

Down-regulation of miR-1181 indicates a dismal prognosis for nasopharyngeal carcinoma and promoted cell proliferation and metastasis by modulating Wnt/ β -catenin signaling

X. HUA¹, K.-C. FAN²

¹Department of ENT, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China

²Department of ENT, The 2nd Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China

Abstract. – OBJECTIVE: Our study aimed to investigate the expression pattern, clinicopathological feature and prognostic role of miR-1181 in nasopharyngeal carcinoma (NPC), and to determine the functional effects and potential mechanism of miR-1181 in NPC.

PATIENTS AND METHODS: The expression levels of miR-1181 were determined in NPC tissues and cell lines by RT-PCR. The clinical data were interpreted by chi-square test, univariate analysis, and multivariate analysis. The effect of PVT1 on proliferation was evaluated by CCK-8 and colony formation assays, and migration and invasion ability were evaluated by transwell and wound-healing assays. The association between miR-1181 and Wnt/ β -catenin pathway was analyzed by Western blot.

RESULTS: We found that miR-1181 expression was significantly down-regulated in both NPC tissues and cell lines. Low expression of miR-1181 was significantly associated with N stage ($p=0.013$), clinical stage ($p=0.037$) and grade ($p=0.033$). Clinical assays showed that patients with low miR-1181 expression had shorter overall survival time than those with high miR-1181 expression ($p=0.0007$). Multivariate analysis revealed that miR-1181 expression was independently associated with the overall survival. Functional investigations indicated that overexpression of miR-1181 suppressed NPC cells proliferation, migration and invasion. Mechanistically, forced miR-1181 expression suppressed the activity of Wnt/ β -catenin pathway.

CONCLUSIONS: Our findings proved that miR-1181 may serve as a candidate prognostic biomarker and target for new therapies in NPC patients.

Key Words

miR-1181, Wnt/ β -catenin pathway, Nasopharyngeal carcinoma, Prognosis, Cell metastasis.

Introduction

Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma derived from epithelial cells lining the nasopharynx, which is frequent in

Southern China, with an incidence of 15-25 per 100,000 people^{1,2}. NPC is closely associated with several factors, such as environmental factors, genetic susceptibility and Epstein-Barr virus (EBV) infection³. Due to its more sensitivity to radiation compared with some other cancers, NPC can be successfully treated if the tumor is locally confined to the nasopharynx at diagnosis⁴. However, 30-40% of NPC patients will develop distant metastasis⁵. Local recurrence and distant metastasis are the important reasons of treatment failure⁶. Thus, exploring potential mechanism of NPC progression and developing novel treatments are required to improve the prognosis for patients with NPC. MicroRNAs (miRNAs) are short noncoding RNAs of approximately 22 nucleotides in length that function in posttranscriptional gene regulatory pathways⁷. It has been confirmed by increasing evidence that miRNAs are closely involved in the regulation of diverse cellular processes including developmental timing, signal transduction, tissue differentiation and apoptosis^{8,9}. Recent studies¹⁰⁻¹² show that miRNAs are involved in the tumorigenesis and progression of almost all tumors, where some can function as tumor promoters or anti-oncogenes according to cells situations. These findings may provide new insights into the molecular mechanisms underlying nasopharyngeal carcinogenesis. MiR-1181 was a recent identified miRNA, which had been reported to be dysregulated in several tumors, such as pancreatic cancer, ovarian cancer and breast cancer¹³⁻¹⁵. However, the expression pattern and potential function of miR-1181 in NPC have not been reported. Wnt/ β -catenin signaling are important in developmental processes, cell growth and differentiation, and they have been confirmed to be involved in the regulation of various tumor progression¹⁶. The epithelial-mesenchymal transition (EMT) is an essential step in invasion and metastasis of human cancers. It has

been confirmed that dysregulation of Wnt/ β -catenin signaling induce EMT¹⁷. However, it remains unclear whether and how other factors are involved in the metastasis of NPC by modulating the Wnt/ β -catenin pathway. In this study, we used bioinformatics and RT-PCR to explore whether miR-1181 was abnormally expressed in NPC, and also studied the prognostic value of miR-1181 in NPC patients. In addition, we conducted *in vitro* assays to explore the potential biological functions of miR-1181 in the proliferation and metastasis of NPC cells. Finally, we attempted to study the potential mechanism of miR-1181 in progression of NPC cells by exploring the association between miR-1181 and Wnt/ β -catenin pathway.

Patients and Methods

Patients and Tissue Samples

Human NPC specimens and corresponding normal tissues were obtained from 143 nasopharyngeal carcinoma patients who were received surgical resection without additional treatments in the Affili-

ated Hospital of Xuzhou Medical University from March 2010 to July 2013. Before surgery, all the patients did not receive any antitumor treatment. The clinical information of the patients with NPC was shown in Table I. This study was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University, and written informed consents were obtained from all patients.

Cell Lines and Cell Transfection

The CNE-2, HONE-1, SUNE-1, HNE-1, C666-1 and CNE-1 cells (NPC cell lines), and NP69 cells (immortalized human nasopharyngeal epithelial cells) which were cultured using RPMI-1640 medium (Yaji Biotech, Minhang, Shanghai, China), were all purchased from Wuhan Procell Co., Ltd. (Wuhan, Hubei, China). For cell transfection, a PepMute transfection kit (SignaGen Biotech, Jinan, Shandong, China) was applied in accordance with the protocols provided by the manufacture. The miR-1181 mimics and negative control miRNA mimics (NC mimic) were all purchased from IGE Biotechnology Co., Ltd. (Guangzhou, Guangdong, China).

