European Review for Medical and Pharmacological Sciences 2021; 25: 3661-3669

MicroRNA-340-5p inhibits the malignant phenotypes of osteosarcoma by directly targeting NRF2 and deactivating the PI3K/AKT pathway

H.-P. FAN[1], S.-Y. WANG[2], Y.-Y. SHI[3], J. SUN[4]

[1]Department of Pediatrics, Yantai Maternity and Child Care Hospital, Yantaishan Hospital, Yantai, China
[2]Department of Hand and Foot Surgery, Qingdao Eighth People's Hospital, Qingdao, China
[3]Department of Oncology, Qingdao Women and Children's Hospital, Qingdao, China
[4]Department of Nephrology Geriatrics, The Third People's Hospital of Qingdao, Qingdao, China

Abstract. – OBJECTIVE: The aim of the study was to examine the effects and potential mechanisms of miR-340-5p in Osteosarcoma (OS) progression.

PATIENTS AND METHODS: qRT-PCR was applied to detect expressions of miR-340-5p and NRF2 mRNA. MTT and transwell assay were carried out to determine the roles of miR-340-5p in OS cells viability, invasion and migration. TargetScan and Luciferase reporter assays were performed to search for the candidate target gene of miR-340-5p. The regulatory roles of miR-340-5p in OS PI3K/AKT pathway and EMT were examined by Western blot.

RESULTS: MiR-340-5p expressions were decreased in OS tissues and cells. Moreover, the decreased miR-340-5p expressions in OS tissues were frequently accompanied by shorter overall survival and malignant clinicopathologic features of OS patients. MTT assay showed that miR-340-5p upregulation prominently repressed OS cell proliferation. In addition, miR-340-5p restoration could significantly suppress OS cell invasion and migration as demonstrated by transwell assays. Results also revealed that miR-340-5p could directly target NRF2 and regulate PI3K/AKT pathway and EMT, exerting prohibitory functions in OS.

CONCLUSIONS: MiR-340-5p repressed the malignant phenotypes of OS via targeting NRF2 and regulating PI3K/AKT pathway and EMT. The current study provided preclinical evidence for the potential applications of miR-340-5p/NRF2 axis in OS therapies.

Key Words:
Osteosarcoma, MiR-340-5p, NRF2, PI3K/AKT, EMT.

Introduction

Osteosarcoma (OS) is a common bone malignancy, characterized by malignant cancer cells directly from bone tissues, and mainly harmful to children and adolescents[1]. Currently, standard treatment strategies for OS mainly include surgery, adjuvant chemotherapy and neoadjuvant therapy[2]. However, existing methods have their own shortcomings. Surgery often leads to an amputation[3]. Moreover, albeit adjuvant chemotherapy and neoadjuvant therapy have improved the survival rates of OS patients, they also induce toxic effects that could not be efficiently prevented and 5-year survival rates in relapsed and metastatic patients remain disappointing[4]. Therefore, identification and verification of novel targets are necessary for further clinical application in OS treatment.

MicroRNAs (miRNAs/miRs) are non-coding RNA molecules with a size of about 22-25 nucleotides. They can complementarily base pair with the 3'UTRs of their target genes, directly degrading or inhibiting the translation of the target gene for post-transcriptional regulations[5]. In the past decades, most investigations concentrated on the roles of protein-coding genes in tumorigenesis, and little attention has been paid to the potential functions of miR in cancers. In recent years, evidence revealed that miR was implicated in the initiation and progression of different tumors, such as prostate cancer[6], endometrial cancer[7], breast cancer[8], and so forth. MiR is confirmed to participate in tumor metastasis *via* regulating various target protein genes[9]. Moreover, studies[10,11] showed that miR could either activate or inhibit tumor metastasis. Therefore, as promising metastasis biomarkers and therapeutic targets in tumors, miRs would provide new hope for OS diagnosis and treatments.

Corresponding Author: Jing Sun, MD; e-mail: xiangkangbuxing540@163.com

Multiple miRs have recently been studied in OS. MiR-16-1/2-3p exerted strong anti-metastatic and cancer suppressive functions in OS[12]. MiR-664a inhibition interfered with OS cell migration *via* modulating MEG3[13]. MiR-142-3p served as an anti-tumor miR in OS by targeting HMGA1[14]. Moreover, dysregulation of miR-340-5p has been identified in various malignancies, playing crucial functions. In non-small cell lung cancer, miR-340-5p suppressed cell metastasis and growth *via* regulation of ZNF503[15]. In melanoma cells, the downregulation of miR-340-5p could increase ABCB5 expressions under oxygen-deprived condition[16]. In thyroid carcinoma, miR-340-5p upregulation facilitated tumor progression by downregulating BMP4[17]. However, the specific role and potential mechanism of miR-340-5p in OS have not been fully elucidated.

Patients and Methods

Tissue Specimens and Cell Cultures

All OS tissues and adjacent non-tumor tissues were acquired from patients who underwent surgical resection in the Yantaishan Hospital. All patients provided the informed consent. This study was approved by the Ethics Committee of the Yantaishan Hospital. All tissue specimens were snap-frozen and stored at -80°C for subsequent studies.

