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Circular RNA circ_0046264 Suppresses Osteosarcoma Progression via microRNA-940/Secreted Frizzled Related Protein 1 Axis

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Circular RNAs (circRNAs) feature prominently in regulating tumor progression. The study aims to investigate the role and mechanism of circ_0046264 in osteosarcoma. In this study, dysregulated circRNAs in osteosarcoma tissues and adjacent tissues were screened out by analyzing circRNA microarray (GSE140256). The expressions of circ_0046264 in 58 osteosarcoma tissues and 4 osteosarcoma cell lines were detected by quantitative real-time polymerase chain reaction. Subsequently, the relationship of circ_0046264 expression level and clinical features were analyzed. Ethyldeoxyuridine assay and Transwell assay were employed to detect cell viability, migration and invasion. Dual-luciferase reporter assay was adopted to confirm the targeting relationships between circ_0046264 and microRNA-940 (miR-940), as well as miR-940 and secreted frizzled related protein 1 (SFRP1). SFRP1 expression was determined by western blot. Here, we demonstrated that circ_0046264 was greatly down-regulated in osteosarcoma and was inversely related to tumor size and Ki67 expression. Functional assays validated that circ_0046264 could restrain the proliferation, migration and invasion. Mechanistically, circ_0046264 could adsorb miR-940 and indirectly modulate SFRP1 expression. Furthermore, the transfection of miR-940 mimics or SFRP1 small interfering RNA could reverse the impact of circ_0046264 overexpression on the growth, migration and invasion of osteosarcoma cells. Taken together, circ_0046264 is a tumor suppressor to inhibit the osteosarcoma progression via modulating the miR-940 / SFRP1 axis.

Keywords: circ_0046264; microRNA-940; migration; osteosarcoma; secreted frizzled related protein 1

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Introduction

Osteosarcoma is a common bond malignancy (Longhi et al. 2006; Noone et al. 2017). The prognosis of patients with osteosarcoma is poor and the mortality rate is high, and the mechanism of its pathogenesis is still unclear (Luetke et al. 2014). Deciphering the molecular mechanism of osteosarcoma is helpful to provide new clues for the diagnosis and treatment of osteosarcoma.

Circular RNAs (circRNAs) can stably present in the cells, attributed to its covalent closed-loop structure generated from specific splicing (Ebbesen et al. 2016). Over the past years, circRNAs, reportedly, can participate in the progression of multiple tumors. For example, circRNAs can modulate the downstream target of miRNAs via adsorbing miRNAs as competing endogenous RNAs (Tay et al. 2014). In osteosarcoma, circCCDC66 can adsorb miR-338-3p and elevate PTP1B expression, thus participating in the osteosarcoma progression (Xiang et al. 2020). Circ_0008934 promotes osteosarcoma cell proliferation and migration via targeting miR-145-5p to increase E2F3 expression (Li et al. 2020). CircFAT1 sponges miR-181b and regulates HK2 expression, thereby promoting the metastasis of osteosarcoma cells (Gu et al. 2020), but circRNA functions and mechanisms in osteosarcoma have not yet been fully clarified.

MicroRNAs (miRNAs), recognized as small non-coding RNAs, can combine with the the 3′UTR of target genes

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and modulate their expressions (Anastasiadou et al. 2018). MiR-940, as a tumor-promoter, negatively regulates secreted frizzled related protein 1 (SFRP1), thereby partaking in the progression of pancreatic cancer (Lin et al. 2018) and osteosarcoma (Yang et al. 2016). In osteosarcoma, miR-940 can promote cell migration and invasion via impeding SFRP1 and activating Wnt/*β*-catenin signaling pathway (Yang et al. 2016). However, the mechanism of miR-940/SFRP1 dysregulation in osteosarcoma is indeterminate.

In this work, we screened out circRNA circ_0046264, which was significantly down-regulated in osteosarcoma tissues by analyzing circRNA microarray, and analyzed circ_0046264 expression in osteosarcoma tissues and adjacent tissues by quantitative real-time polymerase chain reaction (qRT-PCR). We next explored the biological function of circ_0046264 and analyzed its regulatory relationship with miR-940 and SFRP1. Overall, our research shows that circ_0046264 functions as a tumor suppressor in osteosarcoma via regulating miR-940 / SFRP1 axis.

Materials and Methods

Microarray analysis

The microarray GSE140256 was adopted to analyze the differentially expressed circRNAs in tissue samples of three osteosarcoma patients, with fold change absolute value > 1 and $P < 0.05$ as the criterion. CircRNA expression among samples were displayed by hierarchical clustering.