Table I. miR-1181 expression in different clinical variables of NPC tissues.

Variable	No. of patients (n)	miR-1181 expression		p-value
		Low	High	
Gender				NS
Male	83	43	40	
Female	60	27	33	
Age				NS
<60	69	31	38	
≥60	74	39	35	
T stage				NS
T1-T2	82	36	46	
T3-T4	61	34	27	
N stage				0.013
N0-N1	94	39	55	
N2-N3	49	31	18	
M stage				NS
M0	86	43	43	
M1	57	27	30	
Clinical stage				0.037
I-II	88	37	51	
III-IV	55	33	22	
Grade				0.033
G1-G2	96	41	55	
G3	47	29	19	
EBV infection				NS
Y	121	59	62	
N	22	11	10	

Real-Time PCR Assays

TRIzol reagent (IGE Biotech, Guangzhou, Guangdong, China) was used to extract the total RNAs. Subsequently, the cDNA synthesis and qRT-PCR detection for mRNA levels of β -catenin, cyclin D1 and c-myc were conducted using Plexor One-Step qRT-PCR System kit (ZeYe Biotech, Jinshan, Shanghai, China). For miR-1181 detection, the YRBIO miRNA qPCR Detection kit (YRBIO, Changsha, Hunan, China) was used. GAPDH was used as internal of β -catenin, cyclin D1 and c-myc mRNA detection, and U6 was utilized as internal control of miR-1181 expression. The fold changes of β -catenin, cyclin D1 and c-myc and miR-1181 were calculated by $2^{-\Delta\Delta Ct}$ method. All the primers used in this research were exhibited in Table II.

Western Blot Assays

The miR-1181 mimic-transfected CNE1 and SUNE1 cells were washed using ice-cold PBS and lysed by a protein extraction kit (ZeYe Biotech, Jinshan, Shanghai, China). Afterwards, the proteins were separated by 8-12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently transferred to Millipore (Billerica, MA, USA) polyvinylidene difluoride (PVDF) membranes (RenoldBio, Suzhou, Jiangsu, China), following being blocking with 5% skim milk. After incubating with primary antibodies for 12 h at 4°C and corresponding secondary antibodies, the proteins were measured by a Bio-Techne FluoChem E system (Minneapolis, MN, USA) using a SignalFire ECL reagent (BN Biotech, Jinshan, Shanghai, China). The primary antibodies used in this research were as follows: anti-N-cadherin antibody (Santa Cruz Biotech, Santa Cruz, CA, USA), anti-vimentin antibody (Protein Tech Group, Wuhan, Hubei, China), anti-cyclin D1 antibody (Protein Tech Group, Wuhan, Hubei, China), anti-c-myc antibody (Santa Cruz Biotech, Santa Cruz, CA, USA), anti- β -catenin antibody (Bostor, Wuhan, Hubei, China), anti-GAPDH antibody (Protein Tech Group, Wuhan, Hubei, China).

Table II. The sequences of primers used in this study.

Name	Sequence
miR-1181: Forward	5'-GCCGAGCCGTCGCCGCCA-3'
miR-1181: Reverse	5'-CTCAACTGGTGTCGTGGA-3'
U6: Forward	5'-CTCGCTTCGGCAGCACA-3'
U6: Reverse	5'-AACGCTTCACGAATTTGCGT-3'

Cell Counting Kit-8 (CCK-8) and Colony Formation Assays

The cell growth curve was evaluated by a CCK-8 Cell Proliferation detection kit (XuanLing Biotech, Jinshan, Shanghai, China). In brief, the miR-1181 transfected CNE1 or SUNE1 cells were planted into 96-well plates, and then examined at 24 h, 48 h, 72 h and 96 h, using a HS-9602 microreader system (HuiSi Technology, Xingtai, Hebei, China) after 10 μ l CCK-8 solution was added into each well. For cell colony formation assays, crystal violet (0.1%; Ruixi Biotech, Xi'an, Shanxi, China) was utilized to stain the colonies after the treated CNE1 or SUNE1 cells (500 cells/well) were cultured in 6-well plates for about 2 weeks. Then, an XSP-63XA microscope (Teelen, Jiading, Shanghai, China) was applied to observe the colonies.

Flow Cytometry Analysis

The cell apoptosis was evaluated by a flow cytometry apoptosis assay kit (BoGoo Biotech., Jiading, Shanghai, China). In short, after the CNE1 or SUNE1 cells were treated with miR-1181 for 24-36 h, the medium was removed and the cells were collected, following being resuspended (at a density of 5×10^6 cells/ml) in binding buffer containing Annexin V-FITC and propidium iodide. After incubating for 10-15 min in the dark at room temperature, these cells were collected and evaluated using a BD Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

EdU Assays

We also examined the cell proliferation using EdU assays. Briefly, CNE1 or SUNE1 cells were planted into in 48-well plates, and transfection of miR-1181 mimics into the cells was then conducted as described above when the cells reached to proper cell confluence. After 48 h, 5-ethynyl-2'-deoxyuridine (EdU; 100 μ l, 50 μ M) included in the EdU detection kit (Ribobio Biotech, Guangzhou, Guangdong, China) was added into each well. After culturing for 2-3 h, the cell nuclei were then stained with DAPI (1 μ g/mL) for 15 min. Finally, an XSP-63XA microscope (Teelen, Jiading, Shanghai, China) was applied to observe the florescence.

