MiR-20b-5p Targets KIF23 to Suppress Cell Proliferation and Migration in Osteosarcoma

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Research

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Abstract

Background: MicroRNAs (miRNAs) are closely correlated with the development of cancer. Here, the main goal of this study was to analyze the biological function of miR-20b-5p in osteosarcoma (OS).

Methods: The expression of miR-20b-5p and kinesin family member 23 (KIF23) was determined using quantitative real time PCR and western blot analysis, respectively. The correlation between miR-20b-5p or KIF23 expression and the clinicopathological characteristics of OS was evaluated using Chi-square test. Functional experiments, including CCK-8, flow cytometry and transwell assay were performed to analyze cell proliferation, cell cycle distribution and apoptosis, and migration. The association between miR-20b-5p and KIF23 was confirmed by luciferase reporter assay.

Results: Firstly, we observed that miR-20b-5p expression was significantly decreased, while kinesin family member 23 (KIF23) expression was increased in OS tissues. Moreover, miR-20b-5p expression was inversely correlated with KIF23 expression in OS tissues. Clinically, decreased miR-20b-5p expression was significantly associated with increased metastasis and increased KIF23 mRNA expression level was remarkably correlated with increased tumor size. Overexpression of miR-20b-5p significantly suppressed cell proliferation and migration, as well as caused G0/G1 phase arrest and apoptosis in OS cells (U2OS and MG-63). Mechanistically, miR-20b-5p directly targeted to the 3'-UTR of KIF23 and negatively regulated its expression in OS cells. Moreover, KIF23 knockdown imitated, while overexpression abolished the effects on U2OS cell proliferation, G1/S transition, apoptosis and migration induced by miR-20b-5p overexpression. Furthermore, KIF23 overexpression reversed the miR-20b-5p-induced downregulation of CDK4, Cyclin D1, Bcl-2, PCNA, Ki-67 and N-cadherin, as well as upregulation of Bad and E-cadherin in U2OS cells.

Conclusions: In conclusion, miR-20b-5p directly targeted the 3'UTR of KIF23 mRNA to inhibit the proliferation and migration of human OS cells in vitro, which might be a promising candidate target for OS treatment.

Introduction

Osteosarcoma (OS) is an aggressive malignant neoplasm that is most common in the young (< 20 years old) and elderly (over 60 years old) than individuals of intermediary age (20–60 years old) [1]. It is clinically characterized as pain, swelling and limitation of joint mobility [2]. Despite multimodal treatment strategies, including surgical resection and combination of high-dose methotrexate, doxorubicin and cisplatin, patients with OS continue to have poor survival prognosis [3]. Molecular-targeting therapies have been recently emerged as an attractive treatment strategy for OS [4], which requires us to have a better understanding on the molecular mechanisms underlying the pathogenesis of OS.

MicroRNAs (miRNA) are a group of transcripts with 20–24 nucleotides in length without protein-coding potential [5]. Since is was first reported in 1993, a decades of studies have ensued the miRNAs biogenesis, number and action mechanism [6]. It is now recognized that amplification and overexpression
of oncogenic miRNAs or downregulation and loss of anti-oncogenic miRNAs are linked to the occurrence of nearly all types of human tumors, including OS [7]. What's more, miRNAs regulate the cell proliferation, migration and invasion in OS cells via targeting various mRNAs [8]. MiR-20b-5p as the miR-17 family member is transcribed from the miR-106a ~ 363 cluster, which can form an auto-regulatory feedback loop with E2F1 to regulate the proliferation and differentiation of myoblasts [9]. miR-20b-5p has been implicated as a critical modulator in renal cell carcinoma biology, as reflected by suppression of cellular growth and migration [10]. By regulating MAPK/ERK signaling pathway, miR-20b-5p also exerts its anti-tumor effects in papillary thyroid carcinoma cells [11]. Interestingly, miR-20b-5p has been reported to be downregulated in OS patients with pathologic fractures [12], but its biological function and regulatory mechanisms remain unclear in OS cells.

