Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Circular RNA circ_0096041 promotes osteosarcoma cell proliferation and migration via sponging miR-556-5p and regulating LIN28A expression

Fulin Zhou, Hao Wu, Zhen Yin, Weibo Zhou, Wei Chen*

Department of Orthopedics, The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, Changzhou, Jiangsu 213003, China

Article Info

Article history:

Received: September 21, 2023
Accepted: December 12, 2023
Published: February 29, 2024

Use your device to scan and read the article online

Abstract

Strategies targeting lin-28 homolog A (LIN28A) for the treatment of osteosarcoma are limited, even though salient findings have illustrated the crucial role of LIN28A in bone deformities and cancer. In the present study, we proved circ_0096041, one of the circular RNAs (circRNAs) with significant upregulated expression in osteosarcoma, to be notably engaged in the progression of osteosarcoma. We elucidated that osteosarcoma patients with highly expressed circ_0096041 had relatively worse prognoses. We determined that circ_0096041 potentially sponge miR-556-5p using the Circular RNA Interactome database. Meanwhile, we proved circ_0096041 was associated with miR-556-5p. Furthermore, we determined that miR-556-5p was targeted by LIN28A directly, evidenced by *in silico* analysis using the miRWALK tool and *in vitro* analysis. Functionally, our experimental setting aimed to explore the function of circ_0096041/miR-556-5p/LIN28A axis *in vitro* and *in vivo*. Our findings demonstrated that circ_0096041 boosted the proliferation and migration of osteosarcoma via LIN28A/miR-556-5p axis. *In vivo* models were further established to estimate the metastasis promoted by circ_0096041. This research elucidated the enhanced osteosarcoma progression by circ_0096041 and its potential mechanism, which provided innovative targets for osteosarcoma treatment.

Keywords: circ_0096041, LIN28A, Migration, miR-556-5p, Osteosarcoma, Proliferation

1. Introduction

Osteosarcoma, a high-grade primary osteosarcoma, has tumor stem cells that may originate from mesenchymal stem cells, mostly in young people and children. Although the combination of surgery, radiotherapy and chemotherapy is already used in the treatment, the prognosis of advanced osteosarcoma still remains very poor [1, 2]. A majority of patients suffered from its recurrence on account of potential/present distant metastasis. Although there have been therapeutic breakthroughs for patients with recurrence [3], severe adverse effects urgently call for the need to explore the intricate mechanism of osteosarcoma pathogenesis, progression, and metastasis.

Circ-RNA, as the subclass of endogenous non-coding RNAs, is located in eukaryotic cells and has been confirmed to sponge miRNAs, interact with proteins, and serve as the transcription factor [4]. Meanwhile, circRNAs are associated with specific types of cancer and distinctly regulate carcinogenesis and progression [5]. For instance, various circRNAs, including circ_0096041, have been shown to be upregulated in osteosarcoma [6]. However, the functional characterization and mechanistic of circRNAs in osteosarcoma are largely unknown [5]. On the other hand, miRNAs are vital regulators of oncogenicity; miR-556-5p is involved in regulating different cancer prognoses, such as breast cancer, prostate cancer, and cholangiocarcinoma [7-9].

Highly conserved RNA binding proteins Lin28A and Lin28B shared analogous structure and function. First discovered in *Caenorhabditis elegans*, Lin28A regulates the development duration [10, 11], and Lin28B was first found to be located in hepatocellular carcinoma (HCC) with high expression [12]. Previous experiments elucidated that Lin28A and Lin28B have relatively high expression in numerous cancers [13, 14]. There was also researches showing that Lin28A and Lin28B would serve as oncogenes in specific cancers via inhibiting the microRNA let-7s biogenesis or reserving the oncogenic transcription [15]. It was proposed that highly-expressed Lin28A or Lin28B is associated with malignant tumor and negative prognosis [15]. Recently, LIN28A was reported as a vital regulator of osteogenic differentiation [16]. Although LIN28B was found vital in osteosarcoma tumorigenesis [17], no reports have yet investigated the role of LIN28A in bone cancer. Therefore, we investigated the potential role of LIN28A in the progression of osteosarcoma.

Although significant progress has been made in understanding cancer prognosis and therapeutics, limited tumor

* Corresponding author.
E-mail address: 13641502368@163.com (W. Chen).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.2.16

response rates, absence of novel tumor targets, and other unforeseen hurdles retard the development of efficacious therapeutics that preeminently target osteosarcoma. In this research, we tended to demonstrate the association of circ_0096041 with osteosarcoma progression and explicated the effect of circ_0096041/miR-556-5p/LIN28A axis in osteosarcoma pathogenesis.

2. Materials and methods

2.1. Patients and samples

Osteosarcoma patients (n = 35, age 11-24, mean age 17.35) were recruited at The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University. The study was approved by The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University. All patients were informed about the purpose and methods of this work with written consent. Tumors were acquired by complete resection without chemotherapies and were classified according to the Enneking staging system, including 6 cases in stage 1, 21 cases in stage 2, and 8 cases in stage 3. Tumors and adjacent tissues were collected surgically and placed in liquid nitrogen and stored at -80°C.

2.2. Cell culture

Osteosarcoma cell lines MNNG/HOS, U2OS, and MG63, human osteoblast line hFOB 1.19 were purchased from Procell (Wuhan, China). DMEM culture medium (Thermo Fisher Scientific, USA) + 10% fetal bovine serum (FBS) (#1921005PJ, Gibco, USA) was used for cultivation. All cell lines were examined for Mycoplasma presence.

