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Original Article

miR-29c inhibited adhesion, migration and invasion of osteosarcoma cells

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Abstract: Background: Osteosarcoma (OS) is one of the most frequent bone tumors in young patients worldwide. Emerging studies demonstrate that miRNAs play important roles in human cancer tumorigenesis and progression. Many miRNAs were reported to play either oncogenic or tumor-suppressive roles in osteosarcoma. Methods: In the present study, RT-PCR was performed to detect miR-29c's expression. CCK-8 assays were performed to evaluate changes in cell proliferation. Cell cycle, adhesion, migration, and invasion were detected by flow cytometry assay, fluorescence microscopy, and transwell assay, respectively. The potential targets of miR-29c were identified by using Starbase. A public GEO dataset, GSE42352, was analyzed to identify OS-related genes. In combination with these analyses, a miR-29c regulatory network in OS was constructed. Bioinformatics analysis CRKL was found to be a target of miR-29c. Results: We found that miR-29c is a novel metastasis regulator of OS by inhibiting cell adhesion, invasion, and migration. Furthermore, a miR-29c regulatory network was constructed to clarify the mechanisms of miR-29c underlying OS progression. CRKL was found to be a target of miR-29c in OS. Conclusion: The results showed miR-29c plays important roles in OS and may serve as a biomarker.

Keywords: miR-29c, osteosarcoma, adhesion, migration, invasion

Introduction

Osteosarcoma is a common bone tumor in young patients worldwide. Emerging studies demonstrate miRNAs play important roles in human cancer tumorigenesis and progression. miRNAs could play either oncogenic or tumor-suppressive roles in osteosarcoma. For example, microRNA-544 [1], miR-27-3p [2], microRNA-411 [3], and miRNA-20a [4] promote cell proliferation, however, microRNA-140 [5], miR-141-3p [6], microRNA-124a [7] and miR-365 [8] inhibit tumor progress in osteosarcoma. More research into the function of miRNAs can improve the understanding of osteosarcoma and its related factors.

miR-29c was found to be aberrantly expressed in various cancers, such as colorectal cancer [9], schwannoma [10], and hepatocellular carcinoma [11]. miR-29c is involved in regulating cell proliferation [12], cell cycle [13], glycolysis [14], apoptosis [15], invasion [16], migration [17] and radioresistance [18], in cancer cells. For example, Li et al. reported that miR-29c could inhibit prostate cancer proliferation through inhibiting SLC2A3 [14]. Zhang et al. reported that miR-29c reduced colorectal cancer cell migration by targeting SPARC [19]. Moreover, miR-29c can suppress colon cancer cell invasion and migration through inhibition of PHLDB2 [20]. Recently, a few studies suggested the potential roles of the miR-29 family in osteosarcoma. For instance, Hong et al. found that the serum levels of miR-29a and miR-29b could estimate the prognosis of patients with osteosarcoma [21]. Also, the miR-29 family together with IGF1 3'UTR promoted angiogenesis in osteosarcoma [22]. However, the roles of miR-29c in osteosarcoma remain largely unknown.

Materials and methods

Cell culture and transfection

HOS and MG-63 cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). The RPMI 1640 medium

(Corning, USA; 10% FBS, ScienCell, USA) was used to culture cells in an incubator with 5% CO_2 at 37°C. Mimics for miR-29c and negative control (NC) were designed and purchased from BioTNT (China). The cells were plated in a 6-well plate and incubated for 24 h. Then the cells were subsequently transfected using FuGENE6 (Roche) according to the manufacturer's instructions.

Quantitative RT-PCR (qRT-PCR)

Total RNA for RT-qPCR was extracted using PureYield™ RNA Midiprep System (Promega, USA). FastQuant RT Super Mix (TIANGEN, China) was used to perform reverse transcription (RT). Quant One Step RT-qPCR Kit (SYBR Green) (TIANGEN, China) was used to perform the quantitative RT-PCR (qRT-PCR). U6 was selected as a reference. The PCR primers for miR-29c and U6 were designed and obtained from BioTNT. The primer sequence: miR-29c sense, 5'-TGACCGATTTCTCCTGGTGTTC-3', miR-29c antisense, 5'-GCGAGCACAGAATTAATACGAC-3'; U6 sense, 5'-CTCGCTTCGGCAGCACA-3', U6 antisense, 5'-GCGAGCACAGAATTAATACGAC-3'. The primer sequence for CRKL: forward, 5'-GTGCTTATGACAAGACTGCCT-3'; reverse, 5'-CACTCGTTTTCATCTGGGTTT-3'. The 2-ΔΔCt method was used to analyze the miRNA expression. Each sample was performed in triplicate.

Cell proliferation and cell cycle assay

CCK-8 assays were performed to detect cell proliferation at 0, 24 h, 48 h, 72 h, and 96 h after transfection. Ten μl CCK-8 was added to 100 μl medium, then incubated for 1.5 h. After 1.5 h incubation, the absorbance at 450 nm and 630 nm (reference absorbance) were measured by a microplate reader. Cell cycle assay was performed by fluorescence-activated cell sorting (FACS) flow cytometer (BD Biosciences, USA).

