

RAB31 is targeted by miR-26b and serves a role in the promotion of osteosarcoma

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Abstract. Ras-related protein Rab-31 (RAB31), a small guanosine 5'-triphosphate-binding protein, is a member of the Rab family and has been demonstrated to serve an oncogenic role in several common types of human cancer. However, the function of RAB31 in osteosarcoma (OS) has not been previously studied. The present study identified that the expression levels of RAB31 were significantly higher in OS tissue samples compared with matched adjacent non-tumor tissue samples, and high RAB31 expression was associated with malignant progression and a poor prognosis for patients with OS. Furthermore, it was identified that the expression levels of RAB31 were increased in OS cell lines compared with normal osteoblast cells. Silencing of RAB31 expression significantly inhibited OS cell proliferation, cell cycle progression, migration and invasion, and significantly increased the rate of cell apoptosis. In addition, the present study used a luciferase reporter assay to demonstrate that RAB31 was a direct target gene of microRNA-26b (miR-26b), which is a known tumor suppressor in OS. The expression levels of RAB31 were negatively associated with miR-26b expression in OS cells. Finally, miR-26b was demonstrated to be significantly decreased in OS tissues compared with adjacent non-tumor tissues, and an inverse correlation was observed between the expression levels of RAB31 and miR-26b in OS tissues. In summary, to the best of our knowledge, the present study is the first to report that RAB31 is a target gene of miR-26b, and silencing of RAB31 may inhibit OS growth and progression.

Introduction

In 2015, osteosarcoma (OS) was reported as the most common type of malignancy of the bone, accounting for 5% of all

pediatric tumors and 8.9% of cancer-associated mortality in children worldwide (1,2). OS is predominantly caused by metaphysis of the long bones (3). In 2015, the 5-year survival rate of patients with OS was reported to be 60-70% worldwide, due to improvements in chemotherapy and surgical resection (1-4). However, the prognosis of OS patients with distant metastasis remains poor, with a reported 5-year survival rate of only 20% worldwide in 2015 (1-4). Therefore, there is an urgent requirement to investigate the molecular mechanisms underlying OS progression, which may assist with the development of novel treatment strategies for OS.

The small GTPase proteins of the Rab family have been demonstrated to serve key roles in the development and malignant progression of numerous types of human cancer (5,6). Ras-related protein Rab-31 (RAB31), a member of the Rab5 subfamily, is a lipid-anchored protein consisting of 194 amino acids, which localizes to the cytoplasmic side of the cell membrane and serves essential roles in vesicle and granule targeting (7). Previous studies have reported that RAB31 serves an oncogenic role in human cancer types (8-10). For example, Pan *et al* (8) used glioblastoma and cervical cancer cell lines to investigate the function of RAB31 in cancer progression, which revealed that RAB31 promotes cancer cell proliferation, migration and epithelial-mesenchymal transition, and inhibit cell apoptosis. Knockdown of RAB31 was identified to suppress tumor growth *in vivo*. Furthermore, Sui *et al* (9) demonstrated that Rab31 promotes hepatocellular carcinoma progression by inhibiting cell apoptosis induced by the PI3K/AKT/Bcl-2/Bax pathway. Notably, Grismayer *et al* (10) identified that overexpression of RAB31 in breast cancer cells causes a switch from an invasive to a proliferative phenotype. These findings suggest that the different functions of RAB31 may be tumor-specific. However, to the best of our knowledge, the expression and function of RAB31 in OS has not previously been reported.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that contain 22-25 nucleotides and regulate gene expression by binding to a complementary sequence within the 3'-untranslated region (3'UTR) of their target mRNA, which leads to translation repression or RNA degradation (11,12). By affecting the expression of their target genes, a large number of miRNAs have been demonstrated to serve key roles in various biological processes, including cell survival,

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differentiation, proliferation, cell cycle, apoptosis, migration and invasion (13-15). Recently, numerous miRNAs have been identified to be dysregulated in OS and to serve a promoting or suppressive role (16-19). For example, miR-195-5p inhibits OS cell proliferation and invasion by suppressing the expression of naked cuticle homolog 1 (19).

Previously, miR-26b has been reported to function as a tumor suppressor (20-23). Li *et al* (22) reported that miR-26b inhibits the metastasis of colorectal cancer by inhibiting fucosyltransferase 4. miR-26b has also been identified to inhibit proliferation and invasion of neuroglioma cells by downregulating Bcl-2 expression and enhancing caspase-3 activity (23). In OS, miR-26b has been reported to suppress cancer cell proliferation, migration and invasion, and induce OS cell apoptosis by inhibiting glycolysis (20). Furthermore, miR-26b can inhibit OS cell migration and invasion by targeting 6-phosphofructo-2-kinase/fructose-2-6-biphosphatase (PFKFB3), connective tissue growth factor (CTGF) and SMAD family member 1 (Smad1) (21,24). However, to the best of our knowledge, the association between miR-26b and RAB31 has not previously been studied. Therefore, the present study aimed to evaluate the clinical significance of RAB31 expression in OS. In addition, the regulatory role of RAB31 in the malignant phenotype of OS cells was investigated.

Materials and methods

Clinical tissue samples. The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Nanchang University (Nanchang, China) and written informed consent was obtained from all patients. OS tissue samples and matched adjacent non-tumor tissue samples were collected from 54 patients with OS at the Second Affiliated Hospital of Nanchang University between May 2010 and May 2013. The patients included 33 males and 21 females, with a mean age of 18.8 years (range, 10-26 years). The clinical characteristics of the patients are summarized in Table I. No patients received radiotherapy or chemotherapy prior to surgery. All tissues samples were stored at -80°C prior to use.