Participants and tissue samples

Osteosarcoma tissue samples (n = 58) and adjacent tissues (n = 58) that were 3 cm from the edge of tumor from Affiliated Hospital of Binzhou Medical University were confirmed by 2 pathologists and frozen in liquid nitrogen immediately after removal. None of the patients had received radiotherapy, chemotherapy or other treatment before the surgery. This study, with patient's written informed consent, was endorsed by the Ethics Committee of Affiliated Hospital of Binzhou Medical University, and was conducted in accordance with the ethical standards formulated by the Helsinki Declaration of 1964.

Cell culture

Four osteosarcoma cell lines (MG-63, U2OS, HOS and 143B) and normal human osteoblast cell line hFOB1.19 available from Cell Bank of Chinese Academy of Sciences (Shanghai, China) were cultured in Dulbecco's Modified Eagle Medium (Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (Gibco), 100 U/mL penicillin and 0.1 mg/mL streptomycin (Gibco) at 37°C and 5% CO_2.

Cell transfection

Circ_0046264 overexpression plasmid (OE-circ_0046264), empty plasmid, miR-940 mimics and negative control miR-NC, small interfering RNA (siRNA) oligonucleotides targeting human SFRP1 (si-SFRP1), negative control siRNA (si-NC) were available from Genomeditech (Shanghai, China). When the confluency of the cells reached to 60-80%, the above vectors were transfected into OS cell lines by Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) as the manufacturer's introduction.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA extracted from tissues and cell lines by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) were reversely transcribed by ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan), and qRT-PCR was operated adopting SYBR Green Premix Ex Taq TM kit (Takara, Kusatsu, Japan), with Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 as the internal references. The relative RNA expression was calculated by $2^{-\Delta\Delta Ct}$. The primer sequences are detailed in Table 1.

Table 1. The Primer Sequences for quantitative real-time polymerase chain reaction Analysis.

circ_0046264	Forward	5′-CGACAAAGATGGGGTTGTCC-3′
	Reverse	5′-CCAACCTGATCTCGGAACCT-3′
miR-940	Forward	5′-GCATCGTTCCTTCAAGCCGATCT-3′
	Reverse	5′- TGGGTGAGTCGTTCGG-3′
P4HB	Forward	5′- GGAATGGAGACACGGCTTC-3'
	Reverse	5′- TTCAGCCAGTTCACGATGTC-3'
SFRP1	Forward	5′- CCAATCGATGCCCCCATC-3′
	Reverse	5′-GAACGAGGTCAAGCTCTCACA-3′
U6	Forward	5′-GACTATCATATGCTTACCGT-3′
	Reverse	5′-GGGCAGGAAGAGGGCCTAT-3′
GAPDH	Forward	5′-TGACTTCAACAGCGACACCCA-3′
	Reverse	5′- CACCCTGTTGCTGTAGCCAAA-3′

miR-940, microRNA-940; P4HB, prolyl 4-hydroxylase subunit beta; SFRP1, secreted frizzled related protein 1; U6, U6 small nuclear 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Correlation between circ_0046264 and clinicopathological features in osteosarcoma.

Characteristics	n	circ_0046264		*P*
		High expression (n = 29)	Low expression (n = 29)	
Sex				
Male	30	16	14	0.599
Female	28	13	15	
Tumor size (cm)				
< 3	27	18	9	0.017*
≥ 3	31	11	20	
Ki67 expression				
< 50%	26	17	9	0.034*
> 50%	32	12	20	
Staging				
I-II	32	15	17	0.597
III-IV	26	14	12	
Lymph node metastasis				
No	28	16	12	0.293
Yes	30	13	17	

*$P < 0.05$

RNase R treatment and subcellular fractionation

3 U/mg RNase R (Genesee, Guangzhou, China) was incubated with 5 μg RNA at 37°C for 30 min, and then the levels of P4HB and circ_0046264 were detected by qRT-PCR. PARIS™ Kit (Invitrogen) was adopted to separate the nuclear and cytoplasmic fractions of osteosarcoma cells to measure the localization of circ_0046264. The expression patterns of circ_0046264, GAPDH and U6 in cytoplasmic and nucleus fractions were determined by qRT-PCR, with U6 as the nuclear marker and GAPDH as the cytoplasmic marker.

Bioinformatics analysis

By querying CircPrimerTool (http://www.bioinf.com.cn/) and circbase online database (http://www.circbase.org/), the genome sequence and ring structure of circ_0046264 were obtained.

Ethynyldeoxyuridine (EdU) analysis

The EdU kit (KeyGen Biotech, Nanjing, China) was used to measure cell proliferation. Transfected osteosarcoma cells were incubated with 50 μM of EdU at 37°C, fixed with 4% paraformaldehyde for 30 min. Two h later, the aforementioned cells were incubated with glycine for 10 min. In the cells treated with 0.5% Triton X-100, the nucleus was stained with 4′,6-diamidino-2-phenylindole (DAPI) staining solution. Notably, the EdU-positive cells were detected by a fluorescence microscope (Nikon Eclipse Ti Microscope; Nikon, Tokyo, Japan).