In vitro cell adhesion assay

Using 96-well plates we added 50 μl/well mix (25 μl Matrigel and 25 μl serum-free 1640 medium). The transfected cells were cultured in the 96-well plates for 1 hour. Then the cells were harvested and imaged. The cell number was counted in a fluorescence microscope (Olympus, Japan). Each experiment was repeated at least in triplicates.

Cell migration

The plates for cell migration assay were purchased from Corning Life Sciences (Lowell, MA). The matrigel basement membrane matrix was purchased from BD Biosciences (1 mg/ml; Franklin Lakes, NJ). The transfected cells (5×10^4 cells/well) were seeded into the upper chamber (pre-coated matrigel basement membrane matrix) and cultured with RPMI 1640 (2% FBS). The bottom wells were filled with RPMI 1640 (10% FBS). The number of invading cells was calculated after 24 hours of being cultured.

Dual-Luciferase reporter assay

Dual-Luciferase Reporter Assay System (Promega, USA) was used to perform dual-Luciferase reporter assay according to the manufacturer's instructions. In brief, the CRKL 3'-UTR fragment containing the miR-29c seed sequence (5'-GCTGCCAGAGAAAGTTCCTGCTG-3') and its mutant form (5'-GCTGCCAGAG-AAAGTACCACGAG-3') were synthesized and cloned into the vector psiCHECK2 (Promega, USA). All plasmids were verified by DNA sequencing. After 48 h transfection, GloMax 96 Microplate Luminometer was used to detect the Dual-Luciferase.

Western blot analysis

After transfection for 48 h, the cells were harvested by and washed with ice-cold PBS on ice. Aspiration of the PBS, then added 0.5 mL ice-cold lysis buffer, and collected the cell suspension. Centrifugation of the cell suspension at 12,000 rpm for 10 minutes at 4°C, and take the supernatant. The protein content was measured by the Bradford method. Then the protein detection mixture was prepared, boiled the mixture in boiling water for 5 minutes, immediately transferred to ice for 5 minutes, and then centrifuged at 12,000 rpm for 1 minute at room temperature. Load 30 μg of protein into the wells of the 15% SDS-PAGE gel, run the gel for 1.5 h at 100 V. Antibody staining was following the manufacturer's instructions. The primary antibodies were CRKL (1:2000, Genex, USA), GAPDH (1:2000, Abcam, San Francisco, USA. Catalog number: ab8245).