2.3. *In silico* prediction of circRNA and miRNA targets

Circular RNA Interactome was utilized to predict the interactions between miRNA and circRNA [18]. Besides, miRDB and miRWalk [19-21] were taken to analyze the binding of miRNA and target genes.

2.4. MTT assay

Previously description was taken as references [22]. Briefly, 1×10^4/well cells in 0.1 ml DMEM were placed in 96-well plates and treated accordingly for 12, 24, 36, and 48 h. Then, the mixture was treated with MTT solution (10 µl, 5 mg/mL) and incubated at 37 °C for 4 h. Dimethyl sulfoxide solution (150 µl) was added for a 10 min incubation. Optical density (OD, 490 nm) was recorded using a microplate reader (Molecular Devices, USA).

2.5. Migration assay

The migration of U2OS and MG63 cells was determined using modified Boyden chambers (MERCK, Darmstadt, Germany). The upper chamber was filled with 1×10^5 cells suspended in 0.2 mL DMEM while the lower was coated with 600 µL DMEM supplemented with FBS (10%). Each cell group was treated accordingly and incubated for 24 h at 37 °C. The migrated cells were stained and examined using a high-power microscope.

2.6. Western blotting

Total proteins from cells or tissues were extracted using lysis buffer containing protease inhibitor PMSF. Proteins were electrophoresed on SDS-PAGE gel and transferred onto nitrocellulose membranes (PVDF). Non-fat milk (5%) was used for 1 hour blocking. Then the mixture was co-incubated with primary antibodies against LIN28A (#MA1-016, Invitrogen), c-Myc (#MA1-980, Invitrogen), and Ras (#33-7200, Invitrogen) overnight at 4 °C. Secondary antibody against IgG (#MA5-42729, Invitrogen) was added for another one hour at room temperature. ECL kit (#E411-04, Vazyme, Nanjing, China) was employed to visualize protein bands.

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

TRIzol reagent (#15596026, Invitrogen) was used for RNA extraction. RT reaction kit (#K1691, Thermo Scientific) and a real-time PCR system (Agilent, Beijing, China) were used for the cDNA synthesis of 50 µg mRNA. The PCR system included 40 cycles, with each containing 15 s at 93 °C, 10 s at 55 °C, and 20 s at 75 °C. $2^{-\Delta\Delta Cq}$ method was used for calculation, with data normalized to GAPDH [23]. The utilized primer sequences in this study are as follows: circ_0096041, F: 5′-GGGCCCTGCTGGACATCATC-3′; R, 5′-CTGATGGCTGACGGCTGAGG-3′; GAPDH: F: 5′-TCCCCCACCACACTGAATCT-3′; R, 5′-AACAGGAGGAGCAGAGAGCG-3′; miR-556-5p: F: 5′-GATAGTAATAAGAAAGATGAG-3′; R, 5′- TGTTGAAGGTAGTAATAAAAA-3′; U6: F: 5′-CGAGCACAGAATCGCTTCA-3′; R, 5′-CTCGCTTCGGCAGCACATAT-3′; LIN28A, F: 5′-GAGTGAGAGGCGGCCAAAA-3′; R, 5′- TGATGATCTAGACCTCCACAGTTGTAG-3′; cMYC, F: 5′-TGAGGAGACACCGCCCAC-3′; R, 5′-CAACATCGATTTCTTCCTCATCTTC-3′; Ras, F: 5′-GTTCTAATATAGTCACATTT-3′; R, 5′- ACTCATGAAAATGGTCAGAGAAACCTTTAT-3′; β-actin, F: 5′- GCACCACACCTTCTACAATG-3′; R, 5′- TGCTTGCTGATCCACATCTG-3′.

2.8. Luciferase reporter assay

The previous description was used as a reference [24]. Briefly, 1×10^4 cells were cultivated into 96-well plates. Twenty-four hours later, miR-556-5p/NC, along with 100 ng LIN28A/LIN28A-DEL, were transfected into cells. pMIR-REPORT™ vector (#AM5795, Invitrogen) contained cloned 3′-UTR of LIN28A/LIN28A-DEL. Twenty-four hours later, Luciferase Reporter Assay System (Promega) was used to measure relative luciferase activity.

2.9. *In vivo* analysis

Eighteen female nude BALB/c mice (5–6 weeks) were purchased from TROPHIC Animal Feed High-tech Co Ltd (Nantong, China) and used to investigate tumor growth and pancreatic metastasis. MG63 cells (2×10^6) were subcutaneously injected into mice to form the tumor xenografts or injected via spleen in each mouse to form pancreatic metastasis. The animals were allocated into 3 groups (control, circ_0096041, and circ_0096041 + miR-556-5p) at random. Tumor tissues were harvested on day 28 for immunohistochemical analysis. All the procedures were in accordance with relevant guidance [25].

2.10. Hematoxylin and eosin (H&E) staining

Procedures were in accordance with the previous description [26].

2.11. Immunohistochemistry

The paraffin-embedded tumor xenografts were deparaffinized and rehydrated. Heat-induced epitope re-

trieval (HIER) was taken using Tris buffer at pH of 8.0. The samples were treated with 1:20 H_2O_2 (35%) for 20 minutes at room temperature. Anti-LIN28A (#MA1-016, Invitrogen) was then added. The LIN28A expression was determined using DAB kit (#E-IR-R101, Elabscience, Wuhan, China). Hematoxylin was added and the results were examined using bright-field microscope (Olympus, Tokyo, Japan).