Transwell assay

Transwell chamber (BD Biosciences, Bedford, MA, USA) or Transwell chamber pre-coated with Matrigel (Becton Dickinson, Carlsbad, CA, USA) was used to detect cell migration and invasion, with 200 μL of serum-free medium added into the upper chamber and 600 μL of complete medium into the lower. Twenty four h later, the cells in the lower chamber were stained with crystal violet, and counted under the inverted microscope (Olympus, Tokyo, Japan).

Dual-luciferase reporter assay

Wild-type circ_0046264 and wild-type SFRP1 3′UTR sequences containing miR-940 binding sites were inserted into pGL3 promoter vector (Promega, Madison, WI, USA), and the mutant sequences of circ_0046264 and SFRP1 were also inserted into pGL3 promoter vector (Promega). Above luciferase reporters were co-transfected with miR-940 mimics or miR-NC into osteosarcoma cells, and then the cells were cultured for 48 h, and the dual-Luciferase reporter assay system (Promega) was adopted to determine the luciferase activity.

Western blot

Proteins were extracted from tissues and cells by the radio-immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). The protein concentrations were determined by the Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo-Fisher Scientific, Waltham, MA, USA). Then 30 μg protein sample in each group was electrophoresed by sodium dodecyl sulfate-polyacrylate gel electrophoresis (SDS-PAGE) and transferred to polyvinyl fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which was blocked with 5% skimmed milk. Subsequently,

the membranes were firstly incubated with anti-SFRP1 antibody (1:1,000; ab267466, Abcam, Cambridge, UK) and anti-GAPDH antibody (1:1,000; ab8245, Abcam, Cambridge, UK) at 4℃, and secondly with the horseradish peroxidase-conjugated secondary antibody (1:2,000; ab150077, Abcam, Cambridge, UK) for 2 h at ambient temperature. Protein bands were then developed by the ECL luminescence reagent (ThermoFisher Scientific), with GAPDH was used as the internal reference.

Statistical analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was employed for statistical analysis. All experiments were carried out three times, with results expressed as mean-standard deviation. Student's *t* test or one-way ANOVA combined with LSD post-hoc test was adopted for comparison between/among groups, and Pearson correlation was employed for correlation analysis. Statistically, $P < 0.05$ is considered significant.

Results

Circ_0046264 is significantly down-regulated in osteosarcoma tissues and cell lines

Through analyzing microarray expression profile (GSE140256), the differentially expressed circRNAs of osteosarcoma tissues and adjacent tissues were obtained, and it was observed that circ_0046264 was significantly down-regulated (Fig. 1A, B). Then, qRT-PCR indicated that circ_0046264 expression in osteosarcoma tissues was significantly lower compared with paracancerous tissues in 58 patients with osteosarcoma (Fig. 1C). Subsequently, we focused on analyzing the relationship between the expression of circ_0046264 and clinicopathological features of the patients. The samples were divided into high expression group and low expression group according to the median value of circ_0046264. The results showed that the low expression of circ_0046264 was associated with larger tumor diameter and positive Ki67 expression. Additionally, circ_0046264 expression was greatly lower in 4 osteosarcoma cell lines than that in normal human osteoblasts (Fig. 1D). To verify the stability of circ_0046264, total RNA extracted from U2OS and HOS cell lines were treated with RNase R. We found that the linear P4HB in RNase R-treated group was decreased significantly, while circ_0046264 was not digested by RNase R (Fig. 1E, F). To unveil the subcellular localization of circ_0046264, we detected circ_0046264 expression levels in cytoplasm and nucleus of U2OS and HOS cells, respectively, and found that circ_0046264 was mainly located in cytoplasm, suggesting it could probably function as a competitive endogenous RNA (ceRNA) (Fig. 1G). In addition, we obtained the sequence of circ_0046264 through the circPrimer and confirmed the ring structure of circ_0046264, composed of exon 2, 3, and 4 of P4HB gene (Fig. 1H).

Circ_0046264 can inhibit the proliferation, migration and invasion of osteosarcoma cell

In order to explore the function of circ_0046264 in osteosarcoma, circ_0046264 overexpression plasmid was transfected into osteosarcoma cells. It was observed that circ_0046264 overexpression plasmids could significantly increase circ_0046264 expression compared with control group, but there was no obvious changes in P4HB mRNA expression (Fig. 2A). EdU experiment uncovered that circ_0046264 overexpression could significantly inhibit the proliferation of osteosarcoma cells compared with the control group (Fig. 2B). Transwell assay confirmed that the ability of cell migration and invasion in circ_0046264 overexpression group was significantly reduced than that in the control group (Fig. 2C). Taken together, these data suggested that circ_0046264 can inhibit the malignant biological behaviors of osteosarcoma cells.