Wound Healing Assays

The CNE1 or SUNE1 cells after treatment with miR-1181 mimics were planted in 12-well plates, and continued to grow to about 90% cell confluence, followed by generating an artificial

wound by a 200 μ l pipette tip. The wounded areas were then observed using an XSP-63XA microscope (Teelen, Jiading, Shanghai, China) at 0 h and 48 h.

Transwell Invasion Assay

The CNE1 or SUNE1 cells were firstly transfected with miR-1181 mimics as described above. Subsequently, about 4×10^4 cells suspended in medium without serum were transferred into the upper well of the Corning Costar transwell apparatus (KatimesBio, Hangzhou, Zhejiang, China), which were pre-treated with Matrigel. Meanwhile, medium containing 15% fetal bovine serum (FBS), as chemo-attractant, was added into the lower well. After 24 h, crystal violet (0.1%; Ruixi Biotech, Xi'an, Shanxi, China) was applied to stain the transmembrane cells, and the cells were photographed using an XSP-63XA microscope (Teelen, Jiading, Shanghai, China).

Statistical Analysis

Results in this research were analyzed using the Statistical Package version 19.0 (IBM, Armonk, NY, USA). The Student's *t*-test (for 2 groups) or one-way ANOVA (for more than 2 groups) was employed for the comparisons between quantitative variables. We applied the Kaplan-Meier method with the log-rank test to evaluate the overall survival (OS) between these 2 groups. A Cox's regression model was used for univariate and multivariate analysis. A value of $p < 0.05$ was regarded as a statistically significant difference for comparisons between groups.

Results

The Expression of miR-1181 was Down-Regulated in NPC Tissues and Cell Lines

In order to identify dysregulated miRNAs in NPC, we firstly analyzed miRNAs profiles using microarray gene profiling data from GEO (GSE32960). Hierarchical clustering showed systematic variations in the expression of miRNAs between NPC and normal tissue samples (Figure 1A). Then, we observed that miR-1181 expression was significantly down-regulated in NPC tissues compared to normal tissues according to data from GSE32960 ($p < 0.01$, Figure 1B). In order to confirm these results, we performed RT-PCR to detect whether miR-1181 was abnormally expressed in NPC tissues from 143 patients at our hospital. As shown in Figure 1C,

the results showed that miR-1181 was markedly downregulated in NPC samples when compared with that in the adjacent normal tissues ($p < 0.01$). In addition, we also detected the expression of miR-1181 in NPC cell lines using RT-PCR, finding that miR-1181 was lowly expressed in all six BC cell lines (Figure 1D). Taken together, our findings revealed that miR-1181 was lowly expressed in NPC and may play a functional effect in progression of NPC. Because SUNE-1 and CNE-1 displayed a relatively lower expression in all cell lines, we chose them for subsequent cells experiments.

MiR-1181 Expression Was Negatively Associated with Prognosis Of Patients with NPC

In order to explore the clinical significance of miR-1181 in NPC patients, we further divided 143 NPC patients to a high-expression group ($n=73$) and a low expression group ($n=70$) according to the median value of miR-1181 expression levels. As shown in Table I, the results of chi-square test showed that high expression of miR-1181 was significantly associated with advanced N stage ($p=0.0013$), clinical stage ($p=0.037$) and grade ($p=0.033$). However, there was no association between miR-1181 expression and other clinical factors. Then, we performed Kaplan-Meier assays to explore the prognostic value of miR-1181 in NPC patients, finding that patients with low miR-1181 expression had a significantly shorter five-year overall survival than those with high miR-1181 expression ($p=0.0007$). Moreover, we also observed that miR-1181, N stage, clinical stage and grade were unfavorable prognostic factors in NPC patients (Table III). More importantly, multivariate analysis showed that miR-1181 (RR=3.016, 95% CI: 1.216-3.895, $p=0.018$) expression was an independent prognostic indicator for NPC patients in addition to presence of N stage, clinical stage and grade.

MiR-1181 Suppressed Cellular Proliferation and Promoted Apoptosis of NPC Cells

In light of the reduced expression of miR-1181 in NPC tissues and cell lines, we next investigated the effects of miR-1181 on the proliferation and apoptosis of NPC cells. According to the results of qRT-PCR assays, the miR-1181 expressing levels were remarkably upregulated in CNE1 and SUNE1 cells by miR-1181 mimics transfection (Figure 2A). As determined by