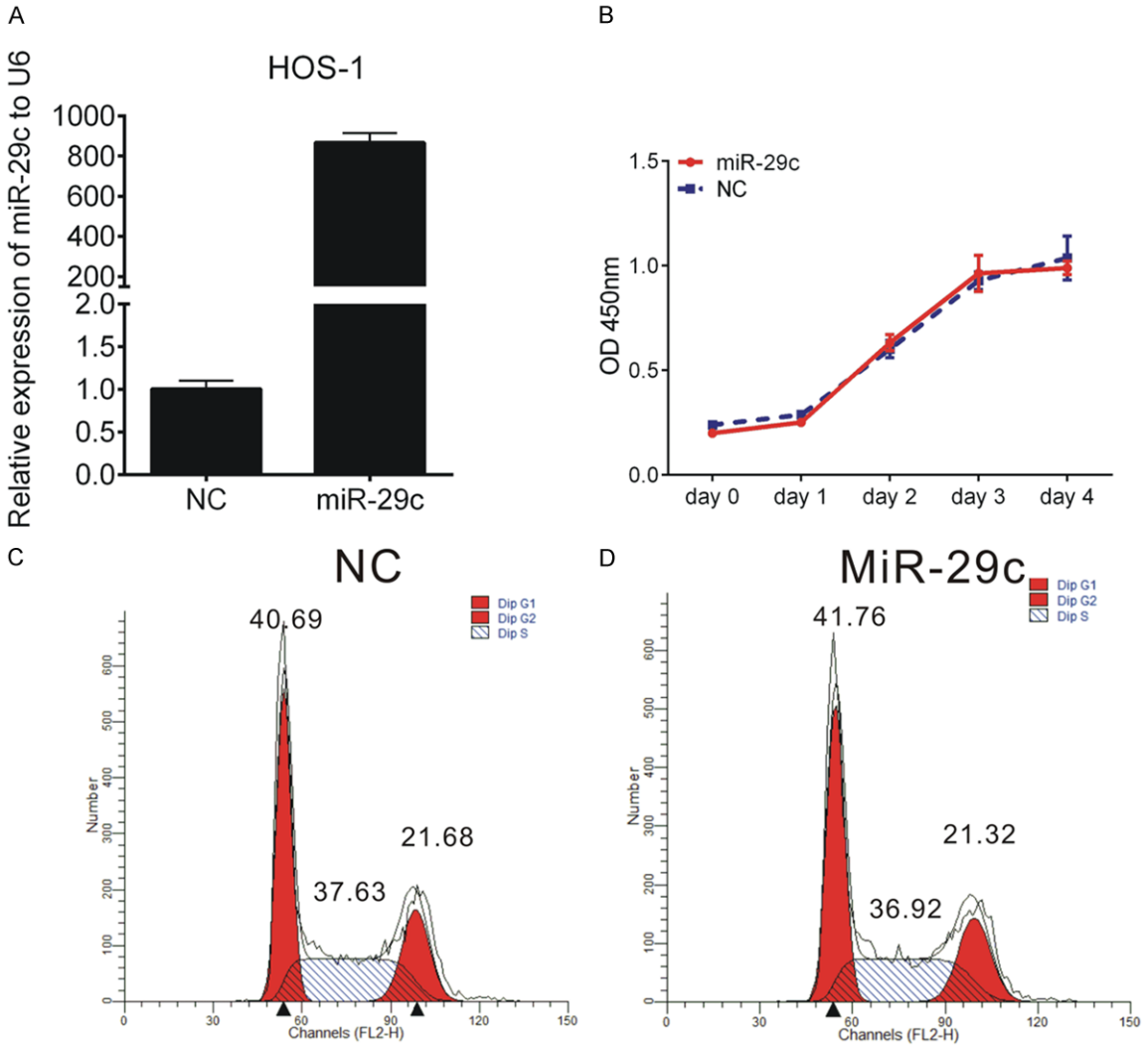

Figure 1. miR-29c did not affect proliferation in OS. A. Transfection efficiency was shown. B. Overexpress miR-29c did not affect proliferation in OS cell line HOS. C, D. Overexpress miR-29c did not affect cell cycle in OS cell line HOS.

Statistical analysis

Mean ± standard deviation (SD) was used to analyze the raw data. The t-test was used to perform the statistical comparison between the two groups. A $P < 0.05$ was selected as significant.

Result

Overexpression of miR-29c did not affect cell proliferation in osteosarcoma

Research has found that miR-29c acts as a tumor suppressor in human cancers. Here, we first evaluate the effect of miR-29c on cell proliferation. By transfecting cells with miR-29c mimics, its expression levels were significantly induced by more than 1000 fold in HOS cells (**Figure 1A**). Then we detected cell growth by using the CCK-8 assay. As shown in **Figure 1B**, the present study observed that miR-29c overexpression had no effect on cell proliferation in HOS cells.

We next check the cell cycle progression following transfecting with either miR-29c or NC. As expected, we found that the percentage of G1, S, and G2 phase cells have no difference between miR-29c and NC groups in HOS cells (**Figure 1C**, **1D**). Our results showed that miR-29c did not affect cell proliferation and cell cycle in the OS.