Circ_0046264 can adsorb miR-940 to modulate SFRP1 expression

Given that circ_0046264 is mainly stably located in cytoplasm, and circRNAs may act as ceRNAs and modulate downstream target expression via adsorbing miRNAs, we then tried to identify the downstream miRNA of circ_0046264. We confirmed that miR-940 contained binding sites complementary to circ_0046264 and SFRP1 by searching StarBase database, and dual-luciferase reporter assay confirmed that miR-940 mimics could significantly reduce the luciferase activity of the reporter containing circ_0046264-WT or SFRP1-WT, while that of the reporter containing circ_0046264-MUT or SFRP1-MUT was not significantly impacted (Fig. 3A, B). qRT-PCR and western blot highlighted that miR-940 was significantly up-regulated in osteosarcoma tissues and cell lines (Fig. 3C, D), while SFRP1 was underexpressed (Fig. 3E, F). Pearson correlation analysis confirmed that miR-940 was negatively correlated with circ_0046264 (Fig. 3G) and SFRP1 mRNA (Fig. 3H), while circ_0046264 was positively correlated with SFRP1 mRNA in osteosarcoma tissues (Fig. 3I). Next, circ_0046264 overexpression plasmid was transfected into osteosarcoma cell line, and qRT-PCR and western blot confirmed that miR-940 expression was decreased significantly by circ_0046264 overexpression, while SFRP1 expression was increased significantly. Co-transfection of circ_0046264 overexpression plasmid and miR-940 mimics in osteosarcoma cell line could significantly reverse the impact of circ_0046264 overexpression plasmid on SFRP1 expression (Fig. 3J, K), thus confirming that miR-940/SFRP1 is the downstream target of circ_0046264 in osteosarcoma, and circ_0046264 can adsorb miR-940 to modulate SFRP1 expression.

MiR-940 mimics or SFRP1 siRNA can reverse the antitumor effect induced by circ_0046264 overexpression in osteosarcoma cells.