Cell culture. The normal human osteoblastic cell line hFOB 1.19 and the human OS cell lines SAOS2, U2OS, MG63 and HOS were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). hFOB 1.19 cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂. OS cells were maintained in DMEM with 10% FBS, 100 U/ml penicillin and 100 U/ml streptomycin at 37°C with 5% CO₂.

Cell transfection. Cells were cultured at 37°C with 5% CO₂ in a 6-well plate at a density of ~1x10⁶ cells/well. When a confluency of 70-80% was reached, the cells were transfected with 100 nM negative control (NC) small interfering (si)RNA (cat. no. AM4611; Thermo Fisher Scientific, Inc.), RAB31 siRNA (cat. no. AM16708; Thermo Fisher Scientific, Inc.), miR-NC (cat. no. 4464058; Thermo Fisher Scientific, Inc.), miR-26b mimic (cat. no. 4464066; Thermo Fisher Scientific, Inc.), NC inhibitor (cat. no. 4464077; Thermo Fisher Scientific, Inc.) or

miR-26b inhibitor (cat. no. 4464084; Thermo Fisher Scientific, Inc.) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following incubation for 48 h at 37°C, subsequent experiments were performed.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from tissue samples and cells (1x10⁶) using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). RT was performed using miScript Reverse Transcription kit (Qiagen GmbH), according to the manufacturer's protocol. qPCR was conducted using QuantiTect SYBR-Green RT-PCR kit (Qiagen GmbH) with an ABI 7500 fast real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions for PCR were as follows: 95°C for 1 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. GAPDH or U6 was used as the internal reference for mRNA and miRNA, respectively. Data were quantified using the 2^{-ΔΔC_q} method (25). The primer sequences were as follows: RAB31 forward, 5'-GGGGTTGGGAAATCAAGCATC-3' and reverse, 5'-GCCAATGAATGAAACCGTTCCT-3'; GAPDH forward, 5'-ACAACCTTTGGTATCGTGGAAGG-3' and reverse, 5'-GCCATCACGCCACAGTTTC-3'. Primers for U6 (cat. no. HmiRQP9001) and miR-16 (cat. no. HmiRQP0356) were obtained from Guangzhou FuluGen, Co., Ltd.

Western blot analysis. Tissue samples and cells (1x10⁷) were solubilized with radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology). The protein concentration was measured using a BCA protein assay kit (Beyotime Institute of Biotechnology). Total protein (50 µg) was separated by 10% SDS-PAGE and then transferred to polyvinylidene difluoride membranes (EMD Millipore). The membranes were blocked with 5% non-fat milk in 0.1% TBS/Tween-20 at room temperature for 1 h, followed by incubation with rabbit anti-human RAB31 (dilution, 1:100; cat. no. ab230881; Abcam), or rabbit anti-human GAPDH (dilution, 1:200; cat. no. ab181602; Abcam) primary antibodies at room temperature for 3 h. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (dilution, 1:5,000; cat. no. ab6721; Abcam) at room temperature for 1 h. Enhanced chemiluminescent reagent (Pierce; Thermo Fisher Scientific, Inc.) was used to detect the signals on the membranes. ImageJ software (v1.46; National Institutes of Health) was used for densitometry analysis.

Cell proliferation assay. Cell proliferation was investigated using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.), according to the manufacturer's protocol. A total of 48 h after transfection, cells (3,000 cells/well) were seeded into 96-well plates and cultured for 0, 24, 48 or 72 h. The optical density value at 450 nm was measured using a microplate reader.

Cell apoptosis assay. The transfected cells were harvested and washed twice with ice-cold PBS. Subsequently, 1x10⁶ cells were resuspended with 300 µl binding buffer. Following staining with Annexin V-fluorescein isothiocyanate and propidium iodide (BD Biosciences), according to the manufacturer's

Table I. Association between RAB31 expression and clinicopathological characteristics of patients with osteosarcoma.

Characteristic	Cases (n=54)	Low RAB31 expression (n=33)	High RAB31 expression (n=21)	P-value
Age, years				0.784
<20	22	14	8	
≥20	32	19	13	
Sex				0.575
Male	33	19	14	
Female	21	14	7	
Tumor size, cm				0.092
<8	24	18	6	
≥8	30	15	15	
Location				0.772
Femur or tibia	37	22	15	
Other	17	11	6	
Lung metastasis				0.001 ^a
No	38	29	9	
Yes	16	4	12	
TNM stage				0.018 ^a
I/IIA	19	16	3	
IIB/III	35	17	18	
Serum lactate dehydrogenase				0.151
Normal	20	15	5	
Elevated	34	18	16	
Serum alkaline phosphatase				0.168
Normal	22	16	6	
Elevated	32	17	15	

^aStatistically significant. RAB31, ras-related protein Rab-31; TNM, tumor-node-metastasis.

protocol, apoptotic cells were analyzed using a flow cytometer and Accuri C6 Software (version 1.0; BD Biosciences).

Wound healing assay. The transfected cells (1×10^6 cells per well) were cultured in 6-well plates for 24 h. Subsequently, an artificial wound was created using a sterile 10 μ l pipette tip in the confluent cell monolayer. Photographs were captured at 0 and 24 h using an inverted light microscope (magnification, x40).