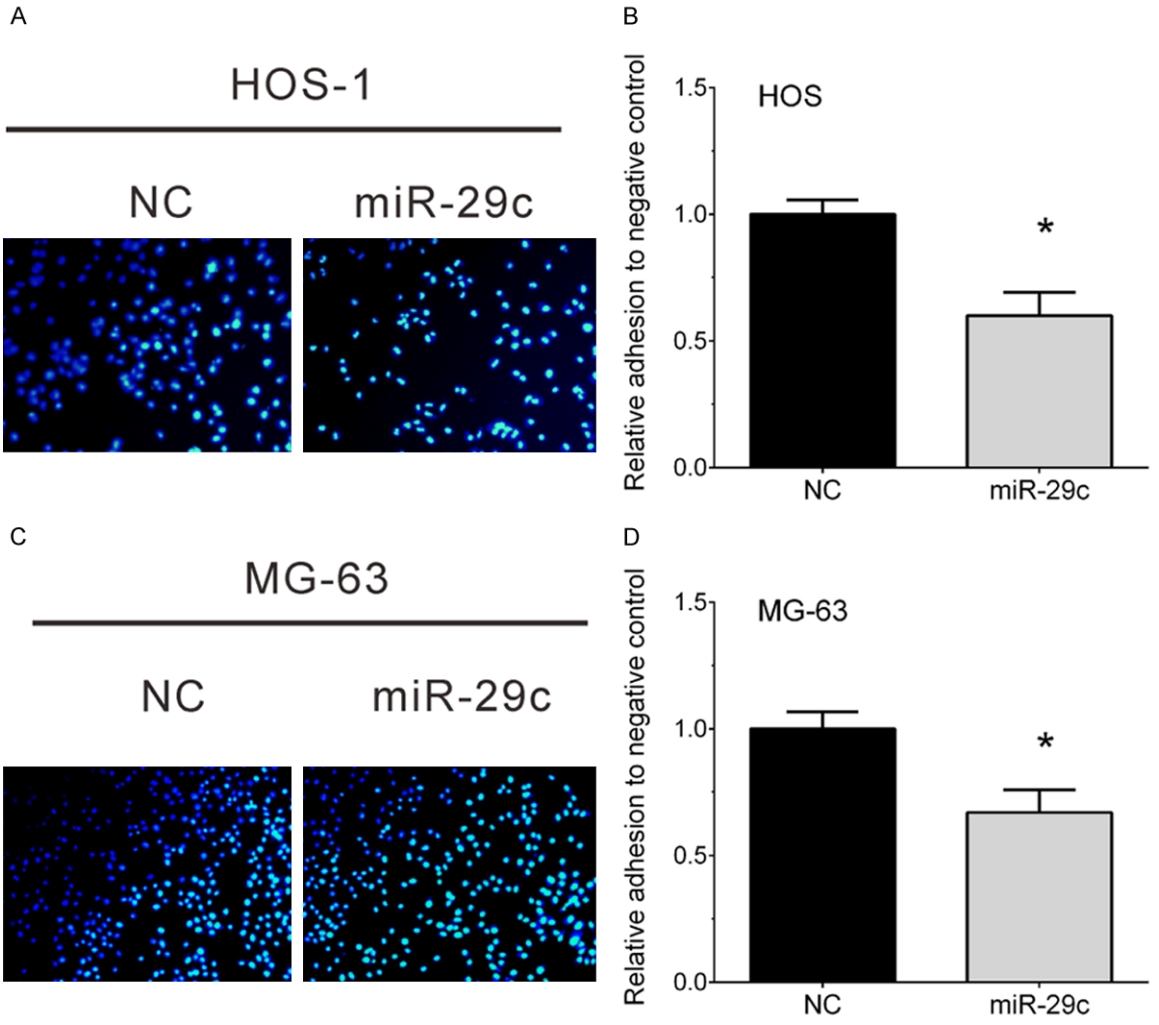

Figure 2. miR-29c inhibits osteosarcoma cells adhesion ability. (A-D) Adhesion of HOS (A, B) and MG-63 (C, D) cells after miR-29c and NC transfection were counted by transwell assay. MiR-29c inhibited cell adhesion of osteosarcoma cells (*, $P < 0.05$).

MiR-29c inhibits HOS and MG-63 cell adhesion ability

Then, we evaluated the effects of miR-29c on metastasis, including cell adhesion, migration, and invasion ability. The cell adhesion was identified by the cell adherence assay. As shown in **Figure 2**, miR-29c notably inhibited the adhesion of HOS and MG-63 cells in comparison with the negative control.

MiR-29c inhibits HOS and MG-63 cell migration and invasion

Furthermore, we explore the roles of miR-29c in regulating OS migration by using a transwell assay. As illustrated in **Figure 3**, we observed that miR-29c overexpression significantly decreased migrating cells of HOS and MG-63 cells compared to the negative control. Our results also showed that miR-29c significantly inhibited cell invasion by 40% and 30% in HOS and MG-63 cells respectively, compared with the NC group (**Figure 4**).

Bioinformatics analysis of miR-29c in OS

Our results showed that miR-29c suppressed OS cell metastasis. However, the mechanisms of miR-29c underlying this progression remained unclear. Previous studies showed a miRNA could bind to a series of targets to regulate cancer progression. Therefore, the present study aims to construct a miR-29c regulatory

