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MicroRNA-373 Promotes Growth and Cellular Invasion in Osteosarcoma Cells by Activation of the PI3K/AKT–Rac1–JNK Pathway: The Potential Role in Spinal Osteosarcoma

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Spinal osteosarcoma (OS) has been proven to be more difficult to treat owing to potently malignant metastasis. The present study aimed to explore the functional role of microRNA (miR)-373 in cell growth and invasion of OS cells, as well as its underlying mechanism. The expression of miR-373 was analyzed in spinal OS tissues and cell lines. MG-63 cells were transfected with the miR-373 mimic or inhibitor and/or treated with the phosphoinositide 3-kinase (PI3K) (LY294002) inhibitor or Ras-related C3 botulinum toxin substrate 1 (Rac) guanosine triphosphate (GTPase) (NSC23766) inhibitor, and then the impact of miR-373 aberrant expression on cell growth and invasion was measured, along with the impact of overexpressing miR-373 on the expression of p53 and PI3K/AKT pathway-related proteins. We found that miR-373 was specifically upregulated in spinal OS tissues ($p<0.01$) and OS cell lines ($p<0.01$ or $p<0.001$). Moreover, miR-373 expression was significantly associated with TNM stage ($p=0.035$) and tumor size ($p=0.002$). Overexpression of miR-373 promoted MG-63 cell viability, migration, invasion, and colony formation (all $p<0.05$), while silencing of miR-373 and LY294002 exerted the opposite effects. Additionally, miR-373 overexpression downregulated p53 as well as its downstream targeted genes and orderly activated the PI3K/AKT–Rac1–JNK signaling pathway. In conclusion, miR-373 promotes growth and cellular invasion in OS cells by activating the PI3K/AKT–Rac1–JNK pathway. Therefore, miR-373 might be a candidate for molecular targeted therapy of spinal OS.

Key words: MicroRNA-373 (miR-373); Metastasis; Spinal osteosarcoma (OS); p53; PI3K/AKT–Rac1–JNK pathway

INTRODUCTION

Osteosarcoma (OS), the most prevalent malignancy of the bone, mainly occurs in adolescents and accounts for 42% of the primary bone sarcoma cases worldwide[1,2]. OS arises from stromal cell lines, and its rapid development is achieved by direct or indirect formation of neoplastic osteoid and bone tissues. A growing body of evidence has reported that OS exerts a high degree of malignancy with potent metastasis and poor prognosis[3,4]. Spinal OS accounts for 0.85%–3% of all OS patients[5]. Compared with OS of the extremities, spinal OS possesses different clinical characteristics such as older age (mean age of 38 years) and nervous system lesions[6]. It has frequently been proven to be more difficult to treat and have a worse prognosis relative to OS of the extremities because of its complex and critical involvement of anatomic sites[7]. There is a lack of effective options in the established therapy for spinal OS, and the long-term prognosis for patients has been dismal so far[8,9]. Therefore, to optimize therapeutic strategies for spinal OS, identification of sensitive and specific biomarkers for predicting the prognosis and understanding mechanisms is urgently required.

There is appreciable evidence confirming that micro RNAs (miRNAs), an abundant class of noncoding RNAs, are engaged in numerous biological processes, especially in human cancers, via modulating the expression of target genes or pathways[10–13]. Recently, it has been claimed that a host of miRNAs regulate tumor growth and metastasis

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of OS, such as miR-218, miR-214, miR-33a, miR-143, miR-183, and miR-29b[3,14–18].

Initially identified as the embryonic stem cell (ESC)-specific miRNA, the past decade has seen a noticeable emergence of miR-373 in tumors[19]. It has been validated as an oncogene in many types of cancers including esophageal cancer, hepatocellular carcinoma, breast cancer, gastric cancer, pancreatic cancer, and liver cancer[20–25]. In addition, solid documentation indicates its role as a tumor suppressor[26–28]. However, the role of miR-373 in OS has been unknown.

In this study, we detected miR-373 expression in human spinal OS, matched normal tumor-adjacent tissues, as well as osteoblast and OS cell lines. Moreover, the effects of miR-373 on cell viability, migration, invasion, and colony formation were measured in an OS MG-63 cell line. Furthermore, we explored the potential role of miR-373 in p53 expression and signaling pathways. The role of miR-373 in cell growth and the metastasis of OS cells as well as its underlying mechanism are expected to provide novel avenues for spinal OS.

MATERIALS AND METHODS

Participants and Clinical Samples

All specimens were collected from patients who underwent the resection of spinal primary OS in the hospital from March 2014 to February 2016. Subjects receiving chemotherapy or radiotherapy before surgery were excluded. Tumor stage of the patients was evaluated by TNM classification of the International Union against Cancer (UICC). A pair of OS and normal tumor-adjacent tissues was obtained from each patient. Afterward, tissues were transported to the laboratory and immediately stored at −80°C for further studies. Clinicopathological data of subjects are summarized in Table 1, including age, gender, tumor location, TNM stage, and tumor size. The present study was approved by the ethics committee, and all patients or guardians (for patients who were <18 years old) signed an informed consent.

Cell Culture

The human osteoblastic cell line hFOB1.19 and OS cell lines Saos-2, MG-63, and U2OS (American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin/streptomycin, and 2 mM L-glutamine (Seromed-Biochrom KG, Berlin, Germany). The four cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2 until reaching 70%–80% confluency judged via phase-contrast microscopy (Olympus Optical Co., Tokyo, Japan).