Cell invasion assay. Matrigel (BD Biosciences) was diluted with serum-free DMEM and added to the upper chamber of a 24-well Transwell™ chamber (Corning Inc.), which was then incubated at 37°C for 1 h. Cells were diluted to a density of 2×10^5 cells/ml with serum-free DMEM. Subsequently, 100 μ l cell suspension was added to the upper chamber and 700 μ l DMEM containing 10% FBS was added to the bottom chamber. Following incubation at 37°C for 24 h, the chamber was washed with PBS. The cells were then fixed with 4% paraformaldehyde at room temperature for 30 min, stained with crystal violet at room temperature for 30 min and photographs

were obtained using an inverted light microscope (magnification, x400).

Bioinformatics analysis and luciferase reporter assay. TargetScan software version 7.1 was used for bioinformatics analysis (26). The wild-type (WT) or mutant type (MUT) 3'-UTR of RAB31 was inserted downstream of the luciferase reporter gene in the pMIR-REPORT vector (Thermo Fisher Scientific, Inc.), generating WT-RAB31 and MUT-RAB31. MG63 and U2OS cells were co-transfected with miR-26b mimics/miR-NC, WT-RAB31/MUT-RAB31 and pRL-SV40 (Promega Corporation) expressing *Renilla* luciferase. Following 48 h incubation at 37°C with 5% CO₂, the luciferase activity was measured using the Dual-Luciferase® Reporter Assay System (Promega Corporation).

Statistical analysis. All data are presented as the mean \pm standard deviation from a minimum of three independent experiments. Statistical analysis was performed using SPSS 20.0 (IBM Corp.). Student's t-test was used for comparisons between two groups. Multiple comparisons were

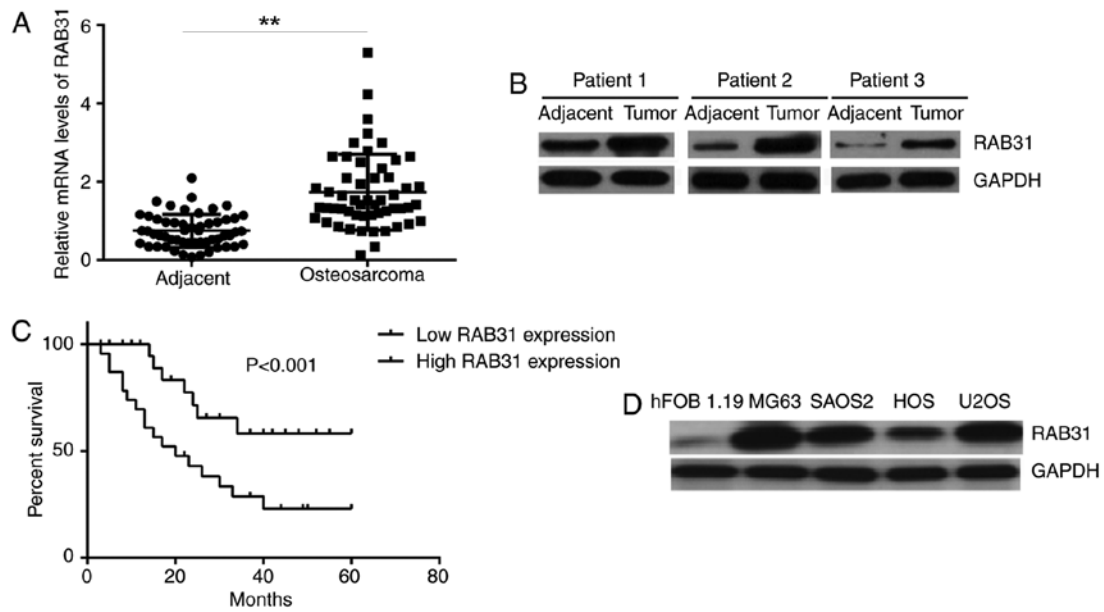


Figure 1. RAB31 expression levels in OS tissues and cell lines. (A) mRNA and (B) protein expression levels of RAB31 were higher in OS tissues compared with adjacent non-tumor tissues. ** $P<0.01$. (C) Patients with OS with high RAB31 expression exhibited a significantly shorter survival time compared with patients with low RAB31 expression. (D) Protein expression levels of RAB31 were higher in OS cell lines compared with the osteoblastic cell line hFOB 1.19. RAB31, Ras-related protein Rab-31; OS, osteosarcoma.

performed using one-way analysis of variance followed by Tukey's test. The patients with OS were divided into a high RAB31 expression group and low RAB31 expression group using the median expression level of RAB31 (1.91) as the cut-off, and χ^2 test was used to perform statistical analysis in Table I. Correlations between the mRNA expression levels of miR-26b and RAB31 were analyzed using Pearson's correlation coefficient. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Increased expression levels of RAB31 are associated with OS progression. First, the mRNA and protein expression levels of RAB31 were examined in tissue samples from OS patients. RT-qPCR and western blot analysis demonstrated that the expression levels of RAB31 were significantly higher in OS tissues compared with adjacent non-tumor tissues (Fig. 1A and B). The patients with OS were then divided into a high RAB31 expression group and low RAB31 expression group using the median expression level of RAB31 (1.91) as the cut-off. As presented in Table I, high expression of RAB31 was identified to be significantly associated with lung metastasis and advanced clinical stage. Furthermore, the patients with high RAB31 expression exhibited a significantly shorter survival time compared with those with low RAB31 expression (Fig. 1C), which suggested that RAB31 upregulation was associated with a poor prognosis for patients with OS. In addition, the protein expression levels of RAB31 were markedly higher in the OS cell lines SAOS2, U2OS, MG63 and HOS compared with the normal human osteoblastic cell line hFOB 1.19 (Fig. 1D). MG63 and U2OS cells exhibited the highest expression levels of RAB31 protein (Fig. 1D); these cell lines were therefore selected for subsequent experiments.