To further confirm whether circ_0046264 inhibits the

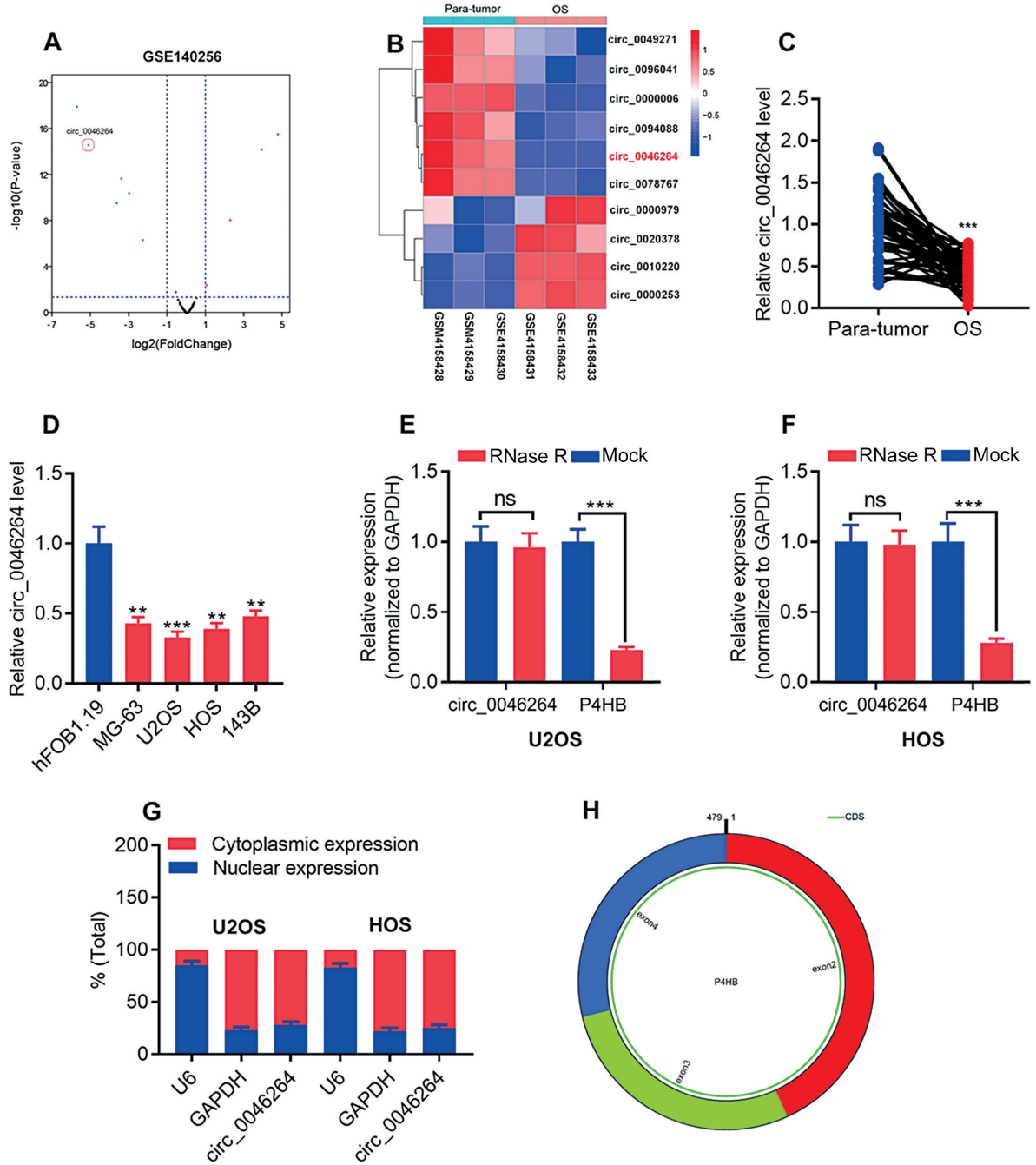

Fig. 1. Circ_0046264 is significantly down-regulated in osteosarcoma tissues and cell lines, and circ_0046264 is mainly located in cytoplasm.

A and B: Volcanic plot and heat map indicated the differences in circRNA expression profiling between osteosarcoma tissues and adjacent tissues in GSE140256. C and D: qRT-PCR was used to detect the expression of circ_0046264 in osteosarcoma tissues and cell lines. E and F: qRT-PCR was used to detect the expression of P4HB and circ_0046264 after the RNA was treated with RNase R. G: qRT-PCR was used to detect the expression of circ_0046264 in osteosarcoma cytoplasm and nucleus. H: Circ_0046264 was derived from exon 2-4 of P4HB gene by back-splicing.

ns, $P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

osteosarcoma progression via targeting miR-940/SFRP1 axis, we transfected circ_0046264 overexpression plasmid and miR-940 mimics, and circ_0046264 overexpression plasmid and SFRP1 siRNA into osteosarcoma cells, respectively, and proved that miR-940 mimics or SFRP1 siRNA could partially reverse the inhibitory effect of circ_0046264 overexpression plasmid on the malignant biological behaviors of osteosarcoma cells (Fig. 4A-D). The aforementioned findings highlighted that circ_0046264 impeded the progression of osteosarcoma via targeting miR-940/SFRP1 axis.