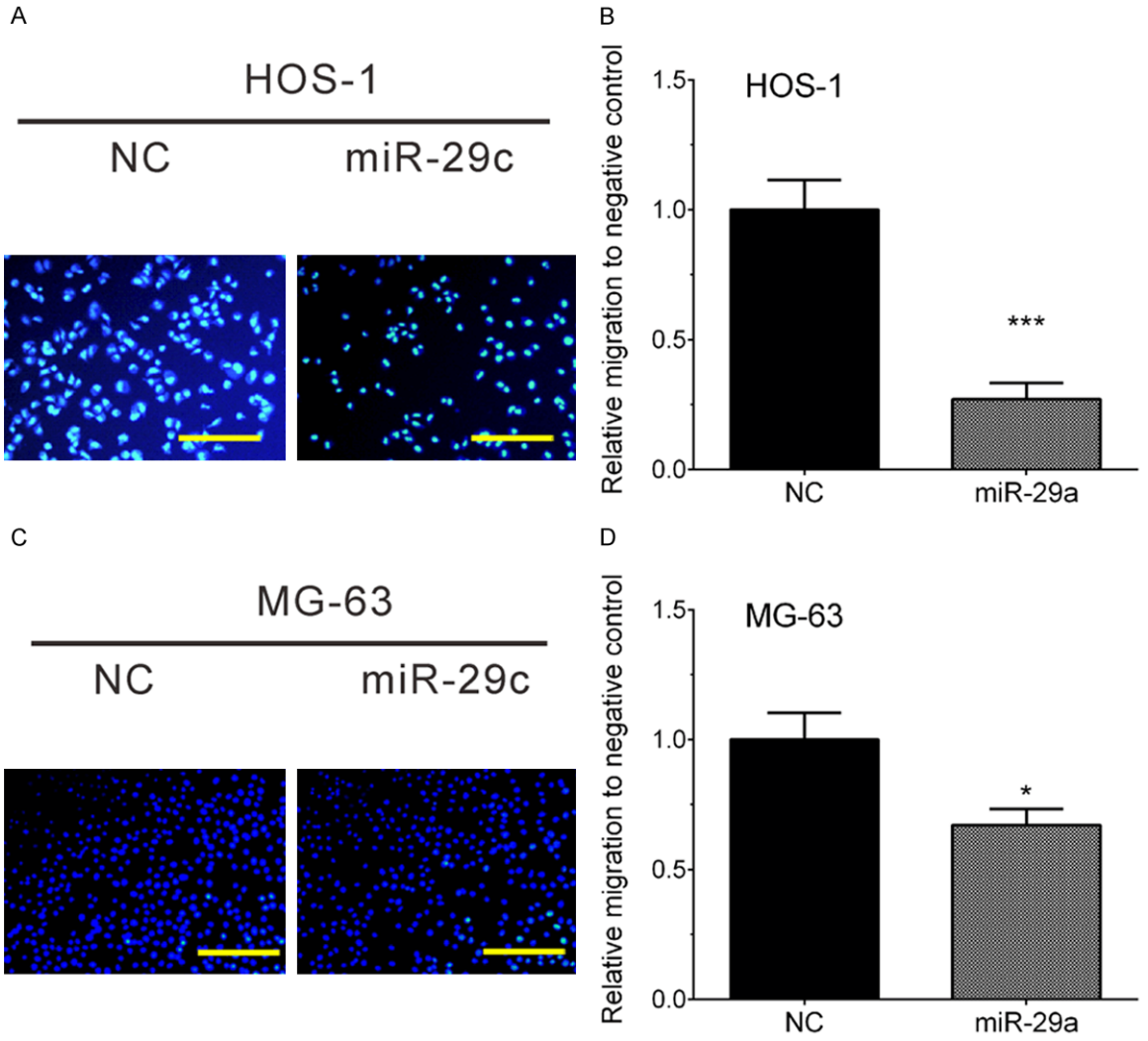

Figure 3. miR-29c inhibits osteosarcoma cell migration. (A, B) Migration of HOS and MG-63 (C, D) cells after miR-29c and NC transfection was counted by transwell assay. MiR-29c inhibited cell migration of osteosarcoma cells (*, $P < 0.05$, ***, $P < 0.001$).

network in OS. Starbase was used to explore the targets of miR-29c. A total of 1646 targets were identified. Next, the present study analyzed a public GEO dataset, GSE42352, to identify OS-related genes. In combination with these analyses, we constructed a miR-29c regulatory network in OS, which included 310 mRNAs.

Bioinformatics analysis showed miR-29c was associated with Sertoli cell proliferation, cellular response to amino acid stimulus, anterior/posterior pattern specification, response to gamma radiation, regulation of cell shape, skeletal system development, skin development, and cell differentiation. Interestingly, the present study observed miR-29c was significantly involved in regulating cancer metastasis-related pathways, such as regulation of cell shape, cell differentiation, TGF beta receptor signaling pathway, and cell adhesion, which was consistent with our previous results (**Figure 5A**). KEGG pathway analysis revealed miR-29c was associated with Protein digestion and absorption, Focal adhesion, microRNAs in cancer, Axon guidance, Platelet activation (**Figure 5B**).

CRKL is a direct target of miR-29c

By analyzing Starbase, we found CRKL may be one of miR-29c's direct targets, and it is involved in the regulation of Focal adhesion