Silencing of RAB31 inhibits OS cell proliferation, cell cycle progression, migration and invasion, and induces cell apoptosis. To inhibit the expression of RAB31, MG63 and U2OS cells were transfected with RAB31 siRNA. As presented in Fig. 2A and B, the mRNA and protein expression levels of RAB31 were significantly lower in MG63 and U2OS cells transfected with RAB31 siRNA compared with the NC siRNA group. A CCK-8 assay was then performed to examine cell proliferation. As presented in Fig. 2C and D, knockdown of RAB31 significantly inhibited OS cell proliferation. The cell cycle distribution and cell apoptosis were then examined by flow cytometry. The data from this experiment indicated that silencing of RAB31 was significantly associated with cell cycle arrest at the G1 stage and significantly promoted OS cell apoptosis (Fig. 2E-H). The aforementioned results suggested that RAB31 may serve a promoting role in OS growth.

To study the potential role of RAB31 in OS metastasis, a wound healing assay and Transwell assay were performed to examine cell migration and invasion, respectively. As presented in Fig. 3, silencing of RAB31 expression significantly inhibited the migration and invasion of MG63 and U2OS cells compared with the NC siRNA group. In summary, RAB31 silencing inhibited OS cell proliferation, cell cycle progression, migration and invasion, and induced cell apoptosis.

RAB31 is a target gene of miR-26b in OS cells. TargetScan software data predicted that RAB31 is a putative target gene of miR-26b (Fig. 4A). To confirm the association between RAB31 and miR-26b, wild-type (WT) and mutant (MT) RAB31 luciferase reporter plasmids were generated (Fig. 3B) and a luciferase reporter gene assay was performed. The data demonstrated that transfection with miR-26b mimic significantly inhibited the luciferase activity in MG63 and U2OS cells transfected with WT RAB31 3'UTR reporter plasmid; however, no effect was observed on the luciferase activity