Transfection and Treatment

MG-63 cells were seeded at 1×10^5 cells/well of six-well plates overnight. The miR-373 mimic, inhibitor, and miR-control (GenePharma Co., Shanghai, P.R. China) were transfected into the MG-63 cell line on the next day using Lipofectamine 3000 reagent (Invitrogen-Life Technologies, Paisley, UK) based on the manufacturer's protocol. Thereafter, cells were exposed to the phosphoinositide 3-kinase (PI3K) LY294002 inhibitor (10 μM; Sigma-Aldrich, St. Louis, MO, USA)[29,30] or the Ras-related C3 botulinum toxin substrate 1 (Rac) guanosine

Table 1. Correlation Between Clinicopathological Features and miR-373 Expression in Patients With Spinal Osteocarcinoma ($n=10$)

		miR-373 Expression		
Features	Cases (n)	Low ($n=4$)	High ($n=6$)	p Value
Age (years)				0.429
≥18	6	3	3	
<18	4	1	3	
Gender				1.000
Male	5	2	3	
Female	5	2	3	
Location				0.429
Proximal	6	3	3	
Distal	4	1	3	
TNM stage				0.035
I+II	4	0	4	
III+IV	6	4	2	
Tumor size (cm)				0.002
<8	4	4	0	
≥8	6	0	6	

triphosphate (GTPase) NSC23766 inhibitor (50 μM; Calbiochem, San Diego, CA, USA)[31,32] for 24 h.

Quantitation of Cell Viability

The effects of aberrant miR-373 on MG-63 cell viability were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[33]. After transfection for 2–3 days, the control, negative control (NC), overexpressing miR-373, and silencing miR-373 cells were added into 0.5 mg/ml of MTT (Sigma-Aldrich). Then blue formazan (Sigma-Aldrich) products were dissolved by 100 μl of dimethyl sulfoxide (DMSO; Lonza, Walkersville, MD, USA) for 1 h. The percentage of living MG-63 cells was quantified on days 1, 2, 3, and 4 by absorbency at 530 nm on the automated plate reader (BioTek Instruments, Winooski, VT, USA).

Migration Assay

For 2-day transfection, MG-63 cells (2×10^6) of control, NC, overexpressing miR-373, and silencing miR-373 were seeded into six-well plates and cultured to 80% confluence. Afterward, the MG-63 cell line was scraped, washed by serum-free medium several times, and observed using a microscope (Olympus, Tokyo, Japan). All cells were refed with 10% FBS medium, and relative wound width was measured on days 1, 2, 3, and 4.

Invasion Assay

The control, NC, overexpressing miR-373, and silencing miR-373 MG-63 cell invasion was assessed after 2 days of transfection using a Transwell system containing an 8-μm pore size polycarbonate filter (Costar, Cambridge, MA, USA). In brief, 600 ml of RPMI-1640 medium of 0.5% FBS was applied to the lower chamber as a chemoattractant. After MG-63 cells were trypsinized (0.25% trypsin; Gibco) and suspended, they were grouped on the upper side of a polycarbonate filter, coated with 5 mg/ml Matrigel. Following incubations in 5% CO_2 at 37°C for 24 h, MG-63 cells invading into the lower compartment were counted with a colorimetric crystal violet assay.

Colony Formation

The control, NC, overexpressing miR-373, and silencing miR-373 MG-63 cells were liquated by trypsin-ethylene diamine tetraacetic acid (EDTA; Gibco) solution, respectively, after 2 days of transfection and diluted to a density of 1×10^4 cells per dish for 14 days. MG-63 cells were then fixed with 4% paraformaldehyde solution and stained with 0.1% crystal violet (Sigma-Aldrich) for 30 min. Pictures of the surviving colonies were captured under ChemiDoc XRS imaging system (Bio-Rad, Hercules, CA, USA).

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's protocol. A total of 2 μg of RNA was used to synthesize poly-oligo(dT) primed complementary DNA (cDNA) with the RevertAid First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany). Furthermore, the relative expression of miR-373 was normalized to the internal control (U6) via the equation $2^{-\Delta\Delta}$Ct. Primers for miR-373 and U6 were made with the miScript Primer Assay Kit (Qiagen, Dusseldorf, Germany). qRT-PCR for p53 from the control, NC, and silencing miR-373 MG-63 cells was carried out using FastStart Universal SYBR Green Master (Roche Diagnostics). The p53 primer sequences were 5′-CCCAAGCAATGGATGATTTGA-3′ (forward) and 5′-GGCATTCTGGGA GCTTCATCT-3′ (reverse)[34].

Western Blot

Standard Western blotting was conducted for protein expression assays from MG-63 cells with miR-373 mimic, inhibitor, and miR-control. Briefly, proteins were isolated with RIPA lysis buffer containing 1 mg of protease inhibitors (Applygen Technologies Inc., Beijing, P.R. China) after 2 days of transfection. The protein content was quantified using Bicinchoninic Acid (BCA) Protein Assay Kit (CoWin Biotech Co., Ltd., Beijing, P.R. China). The following primary antibodies p53 (ab1101), p21 (ab109520), p53 upregulated modulator of apoptosis (Puma; ab9643), B-cell lymphoma-2 associated X (Bax; ab32503), plasminogen activator inhibitor (PAI; ab66705), PI3K (ab86714), AKT (ab8805), Rac1; ab33186), c-Jun N-terminal kinase (JNK; ab124956), and the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were from Abcam (Cambridge, UK). Subsequently, secondary antibodies were marked by horseradish peroxidase for 2 h at 37°C. Samples were then electrotransferred to polyvinylidene fluoride (PVDF) microporous membrane (Millipore, Boston, MA, USA). The bands were visualized by the Odyssey CLx equipment (LI-COR Biosciences, Lincoln, NE, USA).