A human osteoblast cell line hFOB 1.19 and OS cells (U2OS, Saos-2, MG-63, HOS) were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% FBS (Gibco, Grand Island, NY, USA). All cells were maintained at 37°C in a humid chamber with 5% CO_2.

qRT-PCR

Total RNA was isolated from cells and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). 1 µg total RNAs were used as the templates to perform the reverse transcription reaction using the PrimeScript RT-PCR kit (TaKaRa Biotechnology Co., Ltd., Dalian, China) following the manufacturer's instructions. qRT-PCR was conducted using ABI 7900 system (Applied Biosystems, Waltham, MA, USA) with the SYBR Green Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). MiR expression was normalized to U6 and other gene was normalized to GAPDH. $2^{-\Delta\Delta CT}$ was used to calculate the relative quantitative expression level. The primer sequences were shown in Table I.

Cell Transfection

MiR-340-5p mimics, inhibitor or negative controls were transfected into OS cell lines using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). All these transfection procedures followed the manufacturer's protocol. 48 h after the transfections, cells were harvested for the subsequent experiments.

Proliferation Assays

Cell proliferation ability was detected by MTT assays. OS cells with different transfections were seeded into 96-well plates. Then, the cells were incubated for 24, 48 and 72 h, and cell growth was measured following the addition of MTT (5 mg/ml). Thereafter, 150 µl DMSO was used to solubilize the MTT-formazan crystal. OD_{490} was examined by a microplate (Bio-Rad Laboratories, Hercules, CA, USA).

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-340-5p forward	5'-GTTTGTGTGCGTTAGTGAGCG-3'
miR-340-5p reverse	5'-CTTGCCGTTAACTCCAGGGTA-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
NRF2 forward	5'-CAGCCATGACTGATTTAAGCAG-3'
NRF2 reverse	5'-CAGCTGCTTGTTTTCGGTATTA-3'
GAPDH forward	5'-TGCACCACCAACTGCTTAGC-3'
GAPDH reverse	5'-GGCATGGACTGTGGTCATGAG-3'
ERK1/2	ATATCCTTGGCTACTAACTATGGCTACAATGATTCTA

U6: small nuclear RNA, snRNA; NRF2: nuclear factor erythroid 2-related factor 2; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Transwell Assays

Transwell assays were performed to detect the migration and invasion of OS cells. In brief, OS cells were transfected with miR-340-5p mimics or inhibitor into 6-well plates, and then, after 48 h the cells were harvested and re-suspended with FBS-free culture medium. Cells were seeded into the top 24-well transwell chambers (Millipore Corp., Billerica, MA, USA) and medium with 10% FBS was added to the lower chambers as the attractant. For the invasion assay, 2 mg/ml Matrigel (Clontech, Mountain View, CA, USA) was added to the top chamber. For migration assay, the top chamber was not added with Matrigel. They were cultured under the same conditions (37°C, 5% CO_2 for 24 h). After incubation for 48 h (for invasion) or 24 h (for migration), cells that invaded or migrated to the bottom of the membranes were fixed and stained. Images were photographed under a microscope (Olympus, Tokyo, Japan).

Western Blot

Cells were lysed using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). The protein concentration was detected with the BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). SDS-PAGE with 10% gel was used to separate the total proteins, and then, the proteins were transferred to PVDF membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% non-fat milk for 1 h at room temperature, and followed by incubation at 4°C overnight with primary antibodies. Thereafter, the membrane was incubated with HRP-conjugated goat anti-rabbit (1:2000, Abcam, Cambridge, MA, USA) secondary antibody for 2 h at room temperature. The primary antibodies were as follows: antibodies against E-cadherin (1:2000, Abcam, Cambridge, MA, USA), N-cadherin (1:1000, Abcam, Cambridge, MA, USA), Vimentin (1:1000, Abcam, Cambridge, MA, USA), p-AKT (1:1000, Abcam, Cambridge, MA, USA), p-PI3K (1:2000, Abcam, Cambridge, MA, USA), AKT (1:1000, Abcam, Cambridge, MA, USA), PI3K (1:1000, Abcam, Cambridge, MA, USA) and GAPDH (1:1000, Abcam, Cambridge, MA, USA). GAPDH was used as internal control. Finally, the signals were detected using an ECL kit (Millipore, Billerica, MA, USA).

Luciferase Reporter Assay

PMIR-NRF2-3'UTR-Wild/MUT plasmids (RiBoBio, Guangzhou, China) were transfected into OS cells together with miR-340-5p mimics using Lipofectamine 2000. 48 h after transfection, the Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Statistical Analysis

All the above experiments were performed in triplicate. The SPSS 17.0 version (SPSS Inc. Chicago, IL, USA) was used to perform the statistical analysis. The overall survival of OS patients was determined with Kaplan-Meier curve together with log-rank test. Data were tested using Student's *t*-test or one-way ANOVA analysis with Tukey's post-hoc test. $p<0.05$ was considered to be statistically significant difference.

Results

MiR-340-5p Correlated with the Clinical Prognosis and Clinicopathological Features of OS Patients

To verify whether miR-340-5p played crucial roles in OS carcinogenesis, its expressions in OS tissues and cell lines were first measured by qRT-PCR. Findings showed an evident decrease of miR-340-5p expressions in OS tissues compared to the adjacent para-carcinoma tissues (Figure 1A). Moreover, the decreased miR-340-5p expressions were also identified in OS cells (Figure 1B). Then, the patients were assigned into low and high miR-340-5p groups based on the mean level of miR-340-5p to investigate the clinical values about miR-340-5p in OS. In brief, Kaplan-Meier analysis was used to determine the influence of miR-340-5p on the prognosis of OS patients. We found that patients in the low-miR-340-5p group presented a significantly shorter survival rate than those in the high-miR-340-5p group (Figure 1C). Additionally, patients in the low-miR-340-5p group also showed aggressive clinicopathological characteristics (Table II).