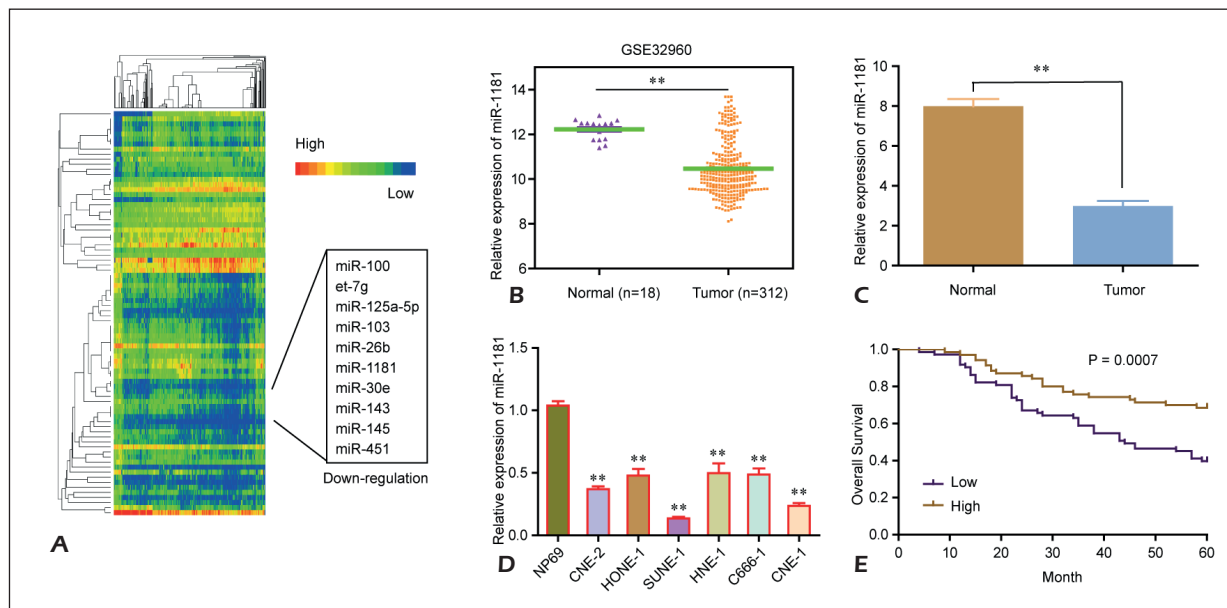


Figure 1. miR-1181 expression is down-regulated in NPC and is correlated with prognosis. **A**, Hierarchical cluster heat map of differentially expressed miRNAs in NPC and normal tissues generated from RNA sequencing data from the GEO database (GSE32960). **B**, The expression levels of miR-1181 in NPC tissues and matched tissues according to GSE32960. **C**, Expression of miR-1181 in NPC tissues and matched normal tissues using RT-PCR. **D**, The expression of miR-1181 in the NP69 cells and NPC cell lines (CNE-2, HONE-1, SUNE-1, HNE-1, C666-1 and CNE-1). **E**, Kaplan-Meier survival analyses of correlations between miR-1181 expression level and overall survival are shown. * $p < 0.05$, ** $p < 0.01$.

CCK-8 assays, transfection of miR-1181 resulted in a notable inhibition of cellular growth in both CNE1 and SUNE1 cells compared with that in the controls (Figure 2B and C). In addition, as demonstrated by EdU assays, the proliferative cells in the miR-1181 mimic-transfected CNE1 and SUNE1 cells group were markedly less than that of control group, which was consistent with the data of CCK-8 assays (Figure 2D and E). Similarly, the number of miR-1181 mimic-transfected CNE1 and SUNE1 cell colonies

were remarkably fewer than that of the controls (Figure 2F). Furthermore, we next transfected miR-1181 mimics into CNE1 and SUNE1 cells to clarify the roles of miR-1181 overexpression in cell apoptosis. As confirmed by flow cytometry assays, ectopic expression of miR-1181 led to dramatically increased apoptotic rates of CNE1 and SUNE1 cells (Figure 2G). Collectively, these data demonstrated that miR-1181 was capable of depressing NPC cell proliferation and accelerating cell apoptosis.

Table III. Univariate and multivariate analysis of overall survival in NPC patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	<i>p</i>	RR	95% CI	<i>p</i>
Gender	1.213	0.672-2.311	0.324	—	—	—
Age	1.548	0.734-1.994	0.173	—	—	—
T stage	1.643	0.844-2.327	0.185	—	—	—
N stage	2.776	1.326-4.554	0.021	2.366	1.169-3.889	0.026
M stage	1.554	0.549-1.994	0.147	—	—	—
Clinical stage	3.216	1.445-4.328	0.008	2.947	1.217-3.953	0.022
Grade	3.056	1.385-3.994	0.014	2.788	1.137-3.775	0.031
EBV infection	1.543	0.845-1.942	0.215	—	—	—
miR-1181 expression	3.344	1.462-4.342	0.006	3.016	1.216-3.895	0.018