Statistical Analysis

Each group was assayed in triplicate, and results are expressed as mean ± standard deviation (SD). Analysis of differences between groups was performed by one-way analysis of variance (ANOVA) with SPSS Statistics 19.0 (IBM Corporation, New York, NY, USA). Correlation between miR-373 expression and clinicopathologic features was analyzed with chi-square test. Differences were considered significant with a value of $p<0.05$.

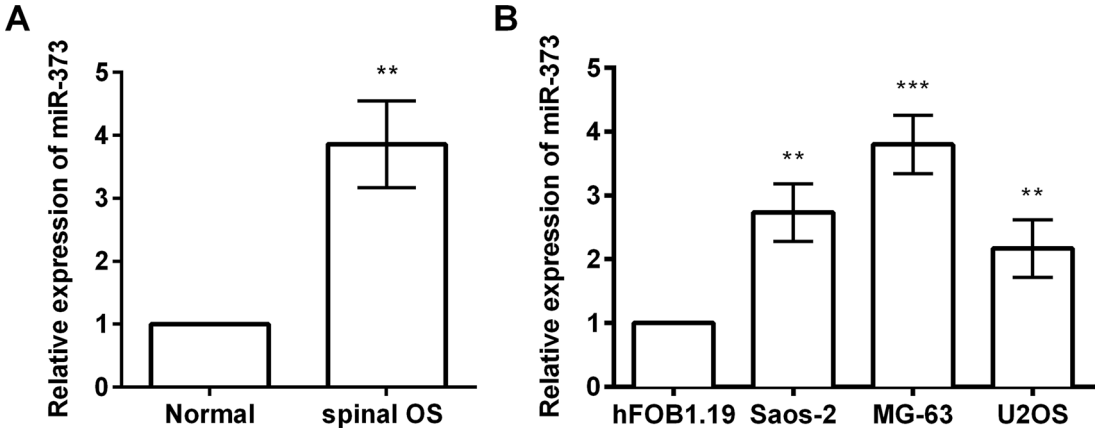

Figure 1. miR-373 is overexpressed in spinal OS tissues and cell lines. (A) The relative expression of miR-373 in normal and tumor tissues. (B) The relative expression of miR-373 in osteoblast cell line hFOB1.19 and OS cell lines Saos-2, MG-63, and U2OS. miR, microRNA; OS, osteosarcoma. $^{**}p<0.01$; $^{***}p<0.001$.

RESULTS

miR-373 Is Overexpressed in Spinal OS Tissues and OS Cell Lines

To investigate the functional role of miR-373 in spinal OS, we first enrolled patients with spinal primary OS ($n=10$). Tumor and adjacent nontumor tissues were collected. Expression levels of miR-373 in tissues were analyzed by qRT-PCR. miR-373 levels in spinal OS were significantly higher than in the normal tumor-adjacent tissues ($p<0.01$) (Fig. 1A). Thereafter, participants with spinal OS were divided into two groups, low ($n=4$) and high ($n=6$), based on the median value (1.93) of miR-373 expression in OS specimens. The correlation between the expression of miR-373 and clinicopathologic features including age, gender, tumor location, TNM stage, and tumor size were explored. The results demonstrated that the expression of miR-373 was significantly associated with TNM stage ($p=0.035$) and tumor size ($p=0.002$). However, no significant associations were observed between the expression of miR-373 and patient's age ($p=0.429$), gender ($p=1.000$), or location ($p=0.429$). Moreover, relative expression of miR-373 of three OS cell lines was further measured. We found that the expression of miR-373 was statistically higher than that in the osteoblast cell line hFOB1.19 ($p<0.01$ or $p<0.001$) (Fig. 1B). The results revealed that miR-373 was highly expressed in spinal OS tissues and OS cell lines and was correlated with the progression and development of spinal OS.

Transfection Efficiency of miR-373 in the MG-63 Cell Line

miR-control, miR-373 mimic, and miR-373 inhibitor were then transfected into the OS cell line MG-63 to explore the potential role of miR-373 in OS. We tested overexpression and silencing efficiency after 2 days of transfection and found a significant increase in miR-373 expression by the miR-373 mimic compared with the miR-control ($p<0.01$) (Fig. 2). Meanwhile, the knockout of miR-373 significantly reduced the mRNA expression of miR-373 ($p<0.01$). Accordingly, overexpression and knockout of miR-373 were effective, and the transferred MG-63 cells could be used in further studies.

miR-373 Promotes Cell Viability, Migration, Invasion, and Colony Formation of MG-63 Cell Line

To test the functional role of miR-373 in human OS cells, the effect of aberrant expression of miR-373 on MG-63 cell viability, migration, invasion, and colony formation was assessed. For cell viability, no difference was witnessed between the control and miR-control. Overexpressing miR-373 enhanced cell viability in a