MiR-340-5p Repressed OS Cell Growth

The potential biological functions of miR-340-5p in OS progression were investigated by transfecting miR-340-5p mimics or inhibitor into OS cells. Among the selected 4 OS cell lines, MG-63 and Saos-2 were chosen for further investigation as they presented relatively low or high endogenic miR-340-5p expressions. MiR-340-5p expression was successfully upregulated or silenced by miR-

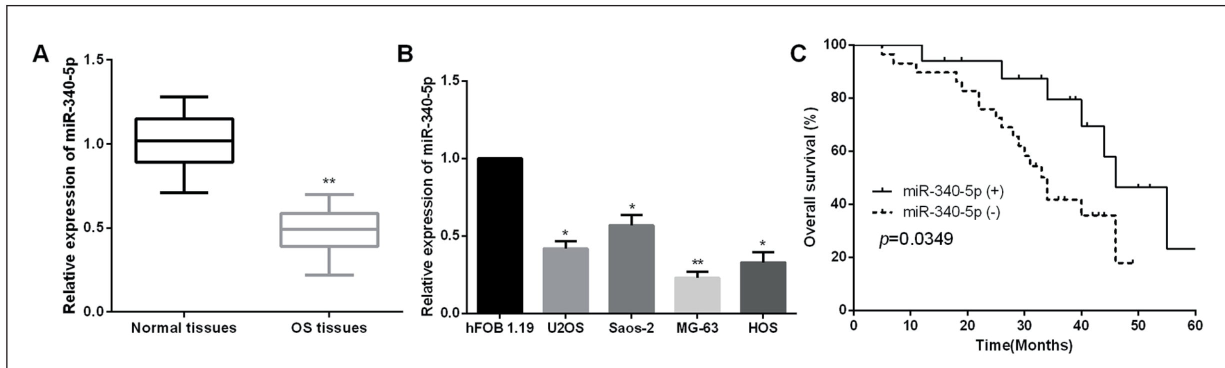

Figure 1. MiR-340-5p expression was decreased in OS and indicated adverse clinicopathological features of OS patients. **A, B,** MiR-340-5p levels in OS tissues or cells were measured by qRT-PCR. **C,** Kaplan-Meier analysis of OS patients with different miR-340-5p expressions.

340-5p mimics or inhibitor as demonstrated by qRT-PCR analysis (Figure 2A and 2B). Thereafter, MTT assays were carried out to explore the influence of miR-340-5p on OS cell proliferation. In MG-63 cells, the proliferation ability was prominently suppressed by miR-340-5p mimic (Figure 2C), while the inhibitor significantly enhanced Saos-2 cell proliferation (Figure 2D).

MiR-340-5p Inhibited OS Cell Migration and Invasion

The effects of miR-340-5p on OS cell invasion and metastasis were then determined by transwell assays. As shown in Figure 3A and 3B, the invasion and migration capacities of MG-63 cells were remarkably restricted by restoration of miR-340-5p. On the other hand, Saos-2 cell invasion and migration abilities were markedly facilitated by miR-340-5p inhibition (Figure 3C and 3D). All the above data indicated that miR-340-5p exerted prohibitory roles in OS.

MiR-340-5p Mediated PI3K/AKT Pathway and EMT of OS Cells

PI3K/AKT pathway plays an important part in tumorigenesis, and induction of EMT is

Table II. Correlation of miR-340-5p expression with the clinicopathological characteristics of the OS patients.

Clinicopathological features	Cases (n = 47)	miR-340-5p # expression		p
		High (n =1 8)	Low (n = 29)	
Age (years)				0.542
> 60	25	9	16	
≤ 60	22	9	13	
Gender				0.454
Male	25	8	17	
Female	22	10	12	
Tumor size (cm)				0.076
≥ 5.0	24	6	18	
< 5.0	23	12	11	
Lymph node metastasis				0.002*
Yes	17	13	4	
No	30	5	25	
TNM stage				0.011*
I +II	22	15	7	
III+IV	25	3	22	
Distant metastasis				0.021*
Yes	27	4	23	
No	20	14	6	

OS: Osteosarcoma; TNM: tumor-node-metastasis. #The mean expression level of miR-340-5p was used as the cutoff. *Statistically significant.