2.12. Immunofluorescence analysis

MG63 cells transfected with corresponding vectors were cultured in a six-well plate on a coverslip until reached a confluency of 60–80%. Cells were prepared as previously described [27]. Primary antibody anti-LIN28A was added for an incubation at 4°C overnight. After being washed 3 times with PBS, the cells were stained with DAPI and goat anti-mouse anti-IgG conjugated with FITC for 45 min at room temperature. The LIN28A was visualized and imaged using a confocal microscope (Olympus, Tokyo, Japan). The images were obtained from five random fields of view.

2.13. Statistical analysis

All experimental data were expressed as means ± SD. The correlation between circ_0096041 and miR-556-5p was calculated using Spearman's correlation coefficient. One-way analysis of variance and the LSD test were taken for evaluation. $P<0.01$ was considered statistically significant. GraphPad version 7.0 (GraphPad, San Diego, CA, USA) was used in the statistical analysis.

3. Results

3.1. Circ_0096041 expression denotes shorter overall survival of osteosarcoma patients and negatively correlates with the expression of miR-556-5p

Previously, 10 circRNAs with differential expression were found in osteosarcoma samples; among them, circ_0096041 expression was significantly expressed [6]. The expression of circ_0096041 is highly expressed in osteosarcoma than in adjacent tissues (Figure 1A). U2OS, MG63 and HOS cells showed higher circ_0096041 expression than HFoB 1.19 (Figure 1B). Highly expressed circ_0096041 was confirmed to be a key factor in patient's poor prognosis (Figure 1C) and advanced stages (Table 1). Patient's gender, age, family history, etc. showed no relevance with circ_0096041. As the *in silico* prediction from the Circular RNA Interactome database suggested, miR-556-5p could sponge circ_0096041 (Figure 1D). Previously, miR-556-5p was shown to be engaged in different types of cancer [8, 9]. Compared with adjacent tissues, the expression of miR-556-5p was decreased in osteosarcoma samples (Figure 1E). These results were in accordance with *in vitro* assays, which showed the expression of miR-556-5p in U2OS and MG63 was apparently decreased compared to hFOB 1.19 (Figure 1F). As shown in correlation analysis (Figure 1G), circ_0096041 was correlated negatively with miR-556-5p in osteosarcoma, with the

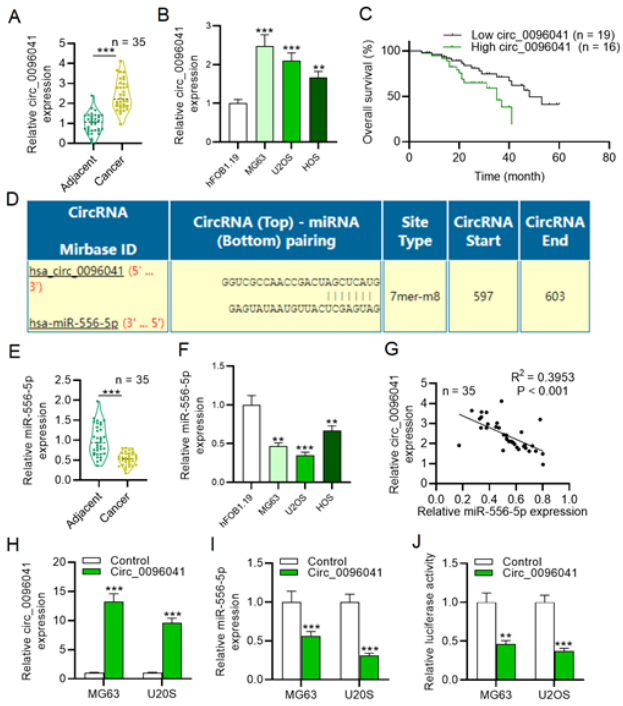

Fig. 1. Circ_0096041 and miR-556-5p expressions are negatively correlated and predict shorter overall survival of osteosarcoma patients. (A) Relative expression of circ_0096041 *in vivo*. ***P<0.001. (B) Relative expression of circ_0096041 *in vitro*. **P<0.01, ***P<0.001. (C) Survivorship curves of circ_0096041. (D) Predicted binding sites between circ_0096041 and miR-556-5p. (E) The expression of miR-556-5p *in vivo*. ***P<0.001. (F) The expression of miR-556-5p *in vitro*. **P<0.01, ***P<0.001. (G) Correlation between circ_0096041 and miR-556-5p. (H) MG63 and U2OS cells were transfected with corresponding vectors. (I) Relative expression of miR-556-5p. (J) Relative luciferase activity. **P<0.01, ***P<0.001 vs control.

Table 1. The relationship between circ_0096041 expression and stages of osteosarcomas.

Variables	Description	N	circ_0096041 expression		X^2	*P*-value
			Low	High		
Gender	Male	15	7	8	0.6140	0.4333
	Female	20	12	8		
Age (years)	<15	19	9	19	0.5928	0.4413
	>15	16	7	9		
Family history	Yes	2	1	1	0.0157	0.9003
	No	33	15	18		
TNM grade	I	5	4	1	6.591	0.037
	II	18	12	6		
	III	12	3	9		

T primary tumor, N regional lymph nodes, M metastasis. *P<0.05.

microRNA-556-5p expression inhibited by circ_0096041 confirming this finding (Figures 1H-J). These results revealed that circ_0096041 promoted osteosarcoma malignancy and inhibited the expression of miR-556-5p.