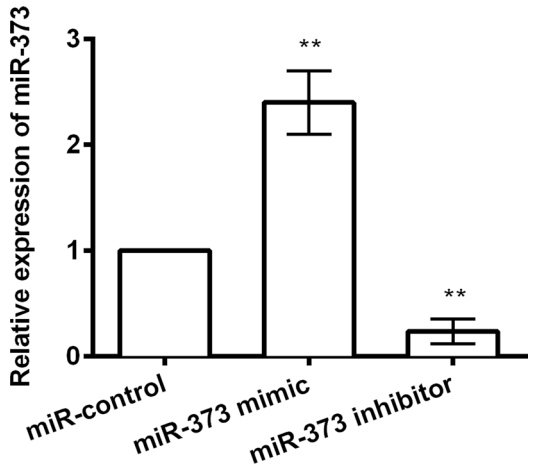

Figure 2. miR-373 expression with miR-control, miR-373 mimic, and miR-373 inhibitor in MG-63 cells. miR, microRNA. $^{**}p<0.01$.

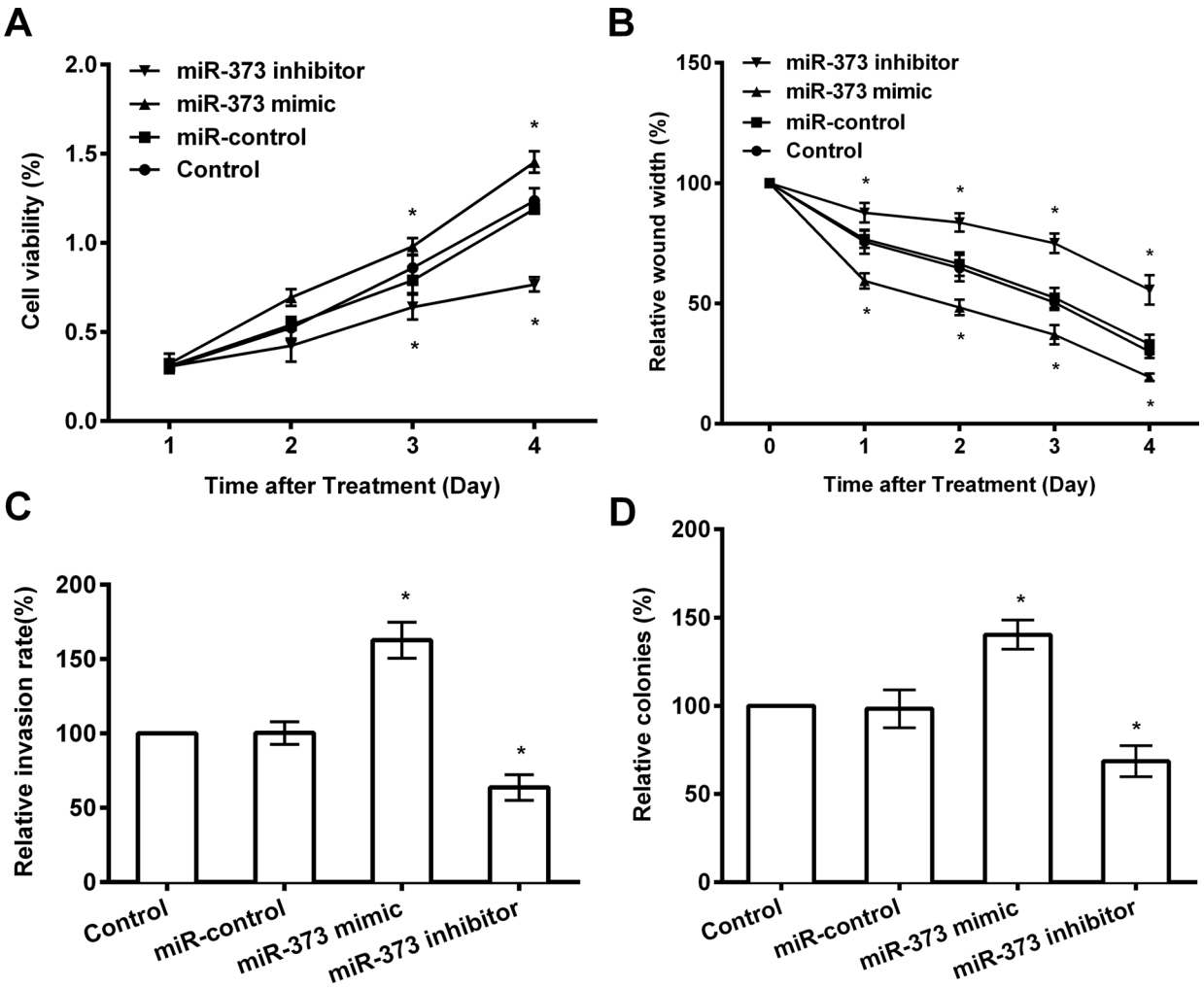

Figure 3. miR-373 promotes cell viability, migration, invasion, and colony formation of MG-63 cells. (A) The effects of aberrant miR-373 on cell viability of the MG-63 cell line. (B) The effects of aberrant miR-373 on cell migration of the MG-63 cell line. (C) The effects of aberrant miR-373 on cell invasion of the MG-63 cell line. (D) The effects of aberrant miR-373 on colony formation of the MG-63 cell line. miR, microRNA. *$p<0.05$.

time-dependent manner and caused a striking increase on days 3 and 4 (both $p<0.05$) (Fig. 3A), while MG-63 viability was remarkably reduced by the miR-373 inhibitor compared with the miR-control on days 3 and 4 (both $p<0.05$).