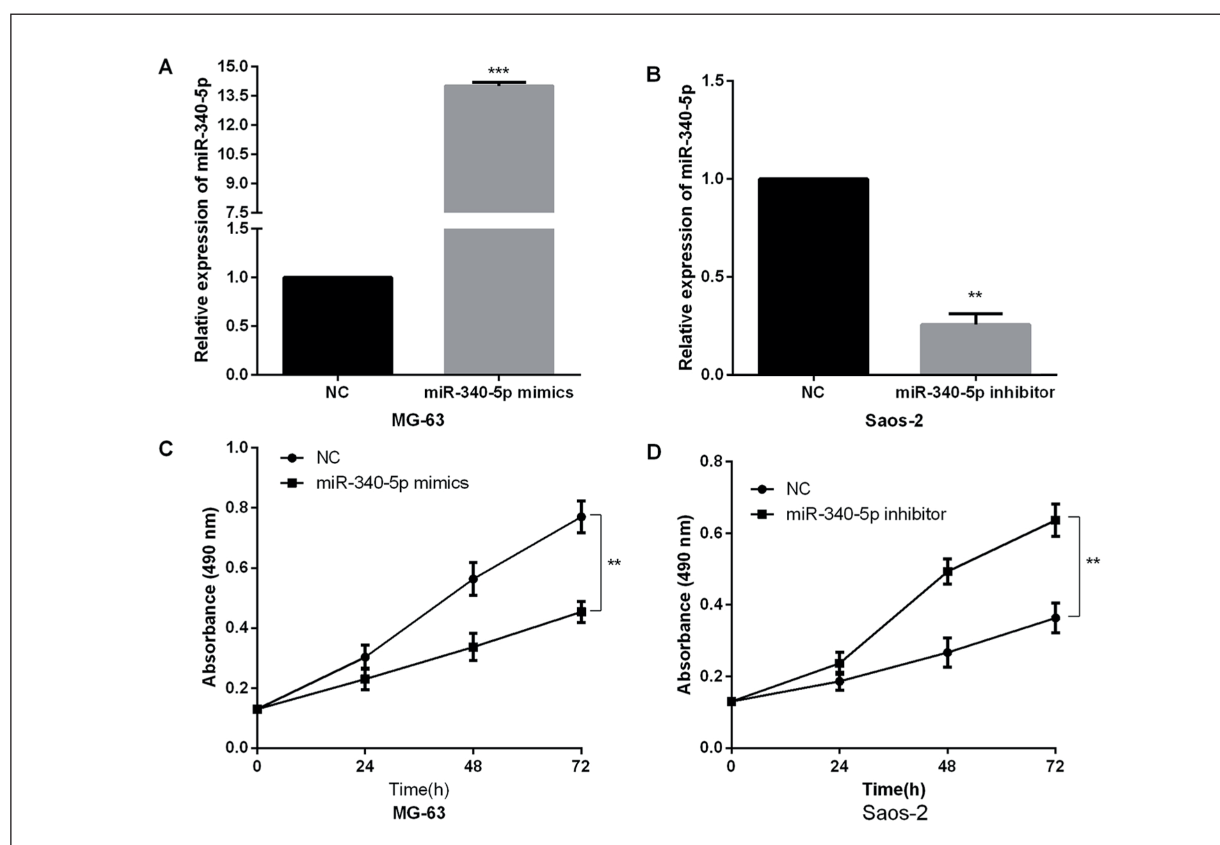

Figure 2. MiR-340-5p overexpression inhibited OS cell viability. **A, B,** The transfection efficiencies of miR-340-5p mimics or inhibitor in OS cells were confirmed by qRT-PCR. **C, D,** The influence of miR-340-5p on OS cell viability was examined by MTT assays.

frequently concomitant with its activations, we investigated whether miR-340-5p mediated OS progression *via* regulation of PI3K/AKT and EMT. Western blot was performed to examine expressions of several key molecules relevant to PI3K/AKT and EMT. Results revealed that miR-340-5p overexpression in MG-63 cells led to dramatically decreased phosphorylation levels of PI3K and AKT expressions, whereas there was no evident variation in PI3K and AKT levels (Figure 4A). On the contrary, p-PI3K and p-AKT were significantly upregulated by miR-340-5p inhibitor in Saos-2 cells (Figure 4B). Furthermore, in miR-340-5p-overexpressed MG-63 cells, there were detectable changes in the expressions of EMT-related proteins, including upregulation of E-cadherin and downregulation of N-cadherin and vimentin (Figure 4C). In Saos-2 cells, the effect of miR-340-5p inhibition on EMT-related protein expression was contrary to miR-340-5p overexpression (Figure 4D).

NRF2 was a Target of miR-340-5p

TargetScan was used to predict downstream target genes of miR-340-5p. Potential binding sites of miR-340-5p on NRF2-3'UTR are shown in Figure 5A. Moreover, Luciferase reporter plasmid containing NRF2-WT/MUT was used to validate the correlation between miR-340-5p and NRF2. As shown in Figure 5B, co-transfection with NRF2-WT and miR-340-5p mimics in OS cells resulted in a significantly decreased Luciferase activity. Additionally, we also found that miR-340-5p could regulate NRF2 expressions *in vitro* (Figure 5C and Figure 5D). The above data together indicated that miR-340-5p/NRF2 was responsible for OS development.