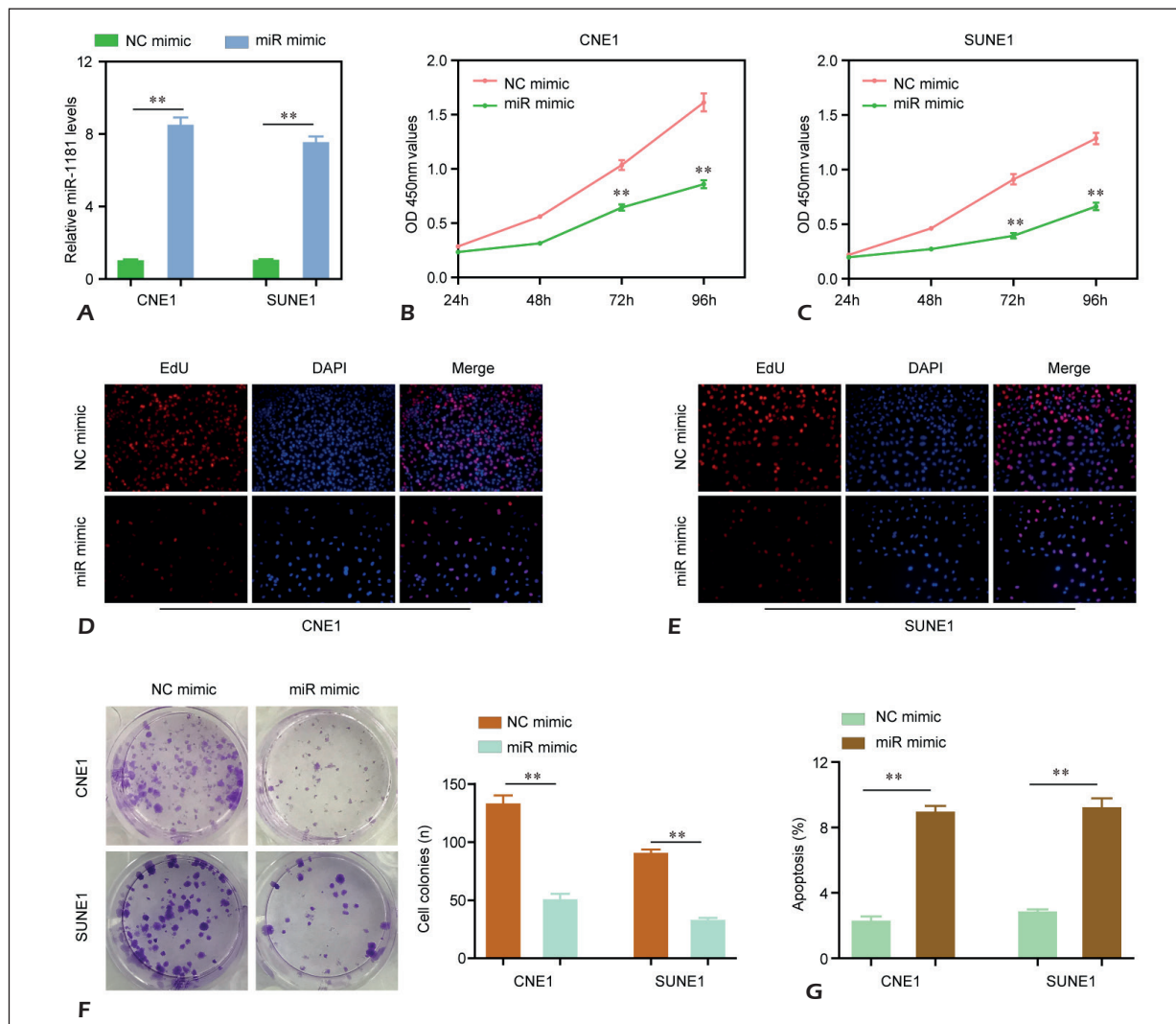


Figure 2. The effects of miR-1181 on the proliferation and apoptosis of CNE1 and SUNE1 cells. **A**, Transfection of miR-1181 mimics increased the miR-1181 expressing levels in CNE1 and SUNE1 cells. (**B** and **C**) CCK-8 assays were utilized to assess the cell proliferation after treatment of miR-1181 mimics at indicated timepoints. (**D** and **E**) EdU assays detected the proliferative CNE1 and SUNE1 cells. The positive cells were red, and the DAPI stained cell nuclei were blue. **F**, Colony formation assay examined the clonogenic capacity of miR-1181 mimic-transfected CNE1 and SUNE1 cells. **G**, Flow cytometry evaluated the apoptosis of CNE1 and SUNE1 cells after transfection of miR-1181 mimics. * $p < 0.05$, ** $p < 0.01$.

miR-1181 Inhibited the Migration and Invasion of NPC Cells

In order to determine the function of miR-1181 on the migration of NPC cells, we conducted wound-healing assays. The results revealed that overexpression of miR-1181 significantly impaired the relative migratory rates of CNE1 and SUNE1 cells (Figure 3A and B). Next, we investigated whether enhancing expression of miR-1181 affected the invasive ability of NPC cells. Therefore, transwell invasion assays were performed using CNE1 and SUNE1 cells. The results showed that transfection of miR-1181

remarkably reduced the invasive cell number, which indicated that overexpression of miR-1181 inhibited the metastatic potentials of NPC cells (Figure 3C). Subsequently, our study evaluated the influence of miR-1181 on proteins relevant to epithelial-mesenchymal transition. After overexpressing miR-1181, the protein expression levels of N-cadherin and vimentin were markedly decreased in CNE1 and SUNE1 cells (Figure 3D). In summary, these data indicated that overexpression of miR-1181 impeded the metastatic potentials of NPC cells via regulating epithelial-mesenchymal transition.