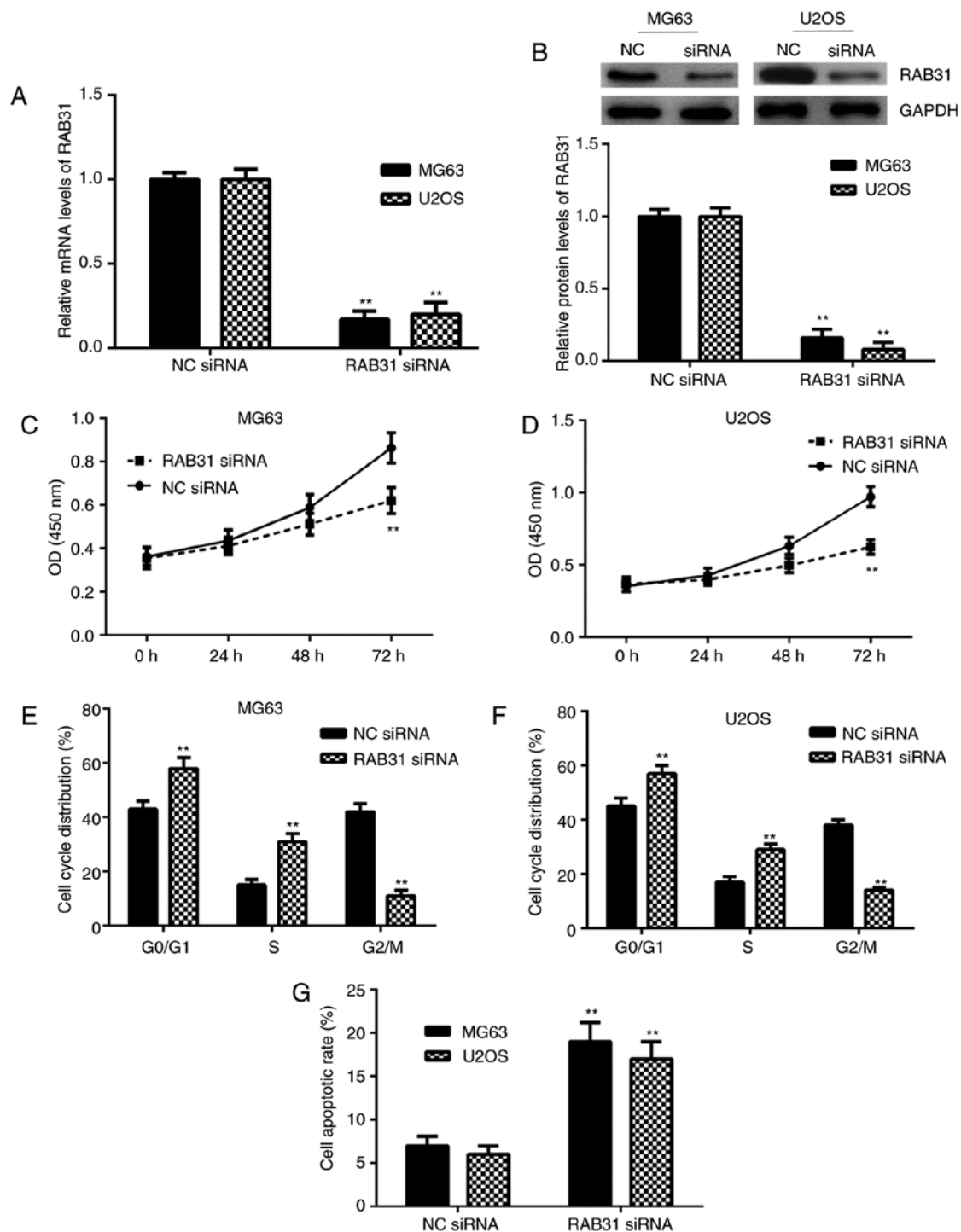


Figure 2. Effects of RAB31 silencing in OS cells. (A) mRNA and (B) protein expression levels of RAB31 were significantly lower in MG63 and U2OS cells transfected with RAB31 siRNA compared with cells transfected with NC siRNA. (C) Knockdown of RAB31 significantly inhibited proliferation of MG63 and (D) U2OS cells. (E) Knockdown of RAB31 caused cell cycle arrest at the G1 stage in MG63 and (F) U2OS cells. (G) Knockdown of RAB31 induced MG63 and U2OS cell apoptosis. Data are presented as the mean \pm standard deviation. ** $P < 0.01$ vs. NC siRNA for the same cell line. RAB31, Ras-related protein Rab-31; siRNA, small interfering RNA; NC, negative control; OD, optical density.

in cells transfected with the MT RAB31 3'UTR reporter plasmid (Fig. 4C and D). This indicates that RAB31 is a target gene of miR-26b in OS cells.

RAB31 is negatively regulated by miR-26b in OS cells. The effects of miR-26b on RAB31 expression were then investigated in OS cells. MG63 and U2OS cells were transfected with miR-26b mimic or miR-NC. Following transfection, miR-26b

expression was significantly upregulated in the miR-26b mimic group compared with the miR-NC group (Fig. 5A). Further experiments revealed that the mRNA and protein expression levels of RAB31 were significantly decreased following miR-26b overexpression (Fig. 5B and C). To further confirm these findings, MG63 and U2OS cells were transfected with miR-26b inhibitor or NC inhibitor. RT-qPCR analysis revealed that miR-26b was significantly downregulated in the miR-26b

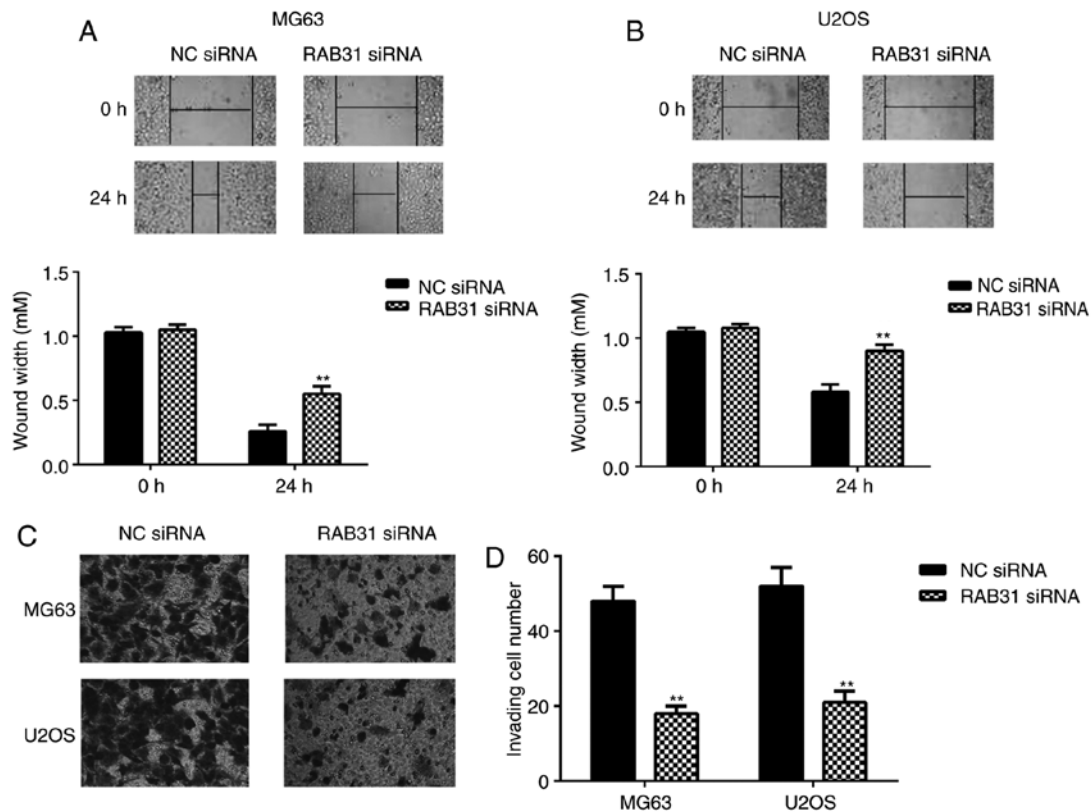


Figure 3. Effects of RAB31 silencing in OS migration and invasion. (A) Silencing of RAB31 expression significantly inhibited the migration of MG63 and (B) U2OS cells compared with the NC siRNA groups. (C) Representative images and (D) quantification showing that silencing of RAB31 significantly inhibited the invasion of MG63 and U2OS cells compared with the NC siRNA groups. Data are presented as the mean \pm standard deviation. ** $P < 0.01$ vs. NC siRNA. RAB31, Ras-related protein Rab-31; siRNA, small interfering RNA; NC, negative control. Magnification for cell migration $\times 40$ and for cell invasion $\times 400$.