Upregulated NRF2 in OS Indicated Poor Outcomes of OS Patients

We further measured NRF2 expressions in OS cells and tissues. As expected, evidently increased NRF2 was identified in OS tissue samples (Figure 6A). Similarly, the upregulated

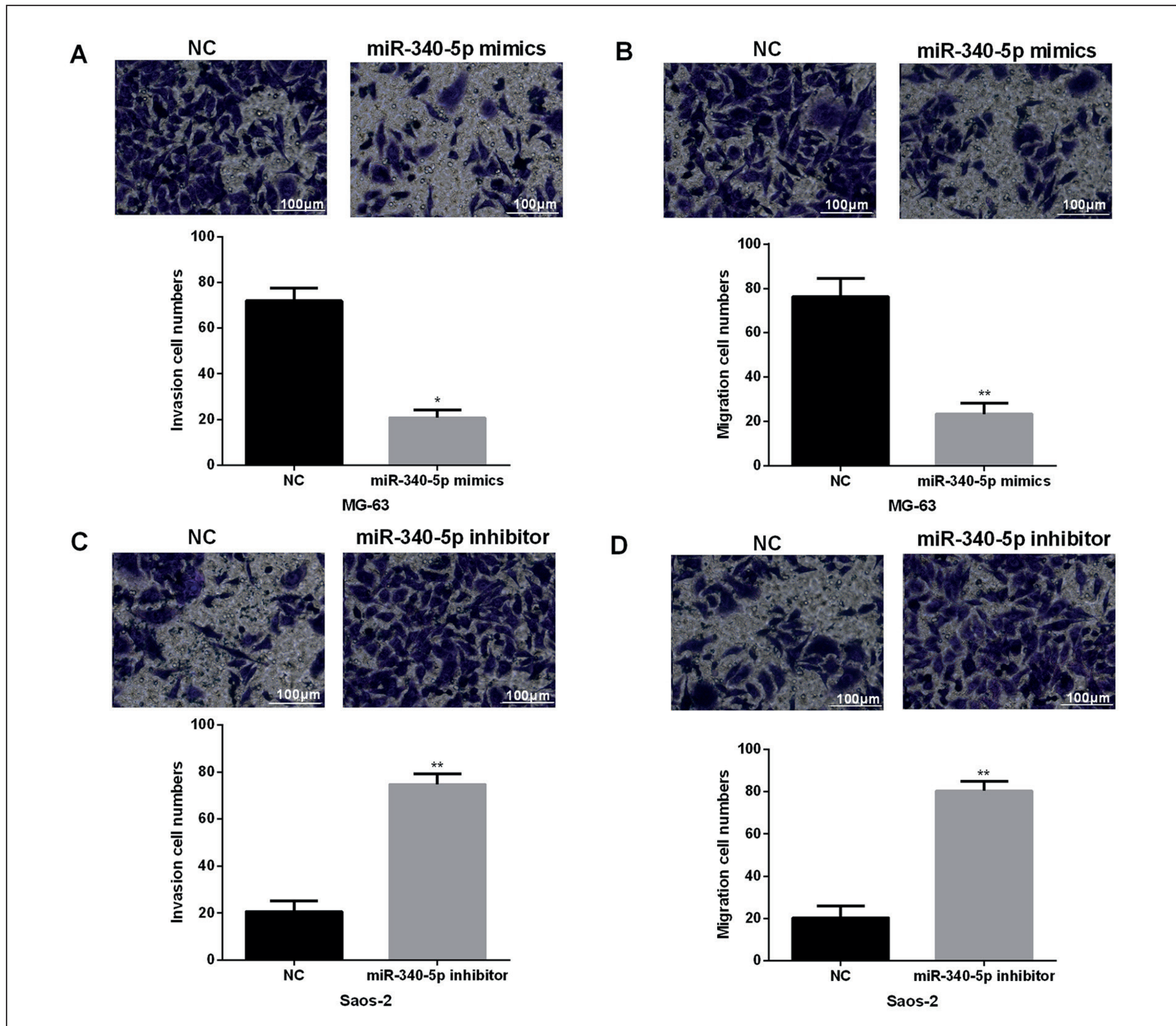

Figure 3. MiR-340-5p upregulation repressed OS cell invasion and migration. **A, B,** MiR-340-5p upregulation inhibited MG-63 cell invasion and migration (magnification: 200×). **C, D,** MiR-340-5p silence facilitated Saos-2 cell invasion and migration (magnification: 200×).

NRF2 was also found in OS cells (Figure 6B). Moreover, Kaplan-Meier analysis was performed to determine the clinical significance of miR-340-5p in OS patients. We found that patients with high NRF2 expression had poor outcomes with a shorter survival rate (Figure 6C).

Discussion

OS has been the most common bone malignancy among adolescents for the past years[18]. In recent years, miRs have attracted widespread attention as they play a crucial role in a variety of cancers. In the current study, we also confirmed that miR-340-5p served as a tumor suppressor in OS. Briefly, miR-340-5p was downregulated in OS, and the decreased miR-340-5p in OS correlated with adverse clinicopathologic features and shorter overall survival of OS patients. Thereafter, MTT and transwell assays were carried out to investigate the influence on OS cell viability, invasion and migration abilities mediated by miR-340-5p. As demonstrated by the results, we could easily find that miR-340-5p restoration dramatically repressed OS cell viability, invasion and migration capacities. Consistent with our findings in the current study, Rongxin et al[19] also revealed that miR-340-5p inhibited OS progression *via* regulating STAT3 and Wnt/β-catenin