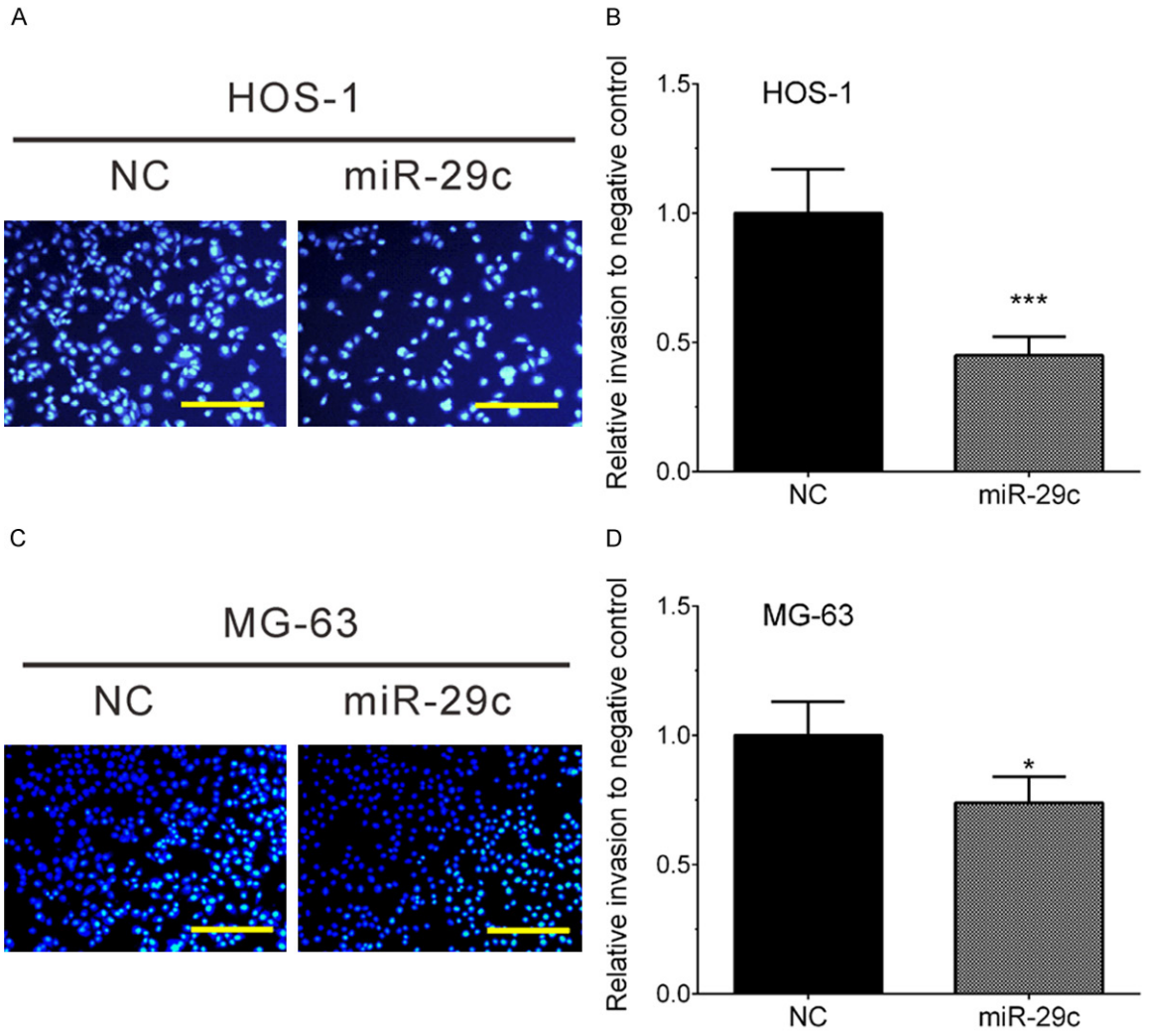

Figure 4. miR-29c inhibits osteosarcoma cell invasion. (A, B) Invasion of HOS and MG-63 (C, D) cells after miR-29c and NC transfection were counted by transwell assay. MiR-29c inhibited cell invasion of osteosarcoma cells (*, $P < 0.05$, ***, $P < 0.001$).

pathway. Bioinformatics analysis showed that miR-29c could directly bind to the 3'UTR of CRKL (**Figure 6A**). Luciferase reporter assays showed the Luciferase expression of the plasmids containing 3'UTR of CRKL was significantly reduced after overexpressing miR-29c. Furthermore, fluorescence intensity was not changed in cells transfected with plasmids containing the CRKL 3'UTR mutation of CRKL (**Figure 6B**).

RT-PCR assay showed that miR-29c could decrease the mRNA levels of CRKL in both HOS-1 and MG-63 cells (**Figure 6C**, **6D**). Western blot assay was further conducted and showed that the protein levels of CRKL in HOS-1 and MG-63 cells were suppressed after overexpressing miR-29c (**Figure 6E**).

Discussion

Previous reports have suggested that metastasis is a significant cause of cancer deaths worldwide. Metastasis is a complex process involved in cancer adhesion, migration, and invasion. miRNAs are highly conserved and widely identified in animals and plants. Emerging studies have shown that miRNAs play crucial roles in cancer metastasis. For example, miR-21, miR-101, and miR-488 could suppress cancer cell metastasis [23-25]. However, miR-103/107, miR-17-5p and miR-21 enhanced tumor metastasis [26-28]. Osteosarcoma lung