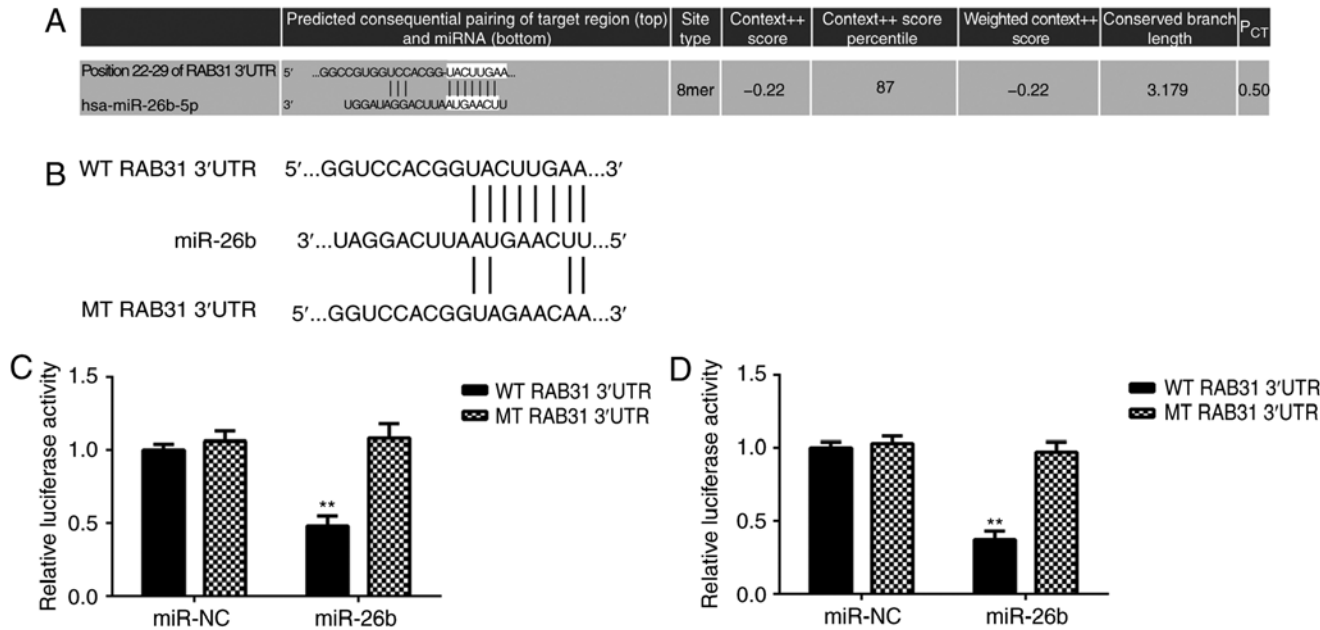


Figure 4. RAB31 is a target gene of miR-26b in OS cells. (A) TargetScan analysis results predicted that RAB31 may be a putative target gene of miR-26b. (B) WT and MT RAB31 luciferase reporter plasmids were generated. (C) Transfection with miR-26b mimic significantly inhibited the luciferase activity in MG63 and (D) U2OS cells expressing the WT RAB31 3'UTR reporter plasmid; however, no effect was observed in cells expressing the MT reporter plasmid. Data are presented as the mean \pm standard deviation. ** $P < 0.01$ vs. miR-NC. RAB31, Ras-related protein Rab-31; miR, microRNA; NC, negative control; WT, wild-type; MT, mutant; 3'UTR, 3'-untranslated region.

inhibitor-transfected group compared with the NC inhibitor-transfected group (Fig. 5D). As presented in Fig. 5E and F,

the mRNA and protein expression levels of RAB31 were significantly increased in the miR-26b inhibitor-transfected