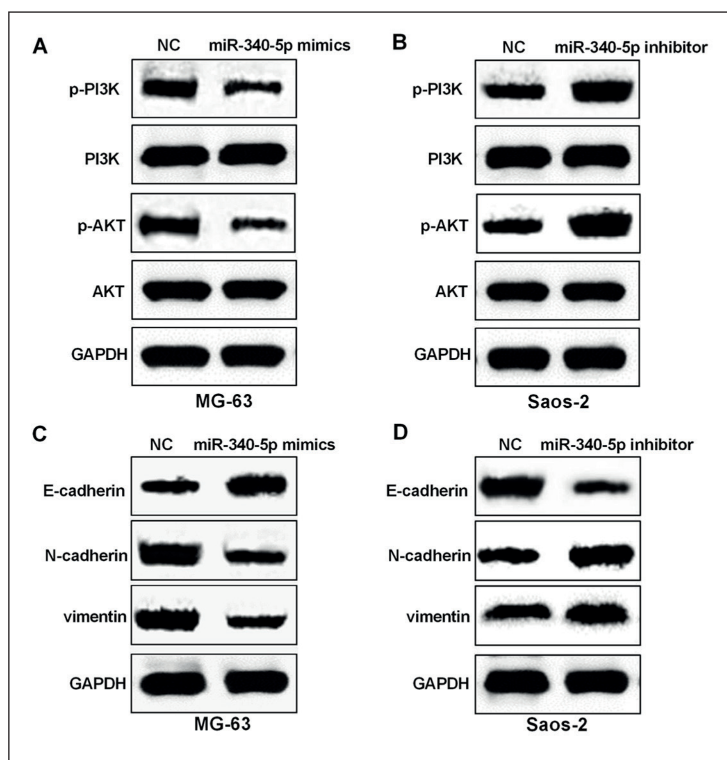

Figure 4. MiR-340-5p modulated OS cell PI3K/AKT and EMT progress. **A, B,** The effect of miR-340-5p on PI3K/AKT pathway of OS cells was analyzed using Western blot. **C, D,** MiR-340-5p influenced OS cell EMT as demonstrated by western blot.

signaling. These findings indicated that miR-340-5p functioned as a tumor suppressor in OS.

EMT has been regarded as a crucial step during the transformations of early-phase cancers into malignant metastatic tumors, being closely related to tumor metastasis[20]. The characteristic downregulations of E-cadherin are considered to be important manifestations of EMT[21]. In addi-

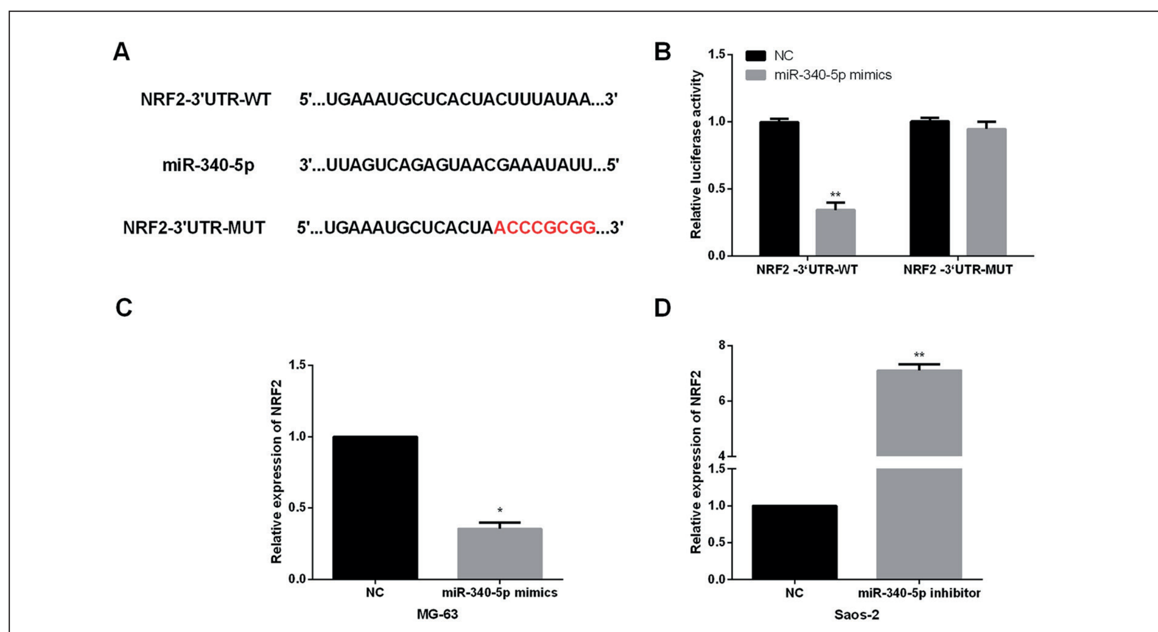

Figure 5. MiR-340-5p regulated NRF2 expression in OS cells. **A,** Potential binding sequences of miR-340-5p in NRF2-3'UTRs. **B,** Luciferase activity of OS cells co-transfected with NRF2-WT/MUT and miR-340-5p mimics. **C, D,** Regulatory functions of miR-340-5p in NRF2 expressions.