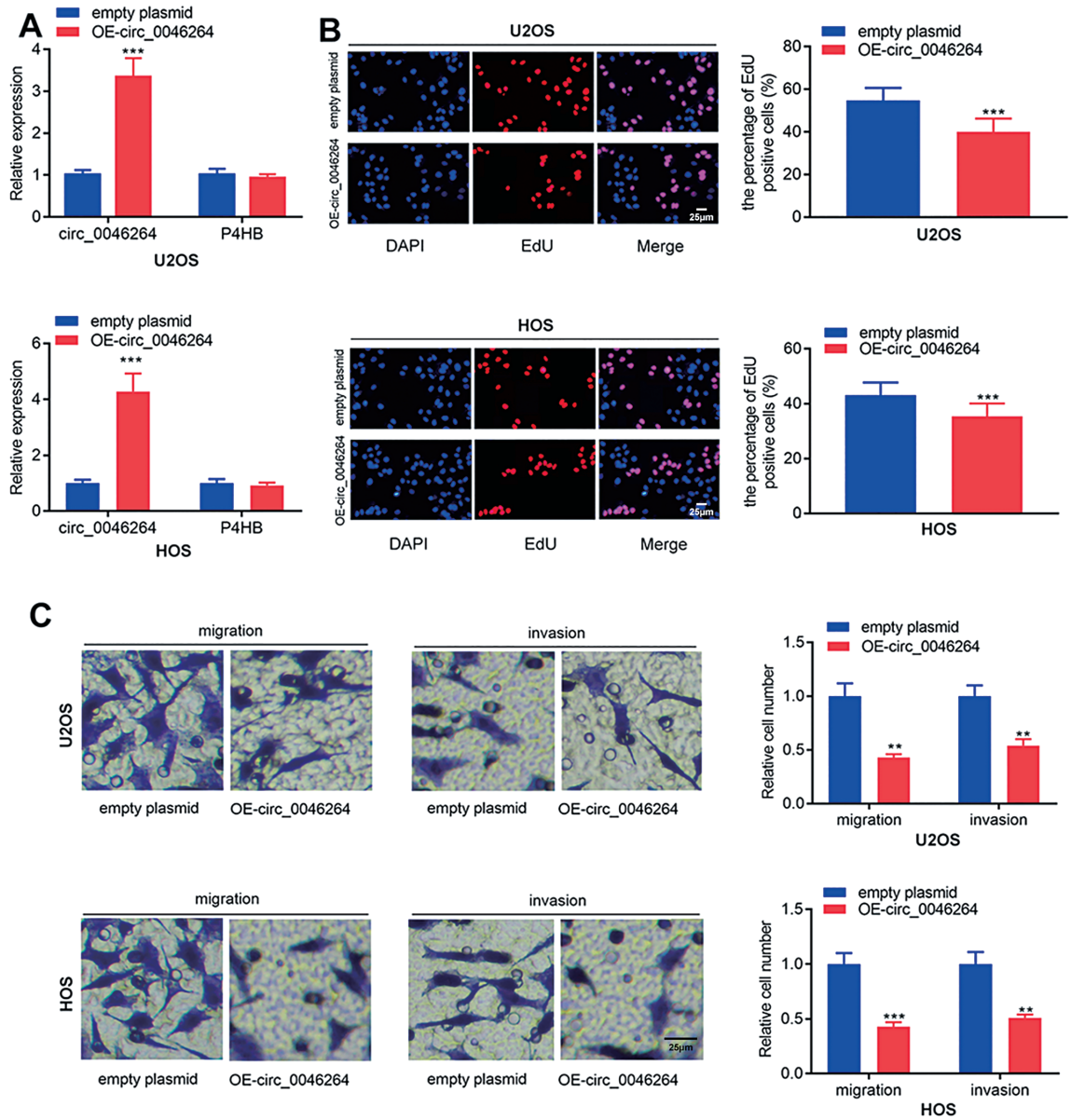

Fig. 2. Circ_0046264 can inhibit the proliferation, migration and invasion of osteosarcoma cells.
A: qRT-PCR was used to detect the expression of circ_0046264 and P4HB mRNA in osteosarcoma cells transfected with circ_0046264 overexpression plasmid. B: EdU assay was used to detect the proliferation of osteosarcoma cells transfected with circ_0046264 overexpression plasmid. C: Transwell assay was used to detect the migration and invasion of osteosarcoma cells transfected with circ_0046264 overexpression plasmid.
$^{**}P < 0.01$; $^{***}P < 0.001$.

Discussion

CircRNA, as an endogenous non-coding RNA, is vital in many biological processes, especially in tumorigenesis and cancer progression (Visci et al. 2020). Certain circRNAs are abnormally expressed in osteosarcoma, and they modulate osteosarcoma progression (Soghli et al. 2020). However, the function of circRNAs in osteosarcoma has not been fully elucidated. In the present study, through analyzing GSE140256, 6 circRNAs (circ_0000006, circ_0046264, circ_0078767, circ_0094088, circ_0096041, circ_0049271) were found to be down-regulated in osteosarcoma tissues, while 4 circRNAs (circ_0010220, circ_0000253, circ_0020378, circ_0000979) were up-regulated. Circ_0046264, reportedly, is significantly lowly expressed in lung cancer, and it can inhibit cancer cell proliferation, migration and invasion, while induce the apoptosis (Yang et al. 2018). In this work, we observed that circ_0046264 was significantly down-regulated in osteosarcoma tissues and cell lines, and it was resistant to RNase R. Besides, we found that the low expression of circ_0046264 was associated with larger tumor diameter and higher Ki67 expression in patients with osteosarcoma. Gain-of-function assays proved that circ_0046264 overexpression could sig-