The kinesin family member 23 (KIF23, also known as CHO1, KNSL5, MKLP1 and MKLP-1), mapped to chromosome 15q23 [13], encodes a member of microtubule-dependent molecular motors that is implicated in the intracellular transport of organelles during cell mitosis in tumor cells [14]. Indeed, amplification of KIF23 has been identified in some types of tumors, including glioma [15], lung cancer [16], hepatocellular carcinoma [17], and malignant pleural mesothelioma [18], which indicate that KIF23 might be an oncogene in human malignant tumors. Recent studies showed that KIF23 can be regulated by Myb-MuvB [19], TCF-4 [20], DREAM and MMB complexes [21]. Interestingly, KIF23 was revealed to be strongly and inversely related to miR-328 expression pattern in glioblastoma [22]. However, the relationship between KIF23 and miRNA family members remains unknown in OS.

Considering the importance of miR-20b-5p and KIF23 in the development of cancer, we sought to explore the expression pattern of these two genes in OS tissues as a complementary tool to enhance our knowledge on OS etiology. Spearman's correlation analysis was carried out to ascertain whether miR-20b-5p expression was correlated with KIF23. Moreover, we further explored whether miR-20b-5p inhibits the proliferation and migration capacity of OS cells through directly targeting KIF23.

**Materials And Methods**

**Clinical tissue collection**

OS tissues and adjacent normal tissues were collected from 35 patients at the First Affiliated Hospital of Jinzhou Medical University (Liaoning Province, China) between 2016 and 2018, which were immediately frozen in liquid nitrogen for subsequent quantitative real time PCR analysis. Meanwhile, the corresponding basic clinicopathological characteristics were recorded. Before surgery, all the patients have not received any radiotherapy or chemotherapy. All participants signed the written informed consent and this work obtained the approval from the Ethics Committee of First Affiliated Hospital of Jinzhou Medical University (Approval number: JM3643; 2017.7.23).

**Cell culture conditions**
Human OS cell lines, including U2OS, MG-63 and Saos-2, as well as osteoblast cell line (hFOB1.19) were obtained from Shanghai Institute for Biological Sciences (Shanghai, China), which were all cultured in DMEM (Solarbio, Beijing, China) supplemented with 10% FBS (Beyotime, China) and maintained at 37 °C in a humidified incubator containing 5% CO₂.

**Cell transfection**

MiR-20b-5p mimics and miRNA negative control (miR-NC), as well as small interfering RNA targeting KIF23 (si-KIF23) and si-NC were provided by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The empty vector (pcDNA3.1) and the plasmids containing KIF23 were purchased from GenePharma Co., Ltd. (Shanghai, China). For miR-20b-5p overexpression, miR-20b-5p mimics or miR-NC at a final concentration of 50 nM was transfected into MG-63 or U2OS cells seeded in a 6-well plate at a density of 5 × 10^5 cells per well. For KIF23 knockdown, si-KIF23 or si-NC was transfected into U2OS cells (5 × 10^5 cells per well). In the rescue experiments, co-transfection of miR-20b-5p mimics and KIF23 was performed in U2OS cells. The above cell transfection was conducted using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After 48 h, transfected cells were harvested for further experiments.

**Quantitative real time PCR**

Tissue samples and cells were pre-treated with an RNA extraction kit (Takara, Beijing, China) to isolate total RNA. Total 2 µg of total RNA was used to synthesize the cDNA using an ABScript II cDNA First Strand Synthesis kit (Invitrogen). Quantitative real time PCR analysis was performed using A SYBR Premix Ex Taq™ Real-Time PCR Kit (Thermo Fisher Scientific, Inc.) on an ABI PRISM 7500 sequence detection system. The relative expression of miR-20b-5p and KIF23 was calculated by the 2^{-ΔΔCq} method with U6 and GAPDH as the internal controls, respectively.

**Cell proliferation assay**

The MG-63 or U2OS cells at a density of 3,000 cells per well were seeded in 96-well plates and cultured in complete media overnight. At 24, 48 and 72 h after seeding, cells were incubated with 10 µl CCK-8 reagent (Beyotime Institute of Biotechnology) at 37 °C for 2 h. Subsequently, the absorbance value was measured at 450 nm at the indicated time points using a microplate reader.