3.2. MiR-556-5p targets LIN28A in osteosarcoma

LIN28A was one of the predicted targets for miR-556-5p by STarMir Database (Figure 2A). Spearman's rank correlation analysis showed that miR-556-5p was negatively correlated with LIN28A (Figure 2B). Luciferase reporter assay manifested that miR-556-5p inhibited the activity of wild LIN28A promoter, compared to those of mutant LIN28A promoter, LIN28A DEL 3′-UTR (the sequences within the predicted binding sites were deleted) (Figures 2C, D). Overexpressed miR-556-5p downregulated LIN28A expression, whereas inhibited miR-556-5p enhanced LIN28A expression in MG63 and U2OS cells (Figures 2E, F). These results suggested that miR-556-5p targeted LIN28A.

3.3. MiR-556-5p inhibits the proliferation and migration of osteosarcoma cells by targeting LIN28A

Previously, it has been proposed that several miRNAs blocked LIN28A expression, suppressing the proliferation and migration of osteosarcoma cells [28]. MiR-556-5p mimic significantly hampered cell proliferation (Figure 3A) and migration (Figure 3B). On the contrary, the miR-556-5p inhibitor significantly activated cell proliferation (Figure 3A) and migration (Figure 3B). These results suggested that miR-578 blocked LIN28A-mediated singling pathways. For instance, it has also been proved that LIN28A contributed to the regulation of bone deformities [16] and regulated c-MYC and Ras proteins [29].

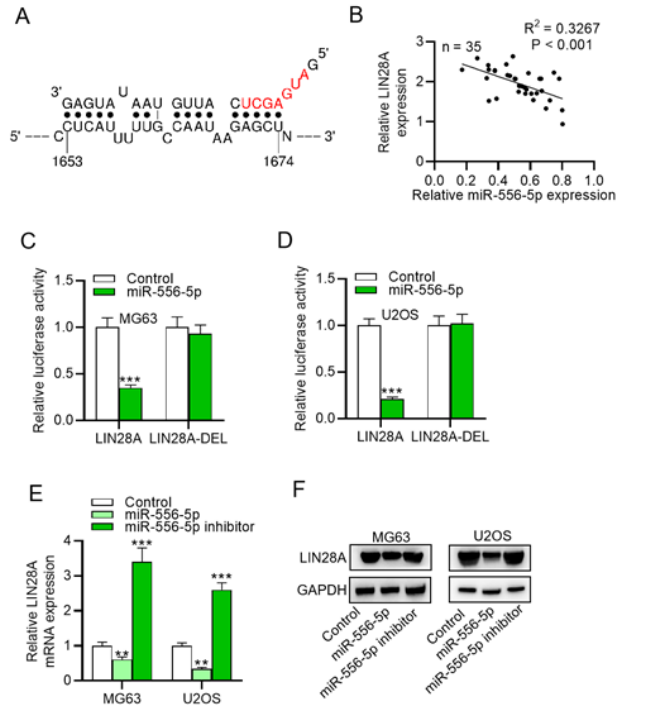

Fig. 2. MiR-556-5p targets LIN28A in osteosarcoma. (A) Binding sites between miR-556-5p and LIN28A, predicted by STarMir Database. (B) Correlation between miR-556-5p and LIN28A. (C,D) Relative luciferase activity. ***P<0.001. (E,F) Relative expression of LIN28A in cells transfected with corresponding vectors, detected by real-time PCR and western blot. **P<0.01.

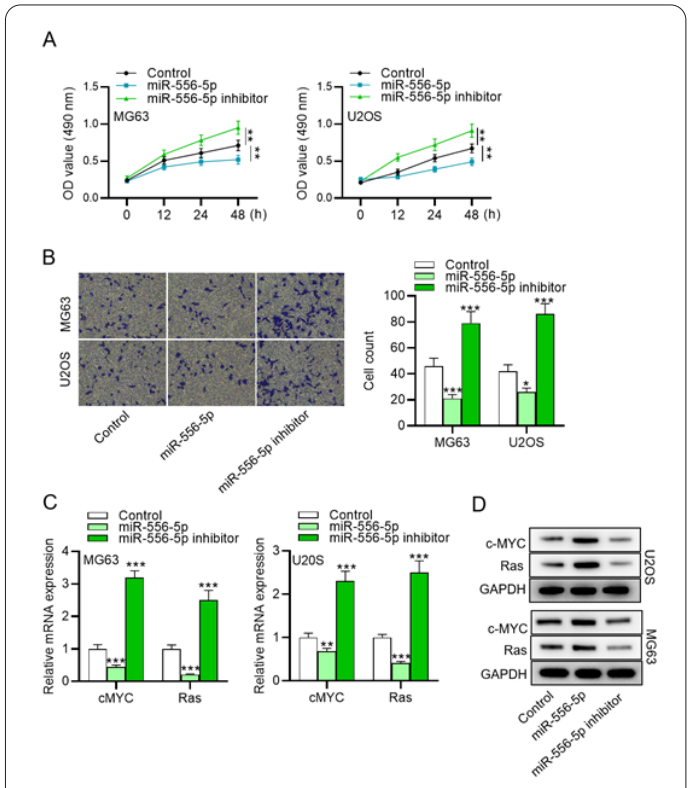

Fig. 3. MiR-556-5p inhibits the growth and migration *in vitro* by targeting LIN28A. (A) Proliferation of cells transfected with control, miR-556-5p, or miR-556-5p inhibitor, detected by MTT assay. (B) Migration of cells transfected with control, miR-556-5p, or miR-556-5p inhibitor, detected by transwell assay. (C,D) Relative protein expression in cells transfected with control, miR-556-5p, or miR-556-5p inhibitor, detected by western blot and real-time PCR. **P<0.01, ***P<0.001.