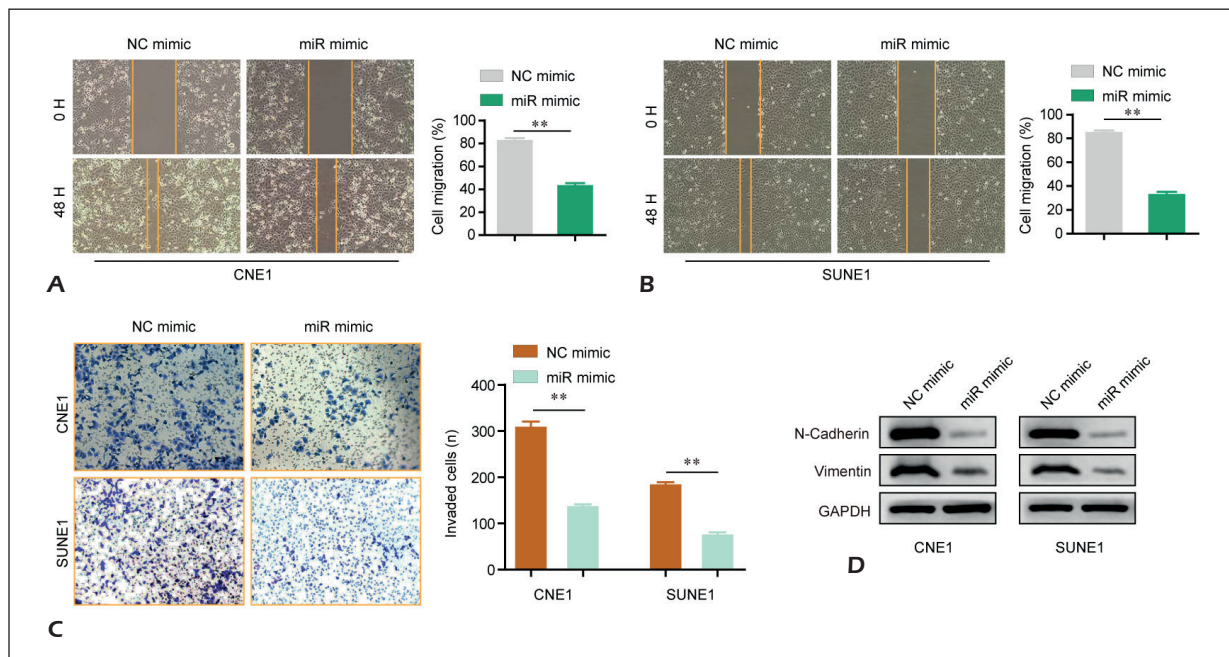


Figure 3. The influence of miR-1181 on the invasion and migration of CNE1 and SUNE1 cells. **(A and B)** The migration of CNE1 and SUNE1 cells were impaired by transfecting miR-1181 mimics. **C**, Transfection of miR-1181 mimics suppressed the invasion of CNE1 and SUNE1 cells. **D**, Transfection of miR-1181 mimics inhibited N-cadherin and vimentin expression in CNE1 and SUNE1 cells. * $p < 0.05$, ** $p < 0.01$.

Enhancing Expression of miR-1181 Impeded the Activation of Wnt/ β -Catenin Signaling in NPC Cells

Having certified that ectopic expression of miR-1181 depressed the development and progression of NPC, we then aimed to investigate its detailed molecular mechanisms. A plethora of studies had shown that the Wnt/ β -catenin signaling was remarkably associated with the growth and metastasis of cancers. Hence, we next focused on Wnt/ β -catenin signaling and transfected miR-1181 mimics into CNE1 and SUNE1 cells to evaluate the expressing alteration of the molecules involved in this signaling. As the data of qRT-PCR assays presented in Figure 4A, forced expression of miR-1181 led to a marked decline of β -catenin, cyclin D1 and c-myc mRNA levels in CNE1 and SUNE1 cells. Additionally, consistent with above results, the data of Western blot assays revealed that the protein expression of β -catenin, cyclin D1 and c-myc was also reduced in CNE1 and SUNE1 cells when they were transfected with miR-1181 mimics (Figure 4B). Therefore, these data suggested that miR-1181 was able to modulate the activity of Wnt/ β -catenin signaling, and the development of NPC could be regulated by miR-1181 via affecting Wnt/ β -catenin signaling.

Discussion

NPC is prevalent in southern China and southern Asia. Despite advances in cancer treatments, the clinical prognosis of NPC patients with metastasis remains relatively poor¹⁸. In order to improve the clinical management of NPC, the early diagnosis and prognosis predication are very critical¹⁹. Up to date, CT and MRI have been used for the evaluation of the tumor and associated lymphadenopathy, and several clinicopathologic features are also used to predict the clinical outcome of NPC patients^{20,21}. However, these methods lack adequate sensitivity and specificity for effective surveillance. Recently, more and more researches have been conducted to explore disease features that can be used for predicting disease outcome and treatment response accurately, but no robust tumor markers have yet been established^{22,23}.

Recently, more and more studies reported that miRNAs may be a candidate factor for predicting prognosis for various tumors, including NPC^{24,25}. Several miRNAs have been confirmed to be independent prognostic factors for the patients with NPC, such as miR-24-3p²⁶, miR-92a²⁷ and miR-223²⁸. In this study, we firstly reported that miR-1181 expression was significantly down-regulated in both NPC tissues and

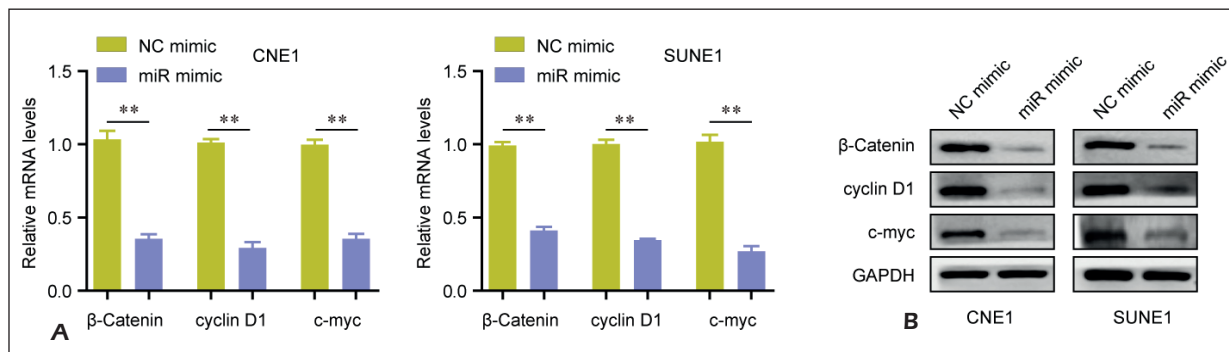


Figure 4. Mir-1181 affected the activity of Wnt/β-catenin signaling in CNE1 and SUNE1 cells. **A**, The mRNA expression of β-catenin, cyclin D1 and c-myc in CNE1 and SUNE1 cells after treatment with miR-1181 mimics were detected by qRT-PCR assays. **B**, The protein levels of β-catenin, cyclin D1 and c-myc in CNE1 and SUNE1 cells were measured by Western blot assays. * $p < 0.05$, ** $p < 0.01$.