**Flow cytometry analysis**

For cell cycle analysis, transfected cells were seeded on 6-cm dishes (1 × 10^5 cells/dish) and collected until 80% confluence. Then, the cells were fixed with 0.8 ml of 70% ethanol for 30 min at 4 °C. After centrifugation, we discarded the ethanol and stained the cells with a propidium iodide (PI, 100 µg/ml) solution containing 10 µg/ml of DNase-free RNase A for 30 min at room temperature. Afterwards, stained cells were analyzed using flow cytometer (Cell Lab Quanta, Beckman Coulter). Similarly, apoptosis was detected by Annexin V-FITC/PI apoptosis detection kit (KeyGEN Biotech, Nanjing, China) following manufacturer’s instructions.
Transwell migration assay

The migration ability of MG-63 or U2OS cells was evaluated by transwell assay using Transwell chambers (BD Biosciences, CA, USA). Approximately $5 \times 10^4$ transfected cells were added to the upper chamber after suspended in 200 μL FBS-free DMEM medium. At the same time, FBS dissolved in 500 μl of culture medium was added to the lower chamber. Being cultivated for 24 h, the cells that migrated on the lower chamber were stained with 0.1% crystal violet. Five randomly fields were selected to count the number of migrated cells was counted using a light microscope.

Luciferase reporter assay

The wild-type (WT) and corresponding mutant (MUT) 3’-UTR of KIF23 in the miR-20b-5p binding sites was amplified by Shanghai GenePharma Co., Ltd. Then, the amplified WT KIF23 and MUT KIF23 were respectively inserted into the psiCHECK-2 vector (Promega Corporation, Madison, WI, USA). Subsequently, the chemically synthesized reporter vectors were co-transfected with miR-20b-5p mimics or miR-NC into MG-63 and U2OS cells using Lipofectamine 2000 reagent. The Dual Luciferase Reporter Assay kit (Promega Corporation) was utilized to analyze the relative luciferase activities after 48 h transfection.

Western blot analysis

RIPA lysis buffer (Beyotime, China) and enhanced BCA Protein Assay Kit were used to extract total protein and quantify protein concentration, respectively. After separated by 10% SDS-PAGE, the protein was transferred onto PVDF membranes (EMD Millipore). Following blocked with 5% fat-free milk prepared in TBST, the membranes were incubated with primary antibodies against KIF23, CDK4, Cyclin D1, Bad, Bcl-2, PCNA, Ki-67, E-cadherin, N-cadherin and GAPDH (all from Abcam, Cambridge, UK) overnight at 4 °C. After washing twice with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h at room temperature, followed by protein detection with an enhanced chemiluminescence kit (Beyotime, China).

Statistical analysis

The data were expressed as mean ± standard deviation (SD). Spearman's correlation analysis was conducted to assess the correlation between miR-20b-5p and KIF23. The correlation between miR-20b-5p or KIF23 expression and the clinicopathological characteristics of OS was evaluated using Chi-square test. The differences between two groups was evaluated by Student’s t-test and differences among more than two groups were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. The statistically significant differences were accepted if a value of $p$ less than 0.05.

Results

MiR-20b-5p was down-regulated in OS tissues and inversely correlated with KIF23 expression
Using quantitative real time PCR analysis, we first examined miR-20b-5p expression in OS and adjacent normal tissues. As presented in Fig. 1A, significantly decreased miR-20b-5p expression levels were found in OS tissues, in comparison with adjacent normal tissues. Next, we determined the expression of KIF23 mRNA and data showed KIF23 was significantly up-regulated in OS tissues, as compared with adjacent normal tissues (Fig. 1B). According to the median value of miR-20b-5p or KIF23 in tumor tissues, we used Chi-square test to analyze the correlation between miR-20b-5p or KIF23 expression and the clinicopathological characteristics of OS. As depicted in Table 1, decreased miR-20b-5p expression was significantly associated with increased metastasis ($p = 0.039$). As shown in Table 2, increased KIF23 mRNA expression level was remarkably correlated with increased tumor size ($p = 0.028$). Furthermore, the expression levels of KIF23 mRNA were inversely correlated with miR-20b-5p in OS tissues (Fig. 1C, $p = 0.0377$). Consistent with the expression of KIF23 mRNA in OS tissues, western blot analysis showed that KIF23 protein expression was obviously upregulated in OS tissues compared with matched adjacent normal tissues by selecting six paired tissue samples (Fig. 1D).