As shown in Figures 3C, D, miR-556-5p significantly increased the expression of cMYC, and Ras in MG63 cells. Similar results were observed in U2OS cells (Figures 3C, D).

3.4. Circ_0096041 promotes osteosarcoma cell proliferation and migration

As shown in Figure 4A, the expression of circ_0096041 boosted the proliferation significantly compared to the negative control. Circ_0096041 group had an increased number of migrated cells compared to control cells (Figure 4B). Western blot and qRT-PCR results indicated that circ_0096041 increased LIN28A, cMYC, and Ras expression significantly in MG63 and U2OS cells (Figures 4C, D). These results demonstrated that circ_0096041 boosted the proliferation and migration by promoting LIN28A, cMYC, and Ras.

3.5. Circ_0096041 promotes the proliferation and migration of osteosarcoma cells via abolishing the inhibition of LIN28A by miR-556-5p

It has been proved that circRNAs/miRNAs/mRNAs axis had a regulatory effect on the initiation and progression of osteosarcoma [30]. Significantly boosted proliferation was observed in circ_0096041 group (Figure 5A) while the proliferation of circ_0096041 + miR-556-5p group was merely affected. Also, the expression of circ_0096041 notably increased the migration of MG63 and U2OS cells while circ_0096041 + miR-556-5p group barely changed (Figure 5B). Western blot and qRT-PCR

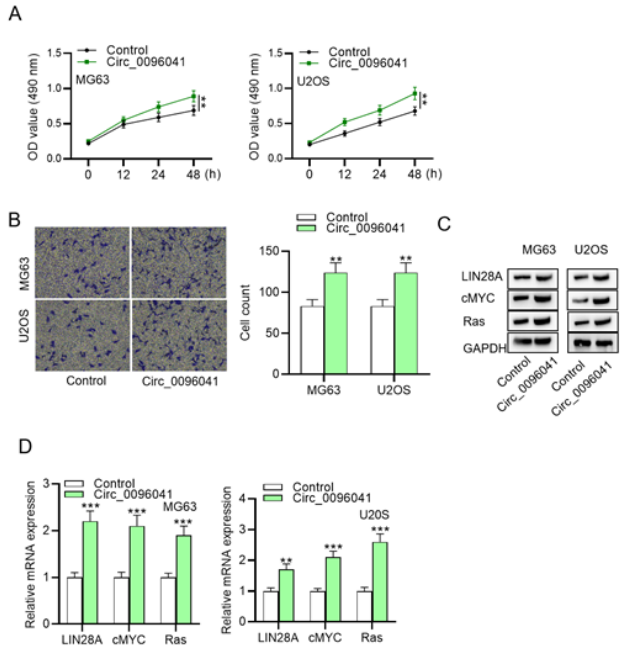

Fig. 4. Circ_0096041 promotes the migration and proliferation *in vitro*. (A) Proliferation of cells transfected with control/circ_0096041, detected by MTT assay. (B) Migration of MG63 and U2OS cells transfected with control/circ_0096041, detected by transwell assay. (C,D) Relative expression in cells expressing control or circ_0096041, detected by western blot and real-time PCR. **P<0.01, ***P<0.001.

results suggested that circ_0096041 + miR-556-5p significantly restored the enhanced LIN28A, cMYC, and Ras expression (Figures 5C, D). The immunofluorescence assay showed that LIN28A expression was increased by circ_0096041 in MG63 cells (Figure 5E) and decreased by additional miR-556-5p. These results suggested that circ_0096041 boosted LIN28A-mediated migration and proliferation by regulating miR-556-5p.

3.6. Circ_0096041 promotes osteosarcoma cell metastasis *in vivo*

Further *in vivo* analysis was conducted to determine the regulation of circ_0096041 on osteosarcoma metastasis. Three weeks later, the animals' pancreatic tissues were harvested. As shown in Figure 6A, in the circ_0096041 group, tumor cells are arranged in a disorder with obvious cell necrosis. Co-expression of circ_0096041 and miR-556-5p significantly reversed this effect. Meanwhile, circ_0096041 enhanced pancreatic metastases while in the combination group, pancreatic metastasis was decreased (Figure 6B). In addition, immunohistochemical analysis confirmed the expression of LIN28A in tumor xenografts (Figure 6C). Overexpressed circ_0096041 elevated LIN28A expression, which was canceled out by additional miR-556-5p. Circ_0096041 evidently promoted the expression of LIN28A, cMYC, and Ras, while additional miR-556-5p significantly obstructed this effect (Figures 6D, E).

4. Discussion

In recent studies, several results have elucidated the regulatory role of circRNA in various biological processes, including the initiation, progression, and metastasis of carcinomas. For instance, it was revealed that circTADA2A directly sponge miR-203a and upregulates the expression level of CREB, resulting in metastasis [31]. CircFAT1 was confirmed to restore Yes-associated protein 1 expression, which was suppressed by miR-375 and progressed tumorigenesis [32]. CircNASP was reported to sponge miR-1253 and regulate forkhead box F1, promoting osteosarcoma from proliferation and invasion [33]. However, there is still considerable room for excavation in the function of the circRNA. In this study, we have explored the regulatory role and mode of circ_0096041 in osteosarcoma.

