to explore the downstream target genes of this lncRNA.

In conclusion, we demonstrate that MIR31HG is highly expressed in NPC, and associated with unfavorable clinicopathological features. Through analyses on dysregulated MIR31HG, we discover that MIR31HG silencing leads to the decrease in NPC cell viability, inhibition on colony formation and promotion on apoptosis through suppressing the PI3K/AKT signaling pathway, which uncovers the carcinogenesis of NPC and provides a novel therapeutic target for patients with NPC.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

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