### Table 1

| Variables      | Cases (n = 35) | miR-23a-3p expression | $P$ value |
|----------------|---------------|-----------------------|-----------|
|                |               | Low (n = 19) | High (n = 16) | (chi-square test) |
| Sex            |               |             |             |                     |
| Male           | 24            | 13          | 11          | 0.983               |
| Female         | 11            | 6           | 5           |                     |
| Age            |               |             |             | 0.072               |
| < 30           | 23            | 15          | 8           |                     |
| ≥ 30           | 12            | 4           | 8           |                     |
| Tumor size (cm)|               |             |             | 0.601               |
| < 4            | 17            | 10          | 7           |                     |
| ≥ 4            | 18            | 9           | 9           |                     |
| TNM stage      |               |             |             | 0.817               |
| I-II           | 29            | 16          | 13          |                     |
| III-IV         | 6             | 3           | 3           |                     |
| Metastasis     |               |             |             | 0.039*              |
| Yes            | 22            | 9           | 13          |                     |
| No             | 13            | 10          | 3           |                     |
### Table 2
Association between KIF23 and clinicopathological features of patients with osteosarcoma

| Variables          | Cases (n = 35) | KIF23 expression | P value 
|--------------------|----------------|------------------|---------
|                    |                | High (n = 18)    | Low (n = 17) | (chi-square test) |
| Sex                |                |                  | 0.053    |
| Male               | 24             | 15               | 9        |
| Female             | 11             | 3                | 8        |
| Age                |                |                  | 0.903    |
| < 30               | 23             | 12               | 11       |
| ≥ 30               | 12             | 6                | 6        |
| Tumor size (cm)    |                |                  | 0.028*   |
| < 4                | 17             | 12               | 5        |
| ≥ 4                | 18             | 6                | 12       |
| TNM stage          |                |                  | 0.330    |
| I-II               | 29             | 16               | 13       |
| III-IV             | 6              | 2                | 4        |
| Metastasis         |                |                  | 0.826    |
| Yes                | 22             | 11               | 11       |
| No                 | 13             | 7                | 6        |

**Up-regulation of miR-20b-5p inhibited cell proliferation and migration in OS**

To investigate the function role of miR-20b-5p in OS in vitro, we compared the expression of miR-20b-5p between OS cell lines and hFOB1.19. The results showed that the expression of miR-20b-5p was significantly decreased in all detected OS cell lines, in comparison with hFOB1.19 cells (Fig. 2A). Among the three OS cell lines, MG-63 and U2OS exhibited relatively lower miR-20b-5p expression, which were thus used for further functional experiments. Next, MG-63 and U2OS cells were transfected with miR-20b-5p mimics or miR-NC to perform gain-of-function assay. Quantitative real time PCR analysis demonstrated MG-63 and U2OS cells transfected with miR-20b-5p mimics presented a significant increase of miR-20b-5p expression (Fig. 2B). CCK-8 assay revealed that miR-20b-5p overexpression remarkably suppressed the proliferation of MG-63 (Fig. 2C) and U2OS (Fig. 2D) cells compared with miR-NC transfection. Flow cytometry analysis further demonstrated that overexpression of miR-20b-5p caused cell cycle G0/G1 phase arrest (Fig. 2E) and promoted apoptosis (Fig. 2F) in both MG-63 and
U2OS cells. Additionally, transwell assay demonstrated that the migratory capacity of MG-63 and U2OS cells was significantly decreased after miR-20b-5p overexpression (Fig. 2G).