Upregulated expression of circ_0096041 was detected in osteosarcoma cells and tissues. The association of circ_0096041 with osteosarcoma stages was determined. Meanwhile, the high expression of circ_0096041 also indicated a poor prognosis in patients with osteosarcoma. Although the evidence associated with the circ_0096041 distributions was lacking, our work indicated that circ_0096041 greatly activated the deterioration of osteosarcoma. The circRNAs/miRNAs/mRNAs axis is known to be an important regulatory network, where miRNAs act as a circRNA sponge, targeting mRNA. In the present study, lowly expressed miR-556-5p was observed in osteosarcoma tissues and cells. Furthermore, a negative correlation exists between miR-556-5p and circ_0096041, indicating that circ_0096041 sponges miR-556-5p. The miR-556-5p was demonstrated to induce apoptosis in can-

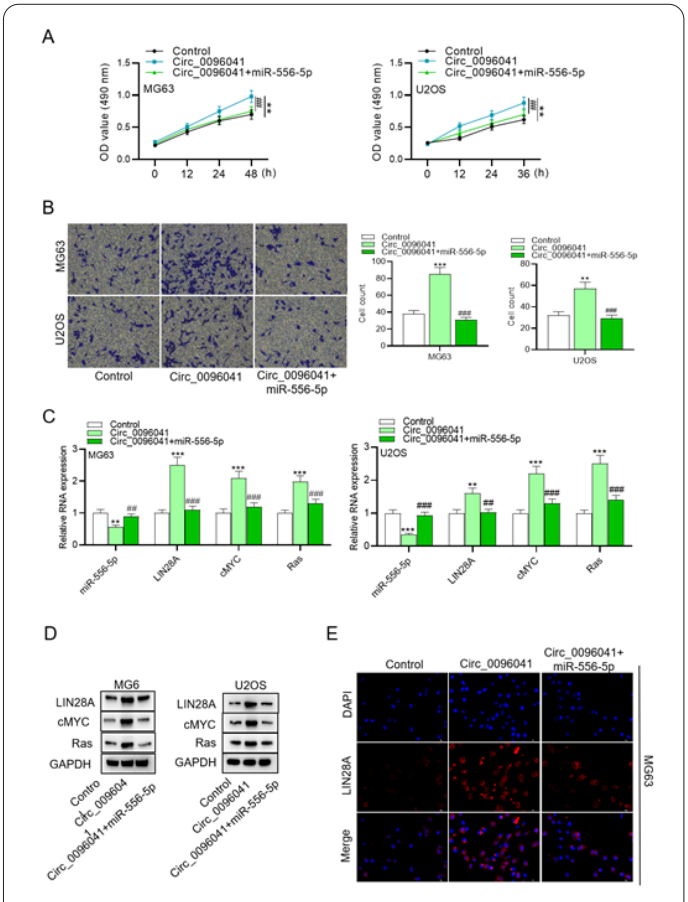

Fig. 5. Circ_0096041 promotes cell migration and proliferation via promoting LIN28A inhibited by miR-556-5p. (A) Proliferation in cells transfected with control/circ_0096041/circ_0096041 + miR-556-5p, detected by MTT assay. (B) Migration of MG63 and U2OS cells transfected with control/circ_0096041/circ_0096041 + miR-556-5p, detected by transwell assay. (C,D) Gene expression in MG63 and U2OS cells, detected by western blot and real-time PCR. (E) Immunofluorescent staining of LIN28A in MG63 cells. Green, LIN28A; Blue, DAPI. **P<0.01, ***P<0.001, ##P<0.01, ###P<0.001.

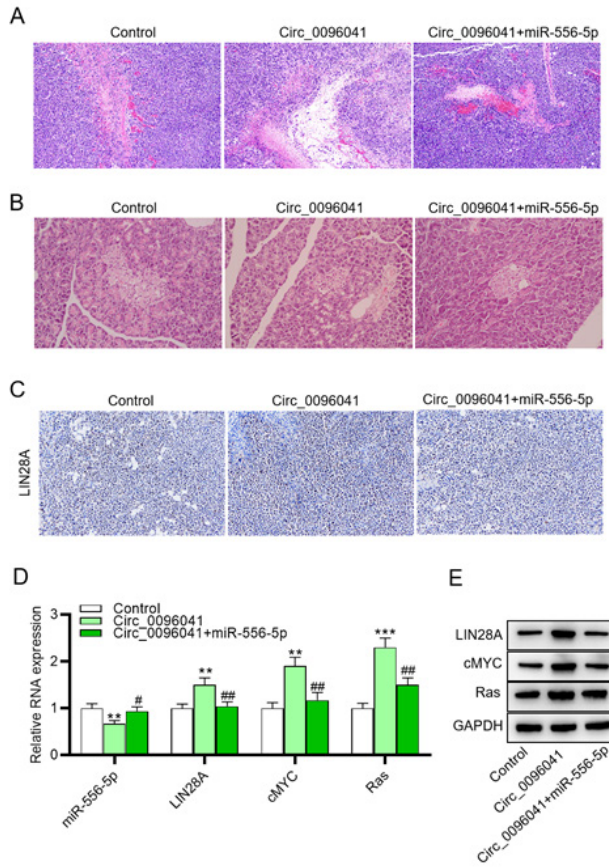

Fig. 6. Circ_0096041 promotes osteosarcoma metastasis *in vivo*. (A and B) The xenograft and pancreatic metastatic foci were validated by hematoxylin and eosin staining. (C) Immunohistochemistry of LIN28A expression in tumor xenografts. (D) Real-time PCR of LIN28A gene expression and its subsequent signaling genes cMyc and Ras. (E) Western blotting analysis of LIN28A protein expression and its subsequent signaling proteins cMyc and Ras. Results are represented as the mean ± SD of three independent experiments. **P<0.01, ***P<0.001 vs control; ##P<0.05, ##P<0.01, vs circ_0096041.