cell lines, indicating that miR-1181 may act as a negative regulator in NPC progression. Then, clinical assays revealed that low miR-1181 expression was associated with advanced N stage, clinical stage, grade and shorter five-year overall survival. Of note, the univariate and multivariate analyses demonstrated that down-regulation of miR-1181 was an independent factor for predicting overall survival in NPC patients, providing important clinical evidence that miR-1181 may be used as a novel tumor biomarker for NPC patients. However, because the samples sizes were relatively small, further studies on more patients are required to confirm our results. As a tumor-related miRNA, miR-1181 has been reported to be involved in the regulation of progression of several tumors. For instance, Wang et al²⁹ showed that miR-1181 suppressed proliferation and invasion via STAT3 in pancreatic cancer. Jiang et al¹³ reported that lower expression of miR-1181 was associated with poorer prognosis in pancreatic cancer patients. In addition, miR-1181 acted as a tumor suppressor in this cancer by suppressing SOX2 and STAT3. In this study, we also performed gain-of-function assays to explore the potential function of miR-1181 in NPC. The results of CCK-8 and Flow cytometry analysis demonstrated that miR-1181 knockdown can induce decreased cell proliferation and increased apoptosis in NPC cells. Moreover, we also observed that down-regulation of miR-1181 suppressed NPC cells migration, invasion and EMT, and promoted apoptosis. Thus, our findings indicated that miR-1181 may be a potential therapeutic target for NPC. The Wnt signaling pathway

is important extracellular signaling molecule family, acting as important regulator in embryonic development and hematopoiesis^{30,31}. Wnt pathway proteins are a group of evolutionarily conserved intracellular signaling molecules that are needed for cell proliferation and differentiation in different tissues^{32,33}. An inappropriate activation of the Wnt/β-catenin signaling pathway play a critical role in regulating oncogenesis and metastasis. β-catenin, the key initial protein in the Wnt signaling, can regulate expression of several transcription factors, and subsequently induce EMT. In fact, a variety of miRNAs have exhibited different regulatory effects on the Wnt/β-catenin signaling pathway^{34,35}. In this study, we found that overexpression of miR-1181 significantly inhibited the expression of cyclin D1, c-myc and β-catenin, suggesting that Wnt/β-catenin pathway was inactivated. Thus, our results provided first evidence that miR-1181 displayed its tumor-suppressive roles by modulating Wnt/β-catenin pathway. Of course, further research is still needed to clarify the deep mechanism of miR-1181 in tumor suppression.

Conclusions

We revealed that the expression of miR-1181 was decreased in human NPC and overexpression of miR-1181 led to the inhibition of NPC metastasis by modulating Wnt/β-catenin pathway. In addition, our clinical data showed that low miR-1181 expression was associated with the malignant status and prognosis in NPC patients. Thus, miR-1181 may represent

a novel prognostic biomarker and therapeutic target in NPC.

Conflict of Interests

The Authors declare that there are no conflicts of interest.