Kif23 Was Directly Targeted By Mir-20b-5p

To elucidate the molecular mechanisms that miR-20b-5p regulated OS cell proliferation and migration, the putative targets of miR-20b-5p were predicted by bioinformatics analysis. As depicted in Fig. 3A, the partially complementary matching the miR-20b-5p seed sequences in the 3'UTR regions of KIF23 were presented. Accordingly, luciferase reporter assay was performed and demonstrated that the luciferase activity of the plasmid carrying the WT KIF23 3'-UTR was significantly reduced, while that carrying the MUT KIF23 3'-UTR did not obviously change by miR-20b-5p overexpression in both MG-63 (Fig. 3B) and U2OS (Fig. 3C) cells. Furthermore, we analyzed the effect of miR-20b-5p on endogenous KIF23 expression in OS cells. Both quantitative real time PCR (Fig. 3D) and western blot analysis (Fig. 3E) proved that the expression levels of KIF23 mRNA and protein were decreased in MG-63 and U2OS cells following overexpression of miR-20b-5p. These results suggest that miR-20b-5p directly targeted the 3'UTR of KIF23 in OS cells.

Silenced Kif23 Restrained Os Cell Proliferation And Migration

Subsequently, loss-of-function assay was carried out to explore the role of KIF23 in OS cells. U2OS cells were selected to transfected with si-KIF23 to achieve KIF23 silenced cells, which was confirmed by western blot analysis (Fig. 4A). CCK-8 assay revealed that silencing KIF23 expression restricted the proliferation of U2OS cells (Fig. 4B). Moreover, knockdown of KIF23 induced cell cycle G0/G1 phase arrest (Fig. 4C) and apoptosis (Fig. 4D) in U2OS cells. In addition, the number of migrated cells was significantly decreased in si-KIF23 group compared with si-NC group in U2OS cells (Fig. 4E). Hence, inhibition of KIF23 exhibited roles similar to those exhibited by miR-20b-5p overexpression in OS cells.

Restoration of KIF23 partially abolished the decreased proliferation and migration caused by miR-20b-5p overexpression

To further confirm whether the suppressive role of miR-20b-5p in OS was achieved by inhibiting KIF23, rescue experiments were performed in U2OS cells by co-transfecting with KIF23 overexpression plasmid and miR-20b-5p mimics. As shown in Fig. 5A, the down-regulation of KIF23 by miR-20b-5p mimics was notably elevated after KIF23 overexpression plasmid. Of note, the restoration of KIF23 abolished the influence of miR-20b-5p overexpression on U2OS cell proliferation (Fig. 5B), G1/S transition (Fig. 5C), apoptosis (Fig. 5D) and migration (Fig. 5E). To further confirm these effects, western blot analysis was performed to analyze the protein expression levels associated with proliferation, G1/S transition, apoptosis and EMT markers. The results demonstrated that KIF23 overexpression reversed the miR-20b-5p-induced downregulation of CDK4, Cyclin D1, Bcl-2, PCNA, Ki-67 and N-cadherin, as well as
upregulation of Bad and E-cadherin (Fig. 6A-B). These results support the conclusion that the suppressive role of miR-20b-5p in OS cells mainly depends on its negative regulation of KIF23 expression.

**Discussion**

Accumulating evidence indicates that miRNAs can bind to multiple genes, including oncogenes and anti-oncogenes, to regulate cellular biology of human cancer cells [23]. Here, we observed the downregulation of miR-20b-5p and upregulation of KIF23 in OS tissues compared with matched normal tissues. Indeed, miR-20b-5p has been shown to be down-regulated in renal cell carcinoma [24], gastric cancer [25], and papillary thyroid carcinoma [11]. Decreased miR-20b-5p expression was associated with increased metastasis. Consistently, a recent study by Ulivi et al. [26] who have shown that circulating high levels of miR-20b-5p is a good predictor for metastatic colorectal cancer patients after bevacizumab treatment. KIF23 has been often found expressed at high levels in multiple types of cancer and considered to be an important oncogene during cancer carcinogenesis [15–17]. Moreover, the expression of miR-20b-5p was inversely correlated with KIF23, indicated that miR-20b-5p/KIF23 signaling axis may affect the biological behavior of OS cells.