The cell migration of MG-63 affected by the aberrant expression of miR-373 was evaluated by wound healing analysis (Fig. 3B). Wound width was decreased in a time-dependent manner. Overexpressing miR-373 accelerated the decrease, and silencing miR-373 alleviated it by day 4 (all $p<0.05$). These data showed that overexpressing miR-373 enhanced the cell migratory ability of the MG-63 cell line, and silencing miR-373 inhibited it.

Furthermore, miR-373 overexpression statistically upregulated the invasion rate, while miR-373 knockout downregulated it (both $p<0.05$) in MG-63 cells (Fig. 3C). Overexpressing miR-373 effectively promoted colony formation in OS MG-63 cells compared to the miR-control, and silencing miR-373 presented the opposite effect (both $p<0.05$) (Fig. 3D). It was speculated that miR-373 might promote MG-63 cell reproduction.

miR-373 Can Downregulate p53 Expression in the MG-63 Cell Line

As a tumor suppressor, p53 has been proven to inhibit cell proliferation and metastasis in the MG-63 cell line[35]. Therefore, to explore the correlation of p53 and miR-373, we evaluated the effect of overexpressing miR-373 in different concentrations and times on p53 levels in the OS cell line MG-63. mRNA and protein expression of p53 was drastically suppressed by overexpressing miR-373 in a dose-dependent fashion, specifically at miR-373 mimic

of 20 nM ($p<0.05$) and 40 nM ($p<0.01$) (Fig. 4A). Additionally, p53 mRNA and protein from MG-63 dosed with 40 nM miR-373 mimic were collected at different time points to determine the time-dependent knockout effect of overexpressing miR-373. miR-373 overexpression could effectively downregulate p53 levels until 72 h ($p<0.01$) (Fig. 4B). Our findings indicated that miR-373 repressed p53 expression in both a dose- and time-dependent manner. Overexpressing miR-373 markedly weakened p53 and downstream targets p21, Puma, Bax, and PAI expressions (Fig. 4C). Above all, miR-373 could downregulate p53, and a p53-related mechanism might be involved in cell apoptosis in MG-63 cells.

miR-373 Activates the PI3K/AKT–Rac1–JNK Pathway in the MG-63 Cell Line

To clarify the molecular mechanisms involved in miR-373-mediated human OS cells, we next investigated the effects of miR-373 on PI3K/AKT, Rac1, and JNK proteins in the MG-63 cell line.

Rising accumulation of p-PI3K and p-AKT was observed in response to overexpression of miR-373, and eases were witnessed in response to knockout of miR-373 (Fig. 5A). Moreover, the protein expression of GTP-Rac1 and p-JNK was upregulated by overexpressing miR-373 and downregulated by silencing miR-373 (Fig. 5B). These findings pointed to the activation of the PI3K/AKT–Rac1–JNK pathway by miR-373. Furthermore, with the PI3K/AKT inhibitor LY294002, the protein expression of GTP-Rac1 and p-JNK was decreased (Fig. 5C). After adding the Rac1 inhibitor NSC23766, there was no impact on PI3K/AKT, but JNK phosphorylation was sharply reduced (Fig. 5D). This reveals that miR-373 orderly activated the PI3K/AKT–Rac1–JNK signaling pathway.

miR-373 Mediates MG-63 Cell Growth and Invasion by Regulation of the PI3K/AKT Pathway

To further investigate whether miR-373 mediates MG-63 cell growth and invasion by regulation of the PI3K/AKT pathway, we administrated the inhibitor of PI3K, LY294002, to the cells with a concentration of 10 μM after transfection with or without the miR-373 mimic.[29,30]. Cell viability, migration, invasion, and colony formation were then analyzed again. The results showed that administration of LY294002 significantly decreased cell viability at days 3 and 4 ($p<0.05$ or $p<0.01$); wound width rate at days 1, 2, 3, and 4 (all $p<0.05$); invasion rate ($p<0.05$); and colony formation rate ($p<0.05$) compared to the control group (Fig. 6A–D). However, these effects were rescued by simultaneous transfection with the miR-373 mimic. These results indicate that miR-373 regulates MG-63 cell growth and invasion by activation of the PI3K/AKT pathway.

DISCUSSION

The current study provides primary research on miR-373 regulating OS cell growth and metastasis. First, miR-373 was highly expressed in tumor tissues compared with normal tissues of spinal OS. miR-373 was also overexpressed in OS cell lines compared with the osteoblast. With transfection of the miR-373 mimic, inhibitor, or miR-control for 2 days, miR-373 can promote cell viability, migration, invasion, and colony formation of the MG-63 cell line. In addition, miR-373 overexpression downregulated p53 and its downstream target genes, and orderly activated the PI3K/AKT–Rac1–JNK signaling pathway. This study lays the groundwork for dissecting the miR-373-mediated mechanism of OS cell growth and metastasis, which provides potential targets for clinical therapy of spinal OS.