cer cells [8, 9]. MiR-556-5p was also proven to modulate cMYC and Ras expression in prostate cancer [29]. It was speculated that the tissue-specific expression of miR-556-5p caused its versatility, regulating different mRNA and protein networks. Alternatively, circ_0096041 acts by modulating the signaling cascades in cancer, both temporally and in space.

It has shown that let-7, as a key RNA-binding protein, underlies tumor growth, invasion and metastasis and regulates carcinogenesis, progression and recurrence of osteosarcoma [13, 15, 16, 34]. The increasing number of studies revealing the role of the LIN28 pathway has also produced corresponding targeted therapies against LIN28A / B for the treatment of recurrent/advanced prostate cancer [29]. However, how LIN28A regulates cancer development and the associated signaling pathways remains largely unknown, hindering the targeted therapeutic advancement of LIN28A. LIN28 is involved in regulating osteogenic differentiation, however, its role in osteosarcoma remains unknown. This study confirmed that circ_0096041 advances osteosarcoma deterioration via the LIN28A/cMYC/Ras axis, compensating for the lack of research on LIN28A-related pathways. Further experiments explored the differential expression of circ_0096041 in different subtypes and osteosarcoma in stage-specific ones, as well as in osteosarcoma tissue and serum. Furthermore, the correlation between circulating LIN28A and circ_0096041 confirmed that circulating LIN28A is strongly associated with the development of metastatic prostate cancer [29].

In conclusion, this study determined the role and mechanism of circ_0096041 as an oncogene in the progression of osteosarcoma. It regulates the proliferation and migratory activities involved in LIN28A regulation by sponging miR-556-5p. These results help to advance the inquiry into the function of circ_0096041 and miR-556-5p in cancer, providing a new explanation for the regulation of the LIN28A signaling cascade.

Informed Consent

The authors report no conflict of interest.

Availability of data and material

We declared that we embedded all data in the manuscript.

Authors' contributions

ZF conducted the experiments and wrote the paper; WH, YZ and ZW analyzed and organized the data; CW conceived, designed the study and revised the manuscript.

Funding

This work was supported by the Changzhou Sci&Tech Program (Grant No. CJ20230065).

Acknowledgement

We thanked the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University approval our study.

References

1. Versleijen-Jonkers YM, Vlenterie M, van de Luijtgaarden AC, van der Graaf WT (2014) Anti-angiogenic therapy, a new player in the field of sarcoma treatment. Crit Rev Oncol Hematol 91(2):172–185. doi: 10.1016/j.critrevonc.2014.02.001
2. Vornicova O, Bar-Sela G (2016) Investigational therapies for Ewing sarcoma: a search without a clear finding. Expert Opin Investig Drugs 25(6):679–686. doi: 10.1517/13543784.2016.1168398
3. Otoukesh B, Boddouhi B, Moghtadaei M, Kaghazian P, Kaghazian M (2018) Novel molecular insights and new therapeutic strategies in osteosarcoma. Cancer Cell Int 18:158. doi: 10.1186/s12935-018-0654-4
4. Shang Q, Yang Z, Jia R, Ge S (2019) The novel roles of circRNAs in human cancer. Mol Cancer 18(1):6. doi: 10.1186/s12943-018-0934-6
5. Wang C, Jing J, Cheng L (2018) Emerging roles of non-coding RNAs in the pathogenesis, diagnosis and prognosis of osteosarcoma. Invest New Drugs 36(6):1116–1132. doi: 10.1007/s10637-018-0624-7
6. Ji X, Shan L, Shen P, He M (2020) Circular RNA circ_001621 promotes osteosarcoma cells proliferation and migration by sponging miR-578 and regulating VEGF expression. Cell Death Dis 11(1):18. doi: 10.1038/s41419-019-2204-y
7. Xu Y, Gao P, Wang Z, Su Z, Liao G, Han Y, et al (2021) CircLAMP1 contributes to the growth and metastasis of cholangiocarcinoma via miR-556-5p and miR-567 mediated YY1 activation. J Cell Mol Med 25(7):3226–3238. doi: 10.1111/jcmm.16392
8. Zhao W, Cao L, Zeng S, Qin H, Men T (2015) Upregulation of miR-556-5p promoted prostate cancer cell proliferation by suppressing PPP2R2A expression. Biomed Pharmacother 75:142–147. doi: 10.1016/j.biopha.2015.07.015