Overexpression of miR-20b-5p suppressed proliferation and migration of OS cells. Accumulating evidence has elucidated the functional role of miR-20b-5p in various diseases, including tumor cells. For instance, miR-20b-5p has been implicated in muscle development through directly binding to the 3′UTR of E2F1 mRNA [9]. KIF23 is a key player in regulating PTEN and BRAC1 levels in breast cancer pathologies [27]. Downregulation of miR-20b-5p promotes papillary thyroid carcinoma cells growth and metastasis through controlling son of sevenless homolog 1 (SOS1) and extracellular signal-regulated kinase 2 (ERK2) [11]. In the present study, KIF23 was confirmed as a direct target of miR-20b-5p by performing bioinformatic analysis and luciferase reporter assay. The loss-of-function assays revealed that silenced KIF23 could imitate the anti-tumor effects of miR-20b-5p in OS cells. According to a recent study by Fischer et al. [21], nearly any repression effect of KIF23 expression was lost after mutation of the cell cycle genes homology region (CHR) element in the KIF23 gene and CHR is the major element mediating p53-dependent repression of KIF23 expression. Here, we showed that reintroduction of KIF23 reversed the effects of miR-20b-5p on U2OS cell proliferation, G1/S transition, apoptosis and migration, indicating that KIF23 might be an oncogene in OS. Although the expression of KIF23 depends on p53 status, the oncogenic role KIF23 in OS cells might be explained by mutation of the CHR element in the KIF23 gene.

Below we will discuss the possible molecular mechanisms responsible for the promotive effects of KIF23 on OS cells proliferation, cell cycle progression, apoptosis and migration. KIF23 as a nuclear protein, has been shown to act as a plus-end-directed motor enzyme involved in controlling microtubule dynamics [28]. Formation of the centralspindlin complex is known to be essential for mitosis [21]. Recent studies have implicated Ki-67 and PCNA as two response factors of KIF23-induced proliferation in gastric cancer cells [29]. In the current study, KIF23 was expressed at a very low level in OS cells with miR-20b-5p overexpression, indicating the alteration of central spindlin complex and abnormal expression of the downstream proliferation-associated molecules, therefore miR-20b-5p can indirectly inhibit OS cells
proliferation. Here, we confirmed that KIF23 overexpression reversed the miR-20b-5p-induced downregulation of PCNA and Ki-67 in U2OS cells. Moreover, restoration of KIF23 abolished the miR-20b-5p-induced downregulation of CDK4, Cyclin D1 and Bcl-2 and upregulation of Bad in U2OS cells. In a previous study, KIF23 was identified as a miR-424-targeted gene [30]. Restoration of miR-424 levels in glioma cells led to strong suppression of epithelial-to-mesenchymal transition (EMT), contributing to decreased migration and invasion [30]. EMT is a central program that allows epithelial acquire mesenchymal to obtain the capacity to induce cancer cells migration and invasion. Here, we further confirmed that KIF23 overexpression reversed the miR-20b-5p-induced upregulation of E-cadherin and downregulation of N-cadherin, resulting in decreased migration in OS cells.

In conclusion, our principal finding is that miR-20b-5p could inhibit OS cell proliferation and migration through targeting KIF23. Despite the precise molecular mechanism regarding miR-20b-5p/KIF23 signaling needs to be further elucidated, the present study may provide novel insights and new therapeutic opportunities in OS.

**Abbreviations**

OS, osteosarcoma; KIF23, kinesin family member 23; miR-NC, miRNA negative control; WT, wild-type; MUT, mutant; SD, standard deviation

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of First Affiliated Hospital of Jinzhou Medical University (Approval number: JM3643; 2017.7.23) and in accordance with Helsinki Declaration.

**Authors’ contributions**

GYZ conceived and designed the study. WZY and WJ mainly performed the experiments and gathered the data. WCB analyzed the data. CYT wrote the paper. ZZ revised the manuscript. All authors read and approved the final manuscript.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data are included in this published article.

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Competing interests

The authors declare that they have no competing interests.