Emerging reports serve as the basis for upregulated miR-373 in tumors. Compared with normal tissues, high levels of miR-373 have been investigated in primary tumor tissues of breast cancer[36], testicular germ cell tumor (TGCT)[37], gastric cancer[28], and esophageal cancer[20]. To date, this is the first study determining the expression of miR-373 in human spinal OS tissues and OS cell lines. We have proven that miR-373 was specifically upregulated in spinal OS tissues and cell lines relative to normal tissues and the osteoblast, respectively, potentially contributing to OS cell growth and metastasis. Since the specific cell line of spinal OS is not available, we used the MG-63 cell line, which possessed the highest level of miR-373 among three cell lines, to assess its effects in OS.

After determining the promotive effects of miR-373 on cell growth and metastasis in the MG-63 cell line, we were interested in its underlying mechanism. p53 has been documented to be a guardian of cell differentiation, which participates in the regulation of various functions, such as metabolism and apoptosis[38,39]. p21, an inhibitor of cyclin-dependent kinase (CDK), has considerable implications for cell cycle regulation[40]. In addition, downregulated Bax was considered to relieve apoptotic cell death through directly suppressing mitochondrial depolarization and a cascade of caspase[41]. Puma is well known to promote cell apoptosis via the p53 pathway, and PAI can induce replicative senescence as a downstream target of p53[42]. Previously, Yang et al. pointed to striking MG-63 cell apoptosis induced by chamaejasmine via upregulating levels of p53, p21, and Bax[43]. In this study, miR-373 repressed p53 expression in both a dose- and time-dependent manner. Also, overexpressing miR-373-induced MG-63 cells exhibited remarkably weakened p21, Puma, Bax, and PAI expressions. Taken together, miR-373 could downregulate p53 as well as its targets in OS cells, which might be involved in p53-related cell apoptotic response.

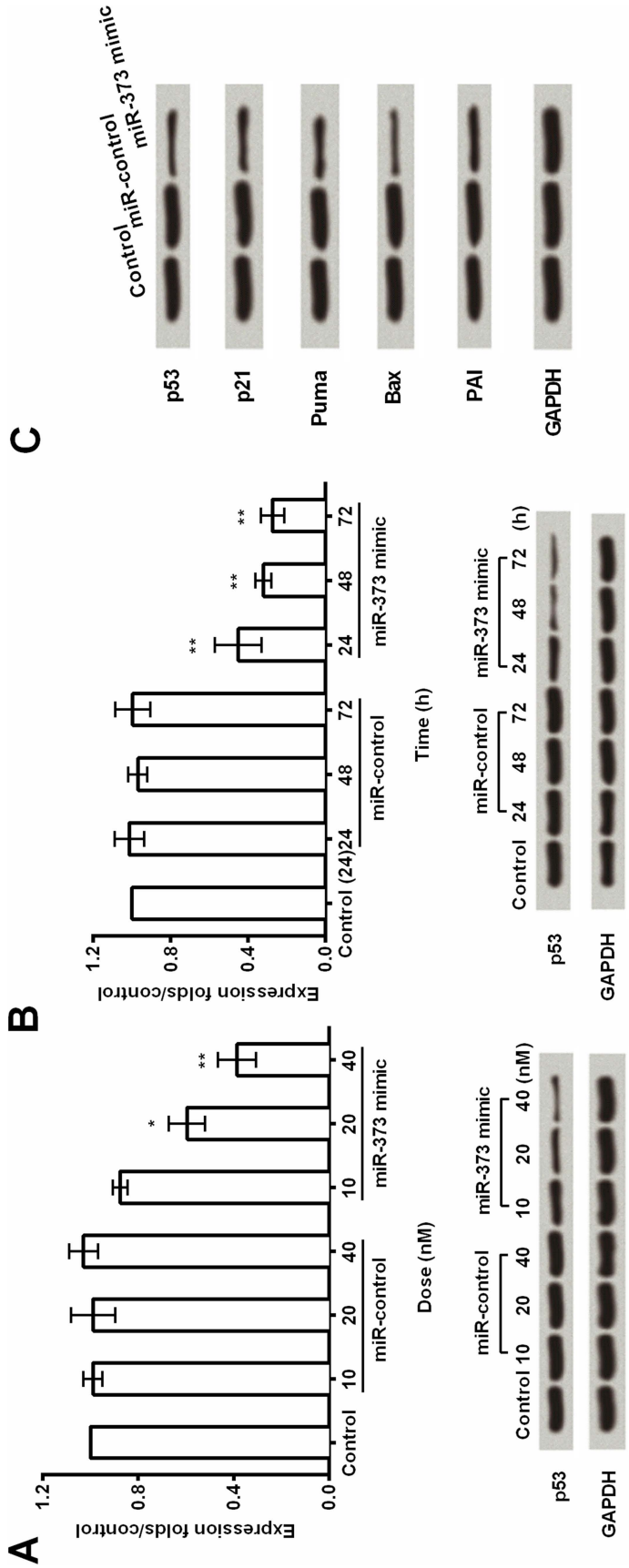

Figure 4. miR-373 can downregulate p53 expression in the MG-63 cell line. (A) The effect of overexpressing miR-373 in different doses on p53 expression. (B) The effect of overexpressing miR-373 for different times on p53 expression. (C) The effect of overexpressing miR-373 on p53 and its target's expression. miR, microRNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Puma, p53 upregulated modulator of apoptosis; Bax, B-cell lymphoma-2 associated X; PAI, plasminogen activator inhibitor. $*p<0.05$; $**p<0.01$.