9. Zhou Y, Liu X, Lan J, Wan Y, Zhu X (2020) Circular RNA circRPPH1 promotes triple-negative breast cancer progression via the miR-556-5p/YAP1 axis. Am J Transl Res 12(10):6220–6234.
10. Horvitz HR, Sulston JE (1980) Isolation and genetic characterization of cell-lineage mutants of the nematode Caenorhabditis elegans. Genetics 96(2):435–454. doi: 10.1093/genetics/96.2.435
11. Ambros V, Horvitz HR (1984) Heterochronic mutants of the nematode Caenorhabditis elegans. Science 226(4673):409–416. doi: 10.1126/science.6494891
12. Guo Y, Chen Y, Ito H, Watanabe A, Ge X, Kodama T, Aburatani H (2006) Identification and characterization of lin-28 homolog B (LIN28B) in human hepatocellular carcinoma. Gene 384:51–61. doi: 10.1016/j.gene.2006.07.011
13. Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, et al (2009) Lin28 promotes transformation and is associated with advanced human malignancies. Nat Genet 41(7):843–848. doi: 10.1038/ng.392
14. Cao D, Allan RW, Cheng L, Peng Y, Guo CC, Dahiya N, et al (2011) RNA-binding protein LIN28 is a marker for testicular germ cell tumors. Hum Pathol 42(5):710–718. doi: 10.1016/j.humpath.2010.09.007
15. Wang T, Han P, He Y, Zhao C, Wang G, Yang W, et al (2016) Lin28A enhances chemosensitivity of colon cancer cells to 5-FU by promoting apoptosis in a let-7 independent manner. Tumour Biol 37(6):7657–7665. doi: 10.1007/s13277-015-4559-8
16. He Q, Yang S, Gu X, Li M, Wang C, Wei F (2018) Long noncoding RNA TUG1 facilitates osteogenic differentiation of periodontal ligament stem cells via interacting with Lin28A. Cell Death Dis 9(5):455. doi: 10.1038/s41419-018-0484-2
17. Mizushima E, Tsukahara T, Emori M, Murata K, Akamatsu A, Shibayama Y, et al (2020) Osteosarcoma-initiating cells show high aerobic glycolysis and attenuation of oxidative phosphorylation mediated by LIN28B. Cancer Sci 111(1):36–46. doi: 10.1111/cas.14229
18. Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M (2016) CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs. RNA Biol 13(1):34–42. doi: 10.1080/15476286.2015.1128065
19. Sticht C, De La Torre C, Parveen A, Gretz N (2018) miRWalk: An online resource for prediction of microRNA binding sites. PLoS One 13(10):e0206239. doi: 10.1371/journal.pone.0206239
20. Liu W, Wang X (2019) Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. Genome Biol 20(1):18. doi: 10.1186/s13059-019-1629-z
21. Wong N, Wang X (2015) miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res 43(Database issue):D146–152. doi: 10.1093/nar/gku1104
22. In: Markossian S, Grossman A, Brimacombe K et al. (eds) (2004) Assay Guidance Manual. Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda (MD).
23. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4):402–408. doi: 10.1006/meth.2001.1262
24. Clément T, Salone V, Rederstorff M (2015) Dual luciferase gene reporter assays to study miRNA function. Methods Mol Biol 1296:187–198. doi: 10.1007/978-1-4939-2547-6_17
25. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011) Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US).
26. Jiashi W, Chuang Q, Zhenjun Z, Guangbin W, Bin L, Ming H (2018) MicroRNA-506-3p inhibits osteosarcoma cell proliferation and metastasis by suppressing RAB3D expression. Aging (Albany NY) 10(6):1294–1305. doi: 10.18632/aging.101468
27. Gu J, Ji Z, Li D, Dong Q (2019) Proliferation inhibition and apoptosis promotion by dual silencing of VEGF and Survivin in human osteosarcoma. Acta Biochim Biophys Sin (Shanghai) 51(1):59–67. doi: 10.1093/abbs/gmy146
28. Sampson VB, Yoo S, Kumar A, Vetter NS, Kolb EA (2015) MicroRNAs and potential targets in osteosarcoma: Review. Front Pediatr 3:69. doi: 10.3389/fped.2015.00069
29. Shrivastava G, Aljabali AA, Shahcheraghi SH, Lotfi M, Shastri MD, Shukla SD, et al (2021) Targeting LIN28: a new hope in prostate cancer theranostics. Future Oncol 17(29):3873–3880. doi: 10.2217/fon-2021-0247
30. Liu W, Zhang J, Zou C, Xie X, Wang Y, Wang B, et al (2017) Microarray Expression Profile and Functional Analysis of Circular RNAs in Osteosarcoma. Cell Physiol Biochem 43(3):969–985. doi: 10.1159/000481650
31. Wu Y, Xie Z, Chen J, Chen J, Ni W, Ma Y, et al (2019) Circular RNA circTADA2A promotes osteosarcoma progression and metastasis by sponging miR-203a-3p and regulating CREB3 expression. Mol Cancer 18(1):73. doi: 10.1186/s12943-019-1007-1
32. Liu G, Huang K, Jie Z, Wu Y, Chen J, Chen Z, et al (2018) CircFAT1 sponges miR-375 to promote the expression of Yes-associated protein 1 in osteosarcoma cells. Mol Cancer 17(1):170. doi: 10.1186/s12943-018-0917-7
33. Huang L, Chen M, Pan J, Yu W (2018) Circular RNA circNASP modulates the malignant behaviors in osteosarcoma via miR-1253/FOXF1 pathway. Biochem Biophys Res Commun 500(2):511–517. doi: 10.1016/j.bbrc.2018.04.131
34. Wang T, He Y, Zhu Y, Chen M, Weng M, Yang C, et al (2016) Comparison of the expression and function of Lin28A and Lin28B in colon cancer. Oncotarget 7(48):79605–79616. doi: 10.18632/oncotarget.12869