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### Figures

**Figure 1**

![Image of Figure 1](image_url)

A. Comparison of relative miR-20b-5p and KIF23 expression in adjacent tissues and OS tissues. **p < 0.001 (n = 35)**

B. Comparison of relative KIF23 expression in adjacent tissues and OS tissues. **p < 0.001 (n = 35)**

C. Correlation between relative miR-20b-5p and KIF23 expression. **r = -0.3527, p = 0.0377**

D. Western blot analysis of KIF23 and GAPDH expression in different samples (N1 to T6).
The inverse correlation between miR-20b-5p and KIF23 mRNA levels was found in OS tissues. (A) Expression level of miR-20b-5p in 35 pairs of OS and normal adjacent tissues were detected using quantitative real time PCR. (B) KIF23 mRNA expression was evaluated by quantitative real time PCR in 35 pairs of OS and normal adjacent tissues. (C) Spearman’s correlation analysis was applied to investigate the correlation between the expressions of miR-20b-5p and KIF23 mRNA in OS tissues. (D) The expression of KIF23 protein was detected in six paired tumor tissues and adjacent normal tissues derived from OS patients using western blot analysis. N: normal; T: tumor

Figure 2

The effects of miR-20b-5p overexpression on the proliferation and migration of OS cells. (A) The expression of miR-20b-5p in three OS cell lines (U2OS, MG-63 and Saos-2) and normal osteoblast cell line hFOB1.19 was detected by quantitative real time PCR analysis. **p < 0.01, ***p < 0.001, compared with hFOB1.19; MG-63 and U2OS cells were transfected with miR-20b-5p mimics or miR-NC, respectively. (B) The expression of miR-20b-5p was determined using quantitative real time PCR analysis. CCK-8 assay was used to analyze cell proliferation in transfected MG-63 (C) and U2OS (D) cells. Flow cytometry analysis was performed to determine the cell cycle progression (E) and apoptosis (F) in both MG-63 and U2OS cells. (G) Transwell assay was conducted to detect cell migration in transfected MG-63 and U2OS cells (magnification, ×100). *p < 0.05, **p < 0.01, ***p < 0.001, compared with miR-NC
KIF23 was the direct target gene of miR-20b-5p in OS cells. (A) The miR-20b-5p targeting sequences in the KIF23 3′-UTR and mutant KIF23 3′-UTR are shown. (B-C) Relative luciferase activity was measured in MG-23 and U2OS cells after co-transfection with psiCHECK-WT-KIF23-3′-UTR or psiCHECK-MUT-KIF23-3′-UTR and miR-20b-5p mimics or miR-NC. (D-E) The KIF23 mRNA and protein levels were detected by quantitative real time PCR and western blot analysis in MG-23 and U2OS cells transfected with miR-20b-5p mimics or miR-NC. **p < 0.01, ***p < 0.001, compared with miR-NC.
Silenced KIF23 restrained the proliferation and migration of OS cells. The si-KIF23 or si-NC was transfected into U2OS cells. (A) Western blot analysis was performed to detect KIF23 protein expression in the transfected U2OS cells. (B) CCK-8 assay was used to analyze cell proliferation in transfected U2OS cells. Flow cytometry analysis was performed to determine the cell cycle progression (C) and apoptosis (D) in transfected U2OS cells. (E) Transwell assay was conducted to detect cell migration in transfected U2OS cells (magnification, ×100). *p < 0.05, **p < 0.01, ***p < 0.001, compared with si-NC.
KIF23 was involved in miR-20b-5p regulation of OS cell proliferation and migration. U2OS cells were co-transfected with miR-20b-5p mimics and KIF23 overexpression plasmid. (A) KIF23 protein expression was restored in miR-20b-5p mimics-transfected U2OS cells through co-transfection with KIF23. (B) CCK-8 assay was used to analyze cell proliferation in transfected U2OS cells. Flow cytometry analysis was performed to determine the cell cycle progression (C) and apoptosis (D) in transfected U2OS cells. (E) Transwell assay was conducted to detect cell migration in transfected U2OS cells (magnification, ×100). **p < 0.01, ***p < 0.001, compared with miR-NC + vector; #p < 0.05, ##p < 0.01, compared with miR-20b-5p mimics + vector.

Figure 6
Detection of protein expression associated with cell proliferation and EMT markers. U2OS cells were co-transfected with miR-20b-5p mimics and KIF23 overexpression plasmid. Western blot analysis was
performed to measure the protein levels associated with G1/S transition and apoptosis (A), as well as proliferation and EMT process (B) in transfected U2OS cells. **p < 0.01, ***p < 0.001, compared with miR-NC + vector; ##p < 0.01, ###p < 0.001, compared with miR-20b-5p mimics + vector