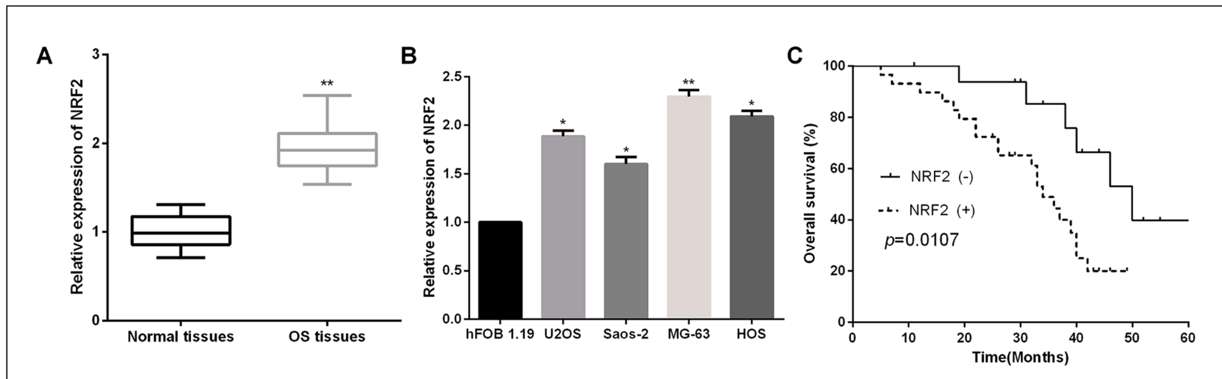

Figure 6. NRF2 overexpression was related to the shorter overall survival of OS patients. **A, B,** qRT-PCR analysis demonstrated prominently increased NRF2 expressions in OS tissue samples and cell lines. **C,** The correlation between NRF2 expressions and the survival rates of patients was determined by Kaplan-Meier analysis.

tion, as a mesenchymal marker, N-cadherin also plays a crucial role in the induction of EMT[22]. In light of the above data and theories, we detected expressions of the tightly relevant proteins using Western blot and findings were in line with the above evidence. Furthermore, abnormal activations of PI3K/Akt are confirmed to be implicated in mediating cell apoptosis and proliferation, especially in tumor progression[23]. Thus, targeting the PI3K/Akt pathway has recently been explored as a therapeutic strategy in tumor treatment. Rafael et al revealed that EMT was frequently accompanied by PI3K/Akt activation[24,25]. Another study demonstrated that Akt could induce EMT. Consistent with these results, our findings showed that key factors of this signaling, p-PI3K and p-Akt were downregulated by miR-340-5p. Collectively, the above data indicated that miR-340-5p exerted inhibitory activities against OS cell proliferation, invasion, metastasis *via* regulating EMT and deactivating PI3K/Akt pathway.

NRF2 (nuclear factor erythroid 2-related factor 2) is one of the basic redox-sensitive leucine zipper transcription factors, playing critical roles in protecting cells from oxidative or electrophilic stress by maintaining redox homeostasis[26]. NRF2 was also identified as an oncogenic transcriptional factor, which played important roles against intracellular or environmental stresses and regulated the antioxidant response element (ARE)-dependent transcription of antioxidant genes in tumor cells, such as NAD(P)H: quinone oxidoreductase 1 (NQO1) and heme oxygenase-1 (HO-1)[27]. Accordingly, tumor cells frequently exhibited overexpressed NRF2, which was related to poor survival outcomes and increased resistance to anti-cancer treatments in patients with tumors[28,29]. Thus, the identification of novel adjuvants to suppress NRF2 might be a promising therapeutic strategy to improve poor outcomes of OS patients.

Conclusions

The main novelty of this research was that we first explored the effect and mechanism of miR-340-5p/NRF2 on OS. MiR-340-5p repressed the malignant phenotypes of OS *via* targeting NRF2 and regulating PI3K/AKT pathway and EMT. Current study provided preclinical evidence for the potential applications of miR-340-5p/NRF2 axis in OS therapies. However, our study mainly focused on the *in vitro* assays, more investigations on the *in vivo* assays of miR-340-5p/NRF2 are necessary in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma overview. Rheumatol Ther 2017; 4: 25-43.
2) Bielack SS, Hecker-Nolting S, Blattmann C, Kager L. Advances in the management of osteosarcoma. F1000Res 2016; 5: 2767.
3) Lamoureux F, Trichet V, Chipoy C, Blanchard F, Gouin F, Redini F. Recent advances in the man-

agement of osteosarcoma and forthcoming therapeutic strategies. Expert Rev Anticancer Ther 2007; 7: 169-181.