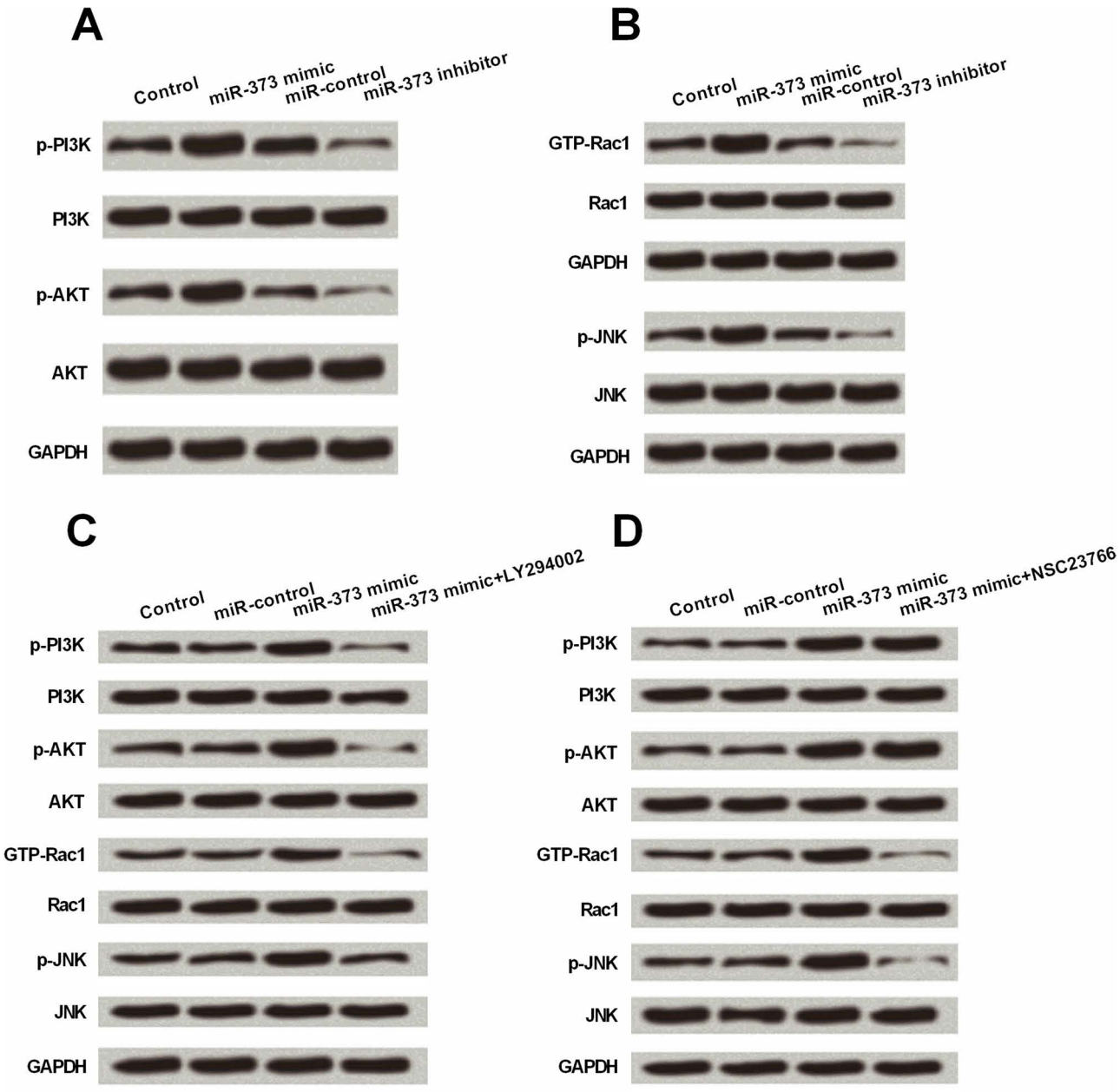

Figure 5. miR-373 activates the PI3K/AKT–Rac1–JNK pathway of the MG-63 cell line. (A) The effect of aberrant miR-373 on the PI3K/AKT pathway of the MG-63 cell line. (B) The effect of aberrant miR-373 on Rac1 and JNK of the MG-63 cell line. (C) The effect of overexpressing miR-373 on the PI3K/AKT–Rac1–JNK pathway with the PI3K/AKT inhibitor LY294002. (D) The effect of overexpressing miR-373 on the PI3K/AKT–Rac1–JNK pathway with the Rac1 inhibitor NSC23766. miR, microRNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PI3K, phosphoinositide 3-kinase; GTP, guanosine triphosphate; Rac1, Ras-related C3 botulinum toxin substrate 1; JNK, c-Jun N-terminal kinase.