References

- 1) TORRE LA, BRAY F, SIEGEL RL, FERLAY J, LORTET-TIEULENT J, JEMAL A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65: 87-108.
- 2) KAMRAN SC, RIAZ N, LEE N. Nasopharyngeal carcinoma. *Surg Oncol Clin N Am* 2015; 24: 547-561.
- 3) DAI W, ZHENG H, CHEUNG AK, LUNG ML. Genetic and epigenetic landscape of nasopharyngeal carcinoma. *Chin Clin Oncol* 2016; 5: 16.
- 4) AYAN I, KAYTAN E, AYAN N. Childhood nasopharyngeal carcinoma: from biology to treatment. *Lancet Oncol* 2003; 4: 13-21.
- 5) BENSOUDA Y, KAIKANI W, AHBEDDOU N, RAHHALI R, JABRI M, MRABTI H, BOUSSEN H, ERRIHANI H. Treatment for metastatic nasopharyngeal carcinoma. *Eur Ann Otorhinolaryngol Head Neck Dis* 2011; 128: 79-85.
- 6) MA BBY, HUI EP, CHAN ATC. Investigational drugs for nasopharyngeal carcinoma. *Expert Opin Investig Drugs* 2017; 26: 677-685.
- 7) FABIAN MR, SONENBERG N, FILIPOWICZ W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* 2010; 79: 351-379.
- 8) JEKER LT, MARONE R. Targeting microRNAs for immunomodulation. *Curr Opin Pharmacol* 2015; 23: 25-31.
- 9) FU LL, WEN X, BAO JK, LIU B. MicroRNA-modulated autophagic signaling networks in cancer. *Int J Biochem Cell Biol* 2012; 44: 733-736.
- 10) RUPAIMOOLE R, SLACK FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov* 2017; 16: 203-222.
- 11) TUTAR Y. miRNA and cancer; computational and experimental approaches. *Curr Pharm Biotechnol* 2014; 15: 429.
- 12) WU RS, QIU EH, ZHU JJ, WANG JR, LIN HL. MiR-101 promotes nasopharyngeal carcinoma cell apoptosis through inhibiting Ras/Raf/MEK/ERK signaling pathway. *Eur Rev Med Pharmacol Sci* 2018; 22: 150-157.
- 13) JIANG J, LI Z, YU C, CHEN M, TIAN S, SUN C. MiR-1181 inhibits stem cell-like phenotypes and suppresses SOX2 and STAT3 in human pancreatic cancer. *Cancer Lett* 2015; 356: 962-970.
- 14) RUAN L, XIE Y, LIU F, CHEN X. Serum miR-1181 and miR-4314 associated with ovarian cancer: MiRNA microarray data analysis for a pilot study. *Eur J Obstet Gynecol Reprod Biol* 2018; 222: 31-38.
- 15) DENG X, ZHAO Y, WANG B. miR-519d-mediated downregulation of STAT3 suppresses breast cancer progression. *Oncol Rep* 2015; 34: 2188-2194.
- 16) CLEVERS H, NUSSE R. Wnt/beta-catenin signaling and disease. *Cell* 2012; 149: 1192-1205.
- 17) GHAAHHARI NM, BABASHAH S. Interplay between microRNAs and WNT/beta-catenin signalling pathway regulates epithelial-mesenchymal transition in cancer. *Eur J Cancer* 2015; 51: 1638-1649.
- 18) TAN WL, TAN EH, LIM DW, NG QS, TAN DS, JAIN A, ANG MK. Advances in systemic treatment for nasopharyngeal carcinoma. *Chin Clin Oncol* 2016; 5: 21.
- 19) NG WT, YUEN KT, AU KH, CHAN OS, LEE AW. Staging of nasopharyngeal carcinoma--the past, the present and the future. *Oral Oncol* 2014; 50: 549-554.
- 20) RONG X, YIN J, WANG H, ZHANG X, PENG Y. Statin treatment may lower the risk of postradiation epilepsy in patients with nasopharyngeal carcinoma. *Epilepsia* 2017; 58: 2172-2177.
- 21) CAPONIGRO F, LONGO F, IONNA F, PERRI F. Treatment approaches to nasopharyngeal carcinoma: a review. *Anticancer Drugs* 2010; 21: 471-477.
- 22) JIANG W, CAI R, CHEN QQ. DNA methylation biomarkers for nasopharyngeal carcinoma: diagnostic and prognostic tools. *Asian Pac J Cancer Prev* 2015; 16: 8059-8065.
- 23) CHEN ZT, LIANG ZG, ZHU XD. A review: proteomics in nasopharyngeal carcinoma. *Int J Mol Sci* 2015; 16: 15497-15530.
- 24) WANG T, LIU X, TIAN Q, LIANG T, CHANG P. Increasing expression of miR-5100 in non-small-cell lung cancer and correlation with prognosis. *Eur Rev Med Pharmacol Sci* 2017; 21: 3592-3597.
- 25) HAYES J, PERUZZI PP, LAWLER S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol Med* 2014; 20: 460-469.
- 26) YE SB, ZHANG H, CAI TT, LIU YN, NI JJ, HE J, PENG JY, CHEN QY, MO HY, JUN C, ZHANG XS, ZENG YX, LI J. Exosomal miR-24-3p impedes T-cell function by targeting FGF11 and serves as a potential prognostic biomarker for nasopharyngeal carcinoma. *J Pathol* 2016; 240: 329-340.
- 27) ZHANG H, CAO H, XU D, ZHU K. MicroRNA-92a promotes metastasis of nasopharyngeal carcinoma by targeting the PTEN/AKT pathway. *Onco Targets Ther* 2016; 9: 3579-3588.
- 28) ZENG X, XIANG J, WU M, XIONG W, TANG H, DENG M, LI X, LIAO Q, SU B, LUO Z, ZHOU Y, ZHOU M, ZENG Z, LI X, SHEN S, SHUAI C, LI G, FANG J, PENG S. Circulating miR-17, miR-20a, miR-29c, and miR-223 combined as non-invasive biomarkers in nasopharyngeal carcinoma. *PLoS One* 2012; 7: e46367.
- 29) WANG J, GUO XJ, DING YM, JIANG JX. miR-1181 inhibits invasion and proliferation via STAT3 in pancreatic cancer. *World J Gastroenterol* 2017; 23: 1594-1601.
- 30) ROSENBLUH J, WANG X, HAHN WC. Genomic insights into WNT/beta-catenin signaling. *Trends Pharmacol Sci* 2014; 35: 103-109.

- 31) VORONKOV A, KRAUSS S. Wnt/beta-catenin signaling and small molecule inhibitors. *Curr Pharm Des* 2013; 19: 634-664.
- 32) HOLLAND JD, KLAUS A, GARRATT AN, BIRCHMEIER W. Wnt signaling in stem and cancer stem cells. *Curr Opin Cell Biol* 2013; 25: 254-264.
- 33) PAI SG, CARNEIRO BA, MOTA JM, COSTA R, LEITE CA, BARROSO-SOUSA R, KAPLAN JB, CHAE YK, GILES FJ. Wnt/beta-catenin pathway: modulating anticancer immune response. *J Hematol Oncol* 2017; 10: 101.
- 34) FENG ZY, XU XH, CEN DZ, LUO CY, WU SB. miR-590-3p promotes colon cancer cell proliferation via Wnt/beta-catenin signaling pathway by inhibiting WIF1 and DKK1. *Eur Rev Med Pharmacol Sci* 2017; 21: 4844-4852.
- 35) HU H, WANG G, LI C. miR-124 suppresses proliferation and invasion of nasopharyngeal carcinoma cells through the Wnt/beta-catenin signaling pathway by targeting Capn4. *Onco Targets Ther* 2017; 10: 2711-2720.