4) Durfee RA, Mohammed M, Luu HH. Review of osteosarcoma and current management. Rheumatol Ther 2016; 3: 221-243.
5) Liu B, Li J, Cairns MJ. Identifying miRNAs, targets and functions. Brief Bioinform 2014; 15: 1-19.
6) Waseem M, Ahmad MK, Serajuddin M, Bhaskar V, Sankhwar SN, Mahdi AA. MicroRNA-183-5p: a new potential marker for prostate cancer. Indian J Clin Biochem 2019; 34: 207-212.
7) Kozak J, Wdowiak P, Maciejewski R, Torres A. Interactions between microRNA-200 family and Sestrin proteins in endometrial cancer cell lines and their significance to anoikis. Mol Cell Biochem 2019; 459: 21-34.
8) García-Vázquez R, Marchat LA, Ruíz-García E, Astudillo-de la Vega H, Meneses-García A, Arce-Salinas C, Bargallo-Rocha E, Carlos-Reyes Á, López-González JS, Pérez-Plasencia C, Ramos-Payán R, Aguilar-Medina M, López-Camarillo C. MicroRNA-143 is associated with pathological complete response and regulates multiple signaling proteins in breast cancer. Technol Cancer Res Treat 2019; 18: 1533033819827309.
9) Zhou JL, Deng S, Fang HS, Yu G, Peng H. Hsa-let-7g promotes osteosarcoma by reducing HOXB1 to activate NF-kB pathway. Biomed Pharmacother 2019; 109: 2335-2341.
10) Zhou W, He L, Dai Y, Zhang Y, Wang J, Liu B. MicroRNA-124 inhibits cell proliferation, invasion and migration by targeting CAV1 in bladder cancer. Exp Ther Med 2018; 16: 2811-2820.
11) Zhao L, Fan W, Fan Y, Gao S. MicroRNA-214 promotes the proliferation, migration and invasion of gastric cancer MKN28 cells by suppressing the expression of Dact2. Exp Ther Med 2018; 16: 4909-4917.
12) Maximov VV, Akkawi R, Khawaled S, Salah Z, Jaber L, Barhoum A, Or O, Galasso M, Kurek KC, Yavin E, Aqeilan RI. MiR-16-1-3p and miR-16-2-3p possess strong tumor suppressive and anti-metastatic properties in osteosarcoma. Int J Cancer 2019; 145: 3052-3063.
13) Sahin Y, Altan Z, Arman K, Bozgeyik E, Koruk Ozer M, Arslan A. Inhibition of miR-664a interferes with the migration of osteosarcoma cells via modulation of MEG3. Biochem Biophys Res Commun 2017; 490: 1100-1105.
14) Xu G, Wang J, Jia Y, Shen F, Han W, Kang Y. MiR-142-3p functions as a potential tumor suppressor in human osteosarcoma by targeting HMGA1. Cell Physiol Biochem 2014; 33: 1329-1339.
15) Lu G, Zhang Y. MicroRNA-340-5p suppresses non-small cell lung cancer cell growth and metastasis by targeting ZNF503. Cell Mol Biol Lett 2019; 24: 34.
16) Wozniak M, Sztiller-Sikorska M, Czyz M. Diminution of miR-340-5p levels is responsible for increased expression of ABCB5 in melanoma cells under oxygen-deprived conditions. Exp Mol Pathol 2015; 99: 707-716.
17) Zhao P, Ma W, Hu Z, Zhang Y, Zhang S, Wang Y. Up-regulation of miR-340-5p promotes progression of thyroid cancer by inhibiting BMP4. J Endocrinol Invest 2018; 41: 1165-1172.
18) Berner K, Johannesen TB, Berner A, Haugland HK, Bjerkehagen B, Bøhler PJ, Bruland ØS. Time-trends on incidence and survival in a nationwide and unselected cohort of patients with skeletal osteosarcoma. Acta Oncol 2015; 54: 25-33.
19. Rongxin S, Pengfei L, Li S, Xiaochen J, Yihe H. MicroRNA-340-5p suppresses osteosarcoma development by down-regulating the Wnt/β-catenin signaling pathway via targeting the STAT3 gene. Eur Rev Med Pharmacol Sci 2019; 23: 982-991.
20) Pearson GW. Control of invasion by epithelial-to-mesenchymal transition programs during metastasis. J Clin Med 2019; 8: 646.
21) Bommi PV, Ravindran S, Raychaudhuri P, Bagchi S. DDB2 regulates Epithelial-to-Mesenchymal Transition (EMT) in oral/head and neck squamous cell carcinoma. Oncotarget 2018; 9: 34708-34718.
22) Dhayat SA, Traeger MM, Rehkaemper J, Stroese AJ, Steinestel K, Wardelmann E, Kabar I, Senninger N. Clinical impact of epithelial-to-mesenchymal transition regulating microRNAs in pancreatic ductal adenocarcinoma. Cancers (Basel) 2018; 10: 328.
23) Polivka J Jr, Janku F. Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. Pharmacol Ther 2014; 142: 164-175.
24) Tian X, Zhou D, Chen L, Tian Y, Zhong B, Cao Y, Dong Q, Zhou M, Yan J, Wang Y, Qiu Y, Zhang L, Li Z, Wang H, Wang D, Ying G, Zhao Q. Polo-like kinase 4 mediates epithelial-mesenchymal transition in neuroblastoma via PI3K/Akt signaling pathway. Cell Death Dis 2018; 9: 54.
25) Rafael D, Doktorovová S, Florindo HF, Gener P, Abasolo I, Schwartz S Jr, Videira MA. EMT blockage strategies: Targeting Akt dependent mechanisms for breast cancer metastatic behaviour modulation. Curr Gene Ther 2015; 15: 300-312.
26) Lu MC, Ji JA, Jiang ZY, You QD. The Keap1-Nrf2-ARE pathway as a potential preventive and therapeutic target: an update. Med Res Rev 2016; 36: 924-963.
27) Levings DC, Wang X, Kohlhase D, Bell DA, Slattery M. A distinct class of antioxidant response elements is consistently activated in tumors with NRF2 mutations. Redox Biol 2018; 19: 235-249.
28) Krajka-Kuźniak V, Paluszczak J, Baer-Dubowska W. The Nrf2-ARE signaling pathway: an update on its regulation and possible role in cancer prevention and treatment. Pharmacol Rep 2017; 69: 393-402.
29) Moon EJ, Giaccia A. Dual roles of NRF2 in tumor prevention and progression: possible implications in cancer treatment. Free Radic Biol Med 2015; 79: 292-299.