Given the fact that OS possesses a potently malignant metastatic ability, targeted therapy of crucial molecules should be an attractive strategy to selectively block metastasis of OS cells. The PI3K/AKT pathway has been proven to be involved in metastatic OS as a promising target, which can activate Rac1 to promote cell survival[44–46]. Rac1 mediates actin polymerization and cell migration, whose mutation can potently activate JNK[47]. Moreover, the JNK pathway holds great potential in regulating cell growth and metastasis[48]. Shi et al. found that basic fibroblast growth factor (bFGF) promoted dermal fibroblast migration via the PI3K/AKT–Rac1–JNK signal pathway[49]. In line with a previous study[45], our data demonstrated that miR-373 activated the PI3K/AKT–Rac1–JNK

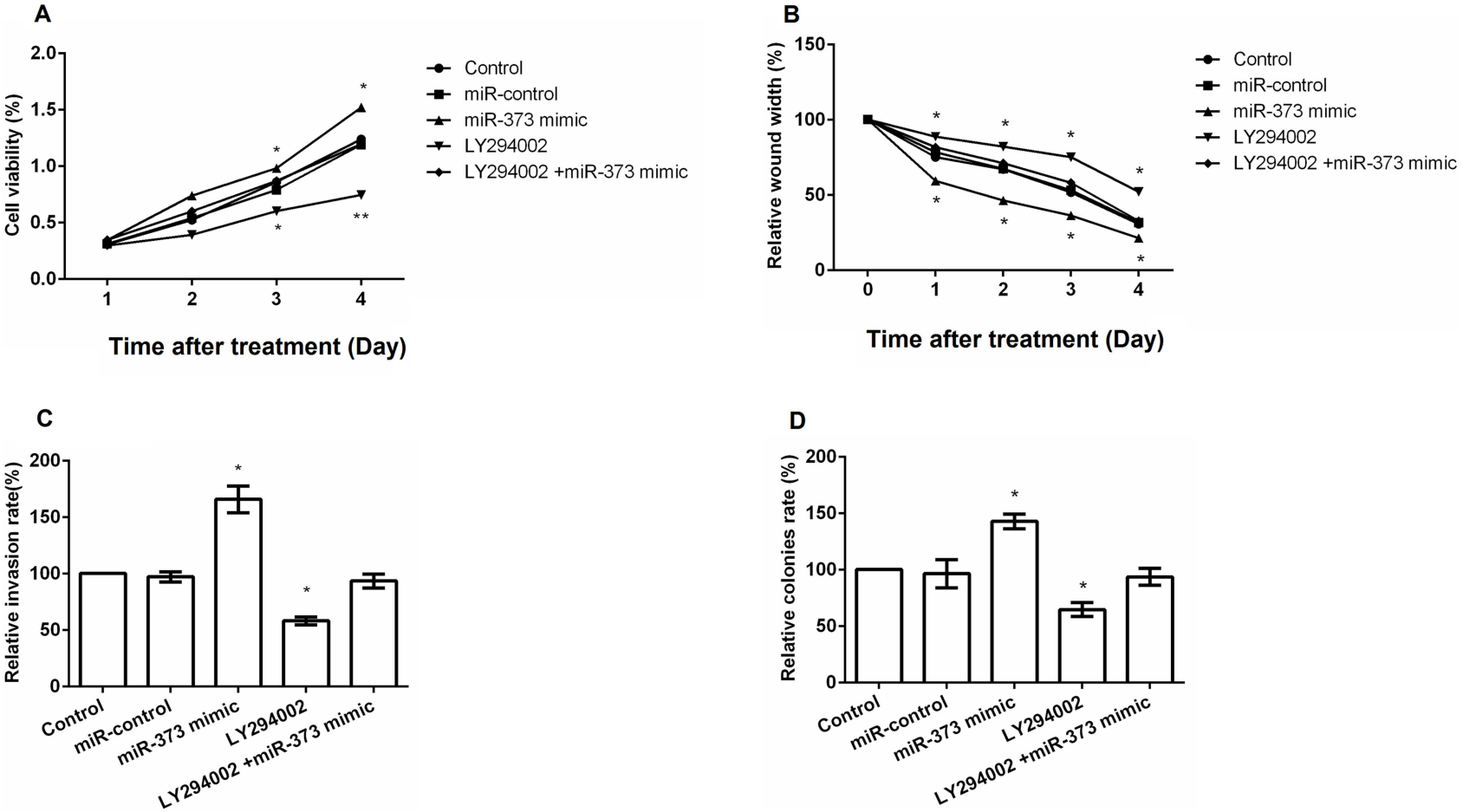

Figure 6. miR-373 mediates MG-63 cell growth and invasion by regulation of the PI3K/AKT pathway. (A) The effect of LY294002 and miR-373 mimic on cell viability. (B) Cell migration. (C) Cell invasion and (D) colony formation of the MG-63 cell line. miR, microRNA. *$p<0.05$; **$p<0.01$.

pathway. Noticeably, with the interference of the PI3K/AKT inhibitor or Rac1 inhibitor, PI3K/AKT, Rac1, and JNK were activated in response to overexpressing miR-373. We further explored whether miR-373 promoted OS cell growth and metastasis via the PI3K/AKT pathway. Interestingly, our data revealed that the inhibitor of PI3K significantly decreased the cell growth and invasion ability, while simultaneous overexpression of miR-373 reduced the effects. These results illustrate a promising mode for the mechanism underlying OS metastasis.

Consequently, miR-373 is specifically upregulated in spinal OS tissues and OS cell lines. Furthermore, miR-373 promotes growth and cellular invasion in OS cells by activating the PI3K/AKT–Rac1–JNK pathway. Thereby, miR-373 might be a candidate for molecular targeted therapy of spinal OS.

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