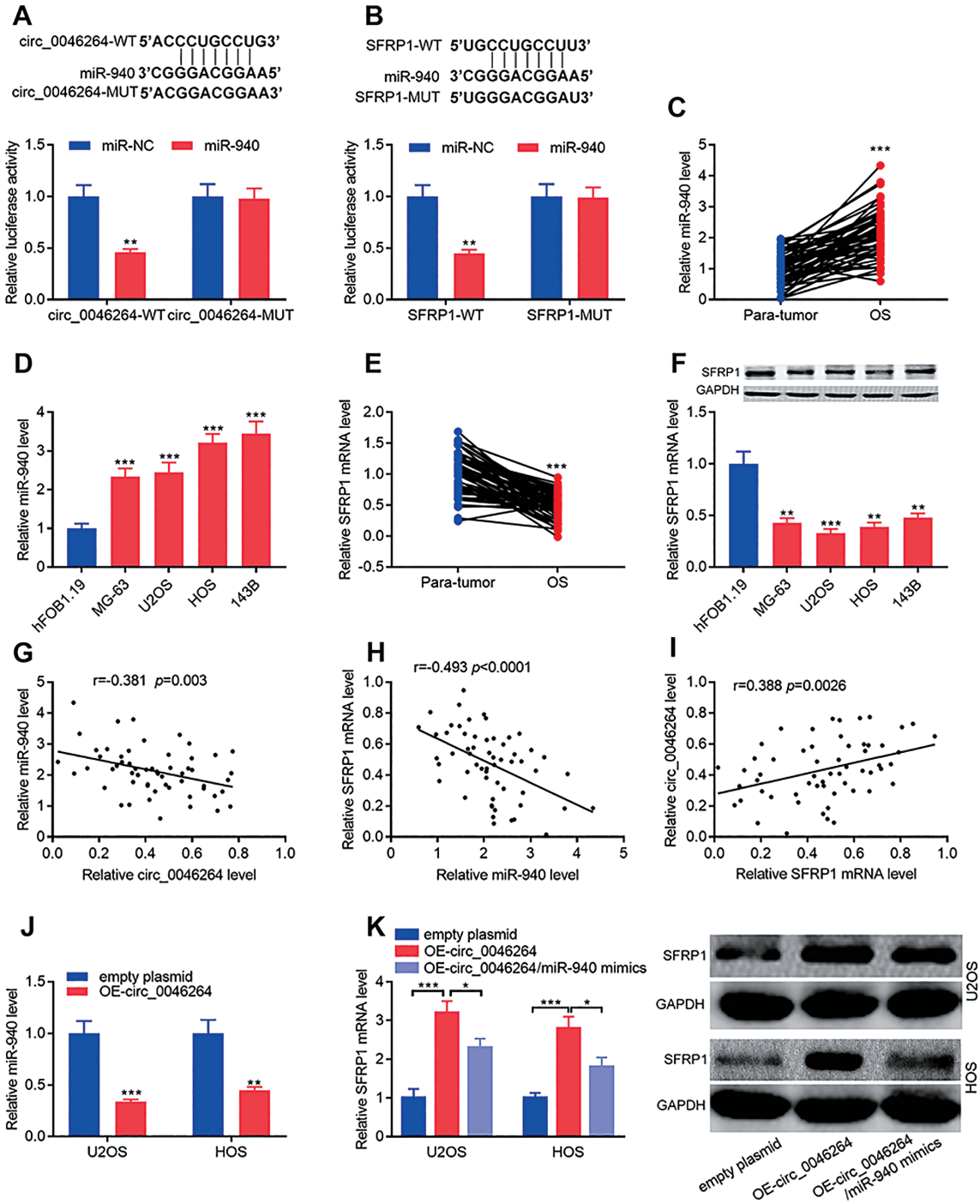

Fig. 3. Circ_0046264 can adsorb miR-940 to regulate the expression of SFRP1.
A and B: StarBase database and dual-luciferase reporter gene assay confirmed that circ_0046264 and miR-940, and miR-940 and SFRP1 mRNA could interact with each other. C and D: MiR-940 expression was detected in osteosarcoma tissues and cell lines by qRT-PCR. E and F: qRT-PCR and western blot were used to detect the expression of SFRP1 in osteosarcoma tissues and cell lines. G and I: Pearson correlation analysis was used to detect the correlation among circ_0046264, miR-940 and SFRP1 mRNA in osteosarcoma tissues. J: qRT-PCR was used to detect the expression of miR-940 in osteosarcoma cells transfected with circ_0046264 overexpression plasmid. K: qRT-PCR and western blot were used to detect the expression of SFRP1 after osteosarcoma cells were co-transfected with circ_0046264 overexpression plasmid and miR-940 mimics.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

nificantly inhibit the viability, migration and invasion of osteosarcoma cells, showing that circ_0046264 partakes in the progression of osteosarcoma as a tumor suppressor.

CircRNAs, a vital part of ceRNA network, can work as molecular sponges of miRNAs to participate in regulating tumor progression (Chen and Yang 2015). Here, we