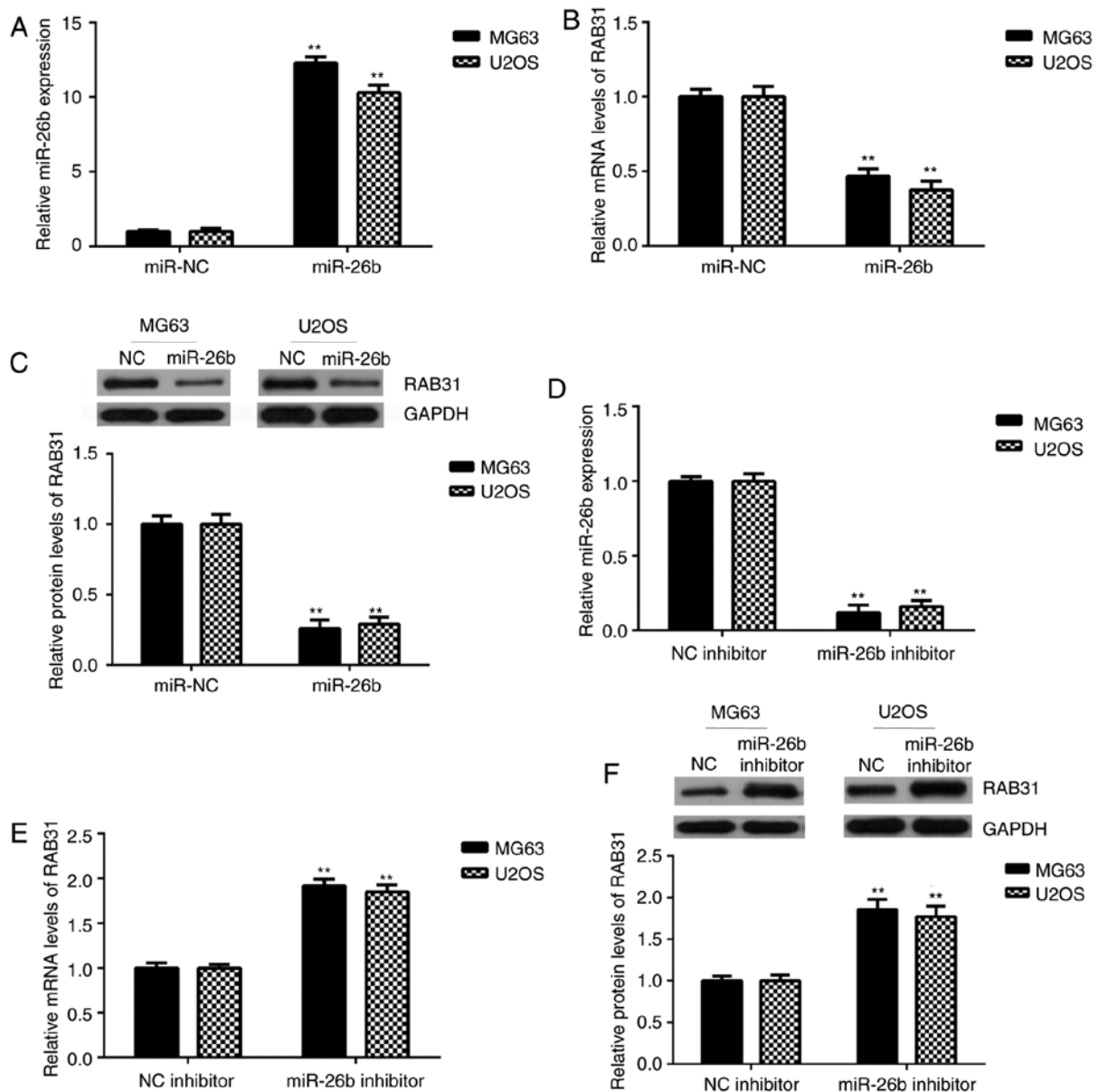


Figure 5. RAB31 is negatively regulated by miR-26b in OS cells. (A) miR-26b was significantly upregulated in MG63 and U2OS cells following transfection with miR-26b mimic compared with the miR-NC group. **P<0.01 vs. miR-NC. (B) mRNA and (C) protein expression levels of RAB31 were significantly decreased following miR-26b overexpression. **P<0.01 vs. miR-NC. (D) miR-26b was significantly downregulated in MG63 and U2OS cells following transfection with miR-26b inhibitor when compared with the NC inhibitor group. **P<0.01 vs. NC inhibitor. (E) mRNA and (F) protein expression levels of RAB31 were significantly decreased following miR-26b inhibition. **P<0.01 vs. NC inhibitor. Data are presented as the mean \pm standard deviation. miR, microRNA; NC, negative control; RAB31, Ras-related protein Rab-31.

group compared with the NC inhibitor-transfected group. These data indicated that RAB31 was negatively regulated by miR-26b in OS cells.

miR-26b expression is inversely correlated with RAB31 expression in OS tissues. RT-qPCR analysis identified that miR-26b levels were significantly lower in OS tissues compared with matched adjacent non-tumor tissues (Fig. 6A). Additionally, an inverse correlation was identified between the miR-26b and RAB31 expression levels in OS tissues (Fig. 6B), which suggested that the increased expression levels of RAB31 in OS tissues may be due to downregulation of miR-26b. Furthermore, miR-26b levels were demonstrated to be decreased in OS cell lines compared with hFOB 1.19 cells (Fig. 6C).

Discussion

To the best of our knowledge, the function of RAB31 in OS has previously been unclear. The present study revealed that RAB31 was upregulated in OS tissues and cell lines, and that high RAB31 expression levels were associated with malignant progression and a poor prognosis for patients with OS. Silencing of RAB31 expression significantly inhibited OS cell proliferation, cell cycle progression, migration and invasion, and induced cell apoptosis. RAB31 was then identified as a direct target gene of miR-26b. Additionally, the expression of RAB31 was negatively regulated by miR-26b in OS cells. Furthermore, miR-26b was downregulated in OS tissues and reversely correlated to the expression levels of RAB31.