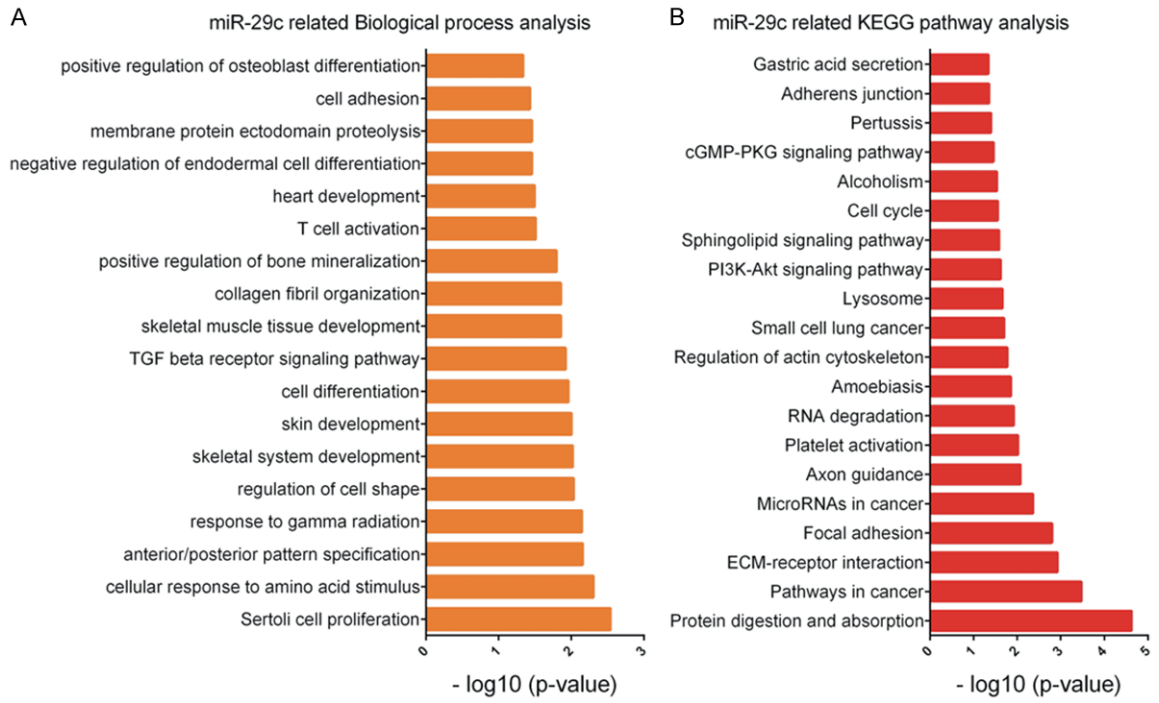

Figure 5. GO (A) and KEGG (B) analysis of MiR-29c in OS.

metastasis is one of the main challenges in treatment, which causes a low 5-year survival rate. In the present study, we demonstrated that miR-29c was a novel cancer metastasis regulator in osteosarcoma.

miR-29c was found to suppress tumor progress in various cancers. miR-29c regulates radioresistance and suppress tumor progress by regulating Bcl-2, Mcl-1, and VEGFA in lung cancer and inhibits cell growth by targeting ITGB1 in pancreatic cancer [29]. In osteosarcoma, limited reports showed miR-29 family was involved in tumor prognosis and angiogenesis [30]. However, the roles of miR-29c in osteosarcoma still need more exploring. In this study, we reported that miR-29c also served as a tumor suppressor in OS, which was consistent with previous study. Overexpress miR-29c had no effect on the proliferation and cell cycle progression in OS but significantly inhibited OS metastasis by suppressing cell adhesion, migration, and invasion.

In this study, our results showed miR-29c suppressed OS cell metastasis. However, the mechanisms of miR-29c underlying this progression remained unclear. A series of miR-29c targets were identified in previous studies, including SLC2A3, SPAR, PHLDB2, LAMTOR3, and LOXL2. Recently, other kinds of noncoding RNAs, including long noncoding RNAs and circular RNAs, could also serve as miR-29c targets. Of note, many studies have revealed that miRNA could bind to a series of targets in the cell to regulate cancer progression. Constructing a miRNA mediated regulatory network could provide system insight to explain how this miRNA regulates tumor progression. In this study, we used four datasets, including TargetScan, Starbase, miRDB, and miRWALK, to identify the potential targets of miR-29c. Then, the present study analyzed a public GEO dataset, GSE42352, to identify OS-related targets of miR-29c and constructed a miR-29c regulatory network in OS, which included 310 mRNAs. Interestingly, the present study observed miR-29c was significantly involved in regulating cancer metastasis-related pathways, such as regulation of cell shape, cell differentiation, TGF beta receptor signaling pathway, and cell adhesion, which was consistent with our previous results.

CRKL is a member of the CRK family. Previous studies have indicated that CRKL was dysregulated in multiple human cancers, such as lung