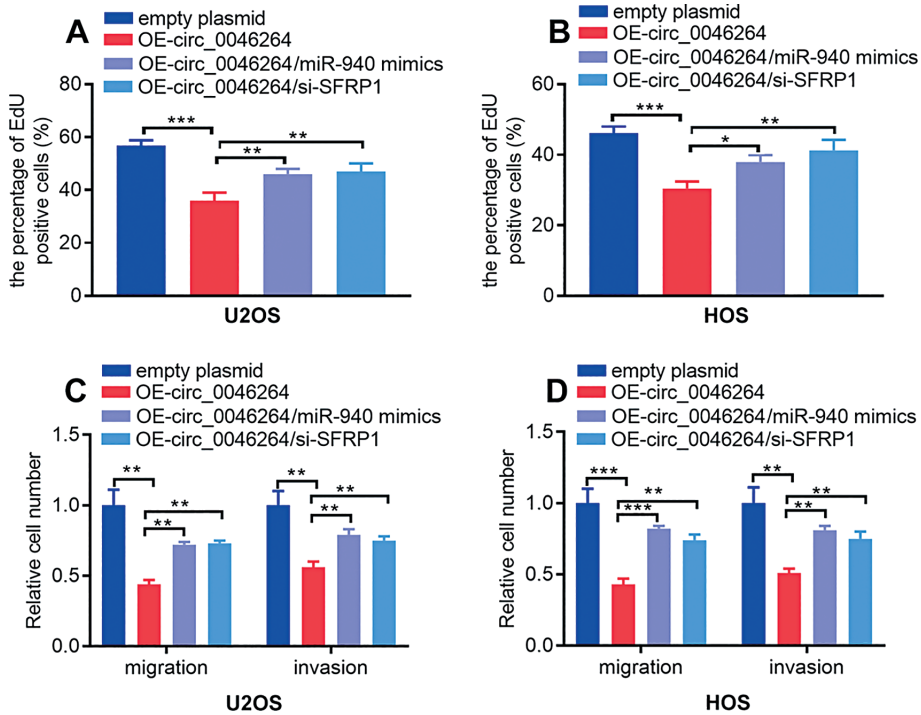

Fig. 4. MiR-940 mimics or SFRP1 siRNA can reverse the anti-tumor effect induced by circ_0046264 overexpression in osteosarcoma cells.
A and B: EdU experiment was used to detect the proliferation of osteosarcoma cells after co-transfection of circ_0046264 overexpression plasmid and miR-940 mimics, or circ_0046264 overexpression plasmid and SFRP1 siRNA. C and D: Transwell assay was used to detect the migration and invasion of osteosarcoma cells after co-transfection of circ_0046264 overexpression plasmid and miR-940 mimics, or circ_0046264 overexpression plasmid and SFRP1 siRNA.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

confirmed that miR-940 contained a binding site complementary to circ_0046264 by Starbase database. Dual-luciferase reporter assay confirmed that miR-940 could bind to circ_0046264 in osteosarcoma cell line. MiR-940, as reported, is significantly up-regulated in many tumors, and can be a tumor-promoter in tumor progression. For example, in breast cancer, miR-940 promotes cell proliferation and invasion via modulating FOXO3 (Zhang et al. 2020). In endometrial carcinoma, miR-940 promotes cell proliferation and metastasis via regulating MRVI1 (Zhou et al. 2019). In gastric cancer, miR-940 expedites cell growth and migration via up-regulating PD-L1 (Fan et al. 2018). Besides, in osteosarcoma, miR-940 is reported to be significantly up-regulated, and promotes the malignant phenotypes of cancer cells by targeting SFRP1. Our research also proved that miR-940 was significantly up-regulated in osteosarcoma tumor tissues and cell lines, and negatively correlated with circ_0046264 expression. Circ_0046264 overexpression could significantly inhibit miR-940 expression. MiR-940 mimics could significantly reverse the impact of circ_0046264 on proliferation, migration and invasion of osteosarcoma cells, highlighting that circ_0046264 partakes in osteosarcoma via adsorbing miR-940.

SFRP1, belonging to secreted glycoprotein sfrp family, can encode a secreted protein containing 314 amino acids (35.4 kDa). SFRP1 protein has two independent domains, namely, carbohydrate-terminal netrin (NTR) and amino-terminal cysteine-rich domain (CRD) (Baharudin et al. 2020). SFRP1 is a tumor suppressor of many tumors. Specifically, in gastric cancer, SFRP1 targets GSK3 β / Rac1 and inhibits TGF-β / Smad3 signaling, thus impeding cell migration and invasion (Peng et al. 2019). SFRP1 can directly bind to the ligand of Wnt protein through its NTR domain, thus restraining the Wnt activity, and reportedly, SFRP1 can inhibit the proliferation, migration and invasion of osteosarcoma cells by inhibiting Wnt / β-catenin signaling (Yang et al. 2016). In this study, we also found that SFRP1 was significantly down-regulated in osteosarcoma tissues and cell lines, which was consistent with the previous report (Yang et al. 2016). We also observed that SFRP1 mRNA was negatively regulated by miR-940 in osteosarcoma cells, and SFRP1 mRNA and miR-940 expression in osteosarcoma tissues were negatively correlated. MiR-940 mimics could partially reverse the impact of circ_0046264 overexpression on SFRP1 expression. Transfection of SFRP1 siRNA into osteosarcoma cells could partially reverse the inhibitory effect of overexpression of circ_0046264 on biological processes. Therefore, we proved that SFRP1 is the downstream target of miR-940 in osteosarcoma, and

circ_0046264 is pivotal in regulating SFRP1 expression through repressing miR-940.

To recapitulate briefly, circ_0046264 is significantly down-regulated in osteosarcoma tissues and cell lines. In terms of function and mechanism, circ_0046264 can repress the proliferation, migration and invasion of osteosarcoma cells via targeting miR-940 / SFRP1 axis. This study confirms the role of circ_0046264 in osteosarcoma for the first time, and further clarifies the molecular mechanism affecting the progression of osteosarcoma.

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Conflict of Interest

The authors declare no conflict of interest.

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