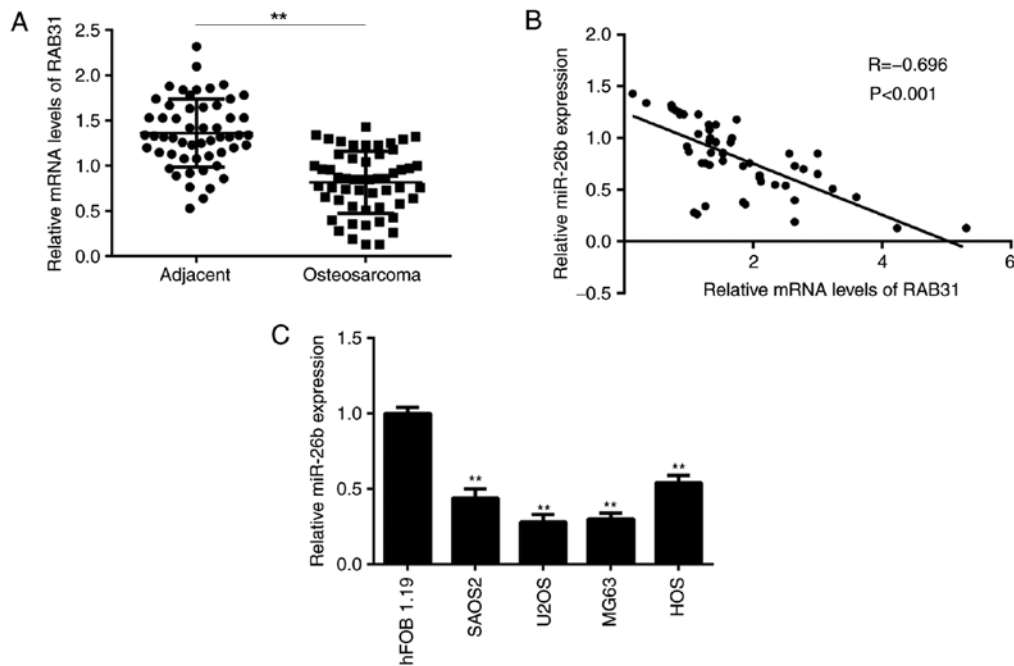


Figure 6. miR-26b expression is inversely correlated with RAB31 expression in OS tissues. (A) miR-26b levels are significantly lower in osteosarcoma tissues compared with adjacent non-tumor tissues. ** $P < 0.01$ vs. adjacent. (B) A significant inverse correlation was identified between miR-26b and RAB31 expression levels in osteosarcoma tissues. (C) miR-26b levels were significantly lower in osteosarcoma cell lines compared with the osteoblastic cell line hFOB 1.19. ** $P < 0.01$ vs. hFOB 1.19. Data are presented as the mean \pm standard deviation. miR, microRNA; RAB31, Ras-related protein Rab-31.

Initially, RAB31 was reported to be upregulated in breast cancer and high expression of RAB31 is significantly associated with worse outcome for patients (27). Furthermore, the mucin 1 C-terminal subunit (MUC1-C) oncoprotein and RAB31 have been demonstrated to function in an autoinductive loop that contributes to overexpression of MUC1-C in breast cancer cells (28). Grismayer *et al* (10) reported that RAB31 overexpression increases breast cancer cell proliferation, reduces adhesion and invasion *in vitro*, and results in decreased lung metastasis formation *in vivo*. In addition, RAB31 has been revealed to promote hepatocellular carcinoma progression by inhibiting tumor cell apoptosis induced by the PI3K/AKT/Bcl-2/Bax pathway (9). However, no previous study has focused on the function of RAB31 in OS.

The present study first examined the expression levels of RAB31 in OS tissues and cell lines. The data demonstrated that RAB31 was significantly upregulated in OS tissues compared with adjacent non-tumor tissues. Furthermore, it was identified that high expression of RAB31 was significantly associated with lung metastasis and advanced clinical stage in patient with OS, which suggested that an upregulation of RAB31 may contribute to OS progression. Additionally, patients with OS exhibiting high RAB31 expression had shorter survival times compared with patients exhibiting low RAB31 expression, which indicates that RAB31 may be used as a novel predictor for the prognosis of patients with OS. Consistent with the clinical data, RAB31 was also significantly upregulated in OS cell lines compared with the normal human osteoblastic hFOB 1.19 cell line. To further clarify the function of RAB31 in OS, two common OS cell lines MG63 and U2OS were used to perform *in vitro* experiments. The results demonstrated that silencing of RAB31 expression significantly reduced the proliferation, cell cycle progression, migration and invasion of

OS cells, and induced cell apoptosis. Therefore, the current results suggested that RAB31 may have a promoting role in regulating the malignant phenotype of OS cells. These findings indicate that targeting RAB31 may be a promising strategy for the treatment of OS.

The mechanism underlying RAB31 expression in OS was further investigated in the current study. As a large number of genes are regulated by miRNAs (11,12), bioinformatics analysis was used to predict the putative miRNAs that may directly target RAB31. Among the predicted miRNAs, miR-26b was selected as it has been reported to function as a tumor suppressor in OS (24). Duan *et al* (24) identified that miR-26b inhibits OS metastasis by targeting CTGF and Smad1. Zheng *et al* (21) reported that miR-26b represses OS cell migration and invasion by inhibiting the expression of PFKFB3. In addition, miR-26b has been demonstrated to inhibit OS cell proliferation, migration and invasion, and induce cell apoptosis by inhibiting PFKFB3-driven glycolysis (20). To further clarify the association between miR-26b and RAB31, the present study performed a luciferase reporter gene assay, which confirmed that RAB31 was a target gene of miR-26b. In addition, it was identified that the expression of RAB31 was negatively regulated by miR-26b in OS cells and that miR-26b levels were inversely correlated with the expression levels of RAB31 in OS tissues. These findings suggested that downregulation of miR-26b may contribute to the upregulation of RAB31 in OS. A limitation of the current study is that the effects of miR-26b on proliferation, apoptosis, migration and invasion were not investigated; this should be performed in future studies.

In conclusion, to the best of our knowledge, the present study was the first to demonstrate that RAB31 was upregulated in OS, was significantly associated with malignant

progression and a poor prognosis for patients with OS, and that RAB31 silencing inhibited the malignant phenotype of OS cells. Furthermore, a novel association between RAB31 and miR-26b, a tumor suppressor in OS, was identified. Therefore, the present results suggested that RAB31 may be a promising therapeutic target for OS.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

QW designed the study and wrote the manuscript. QF collected sample tissues and performed statistical analysis. YX and XL performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Nanchang University (Nanchang, China). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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