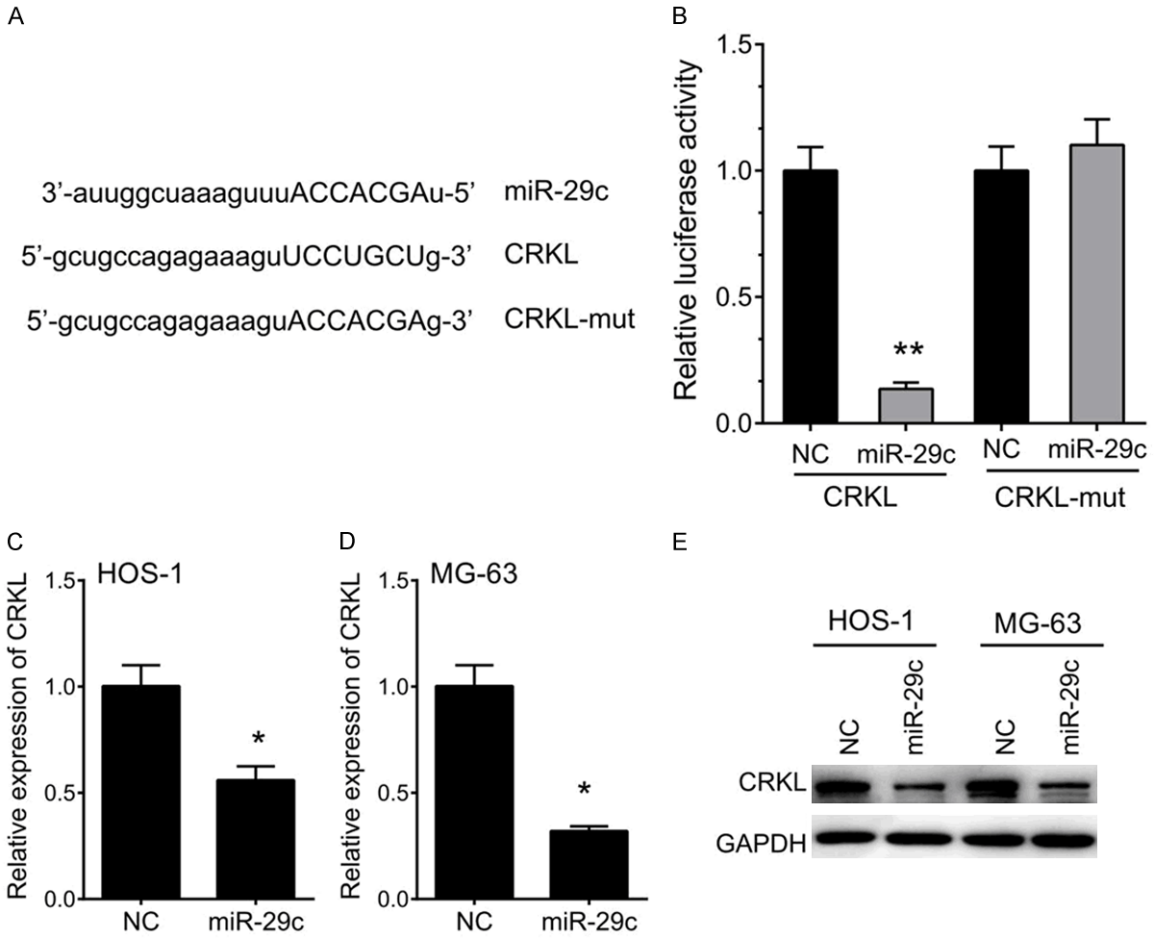

Figure 6. CRKL is a direct target of miR-29c in OS. (A) Bioinformatics analysis showed that miR-29c could directly bind to the 3'UTR of CRKL. (B) Luciferase reporter assays showed overexpress miR-29c significantly reduced the luciferase expression of the plasmids containing 3'UTR of CRKL, but not the plasmids containing the CRKL 3'UTR mutation of CRKL. (C, D) RT-PCR assay showed that miR-29c could decrease the mRNA levels of CRKL in both HOS-1 and MG-63 cells. (E) Western blot assay showed that the protein levels of CRKL in HOS-1 and MG-63 cells were suppressed after overexpressing miR-29c (E).

cancer, endometrial carcinoma, and colorectal cancer. CRKL was found to be an oncogene in cancer cells through regulating cell proliferation and apoptosis. Of note, CRKL was found to be a metastasis promoter in human cancers. For instance, miR-429-CRKL could affect hepatocellular carcinoma migration and invasion by regulating the adhesion ability and cytoskeleton F-actin expression. CRKL promoted breast cancer metastasis through positively regulating the ERK1/2 signaling pathway. In this study, we identified that CRKL was a direct target of miR-29c. Overexpress miR-29c significantly inhibited the expression of CRKL. These results suggested that miR-29c suppressed OS migration and invasion through CRKL.

Conclusion

In conclusion, we demonstrated that miR-29c is a novel metastasis regulator of OS by inhibiting cell adhesion, invasion, and migration. Furthermore, a miR-29c regulatory network was constructed to clarify the mechanisms of miR-29c underlying OS progression. Moreover, we found that CRKL was a target of miR-29c. These results suggest that miR-29c plays important roles in OS and may serve as a biomarker for OS.

Disclosure of conflict of interest

None.

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References

[1] Chen M, Liu YY, Zheng MQ, Wang XL, Gao XH, Chen L and Zhang GM. microRNA-544 promoted human osteosarcoma cell proliferation by downregulating AXIN2 expression. Oncol Lett 2018; 15: 7076-7082.

[2] Ye P, Ke X, Zang X, Sun H, Dong Z, Lin J, Wang L, Liu W, Miao G, Tan Y, Tong W, Xiao H and Gao L. Up-regulated MiR-27-3p promotes the G1-S phase transition by targeting inhibitor of growth family member 5 in osteosarcoma. Biomed Pharmacother 2018; 101: 219-227.

[3] Xu N, Yang W, Liu Y, Yan F and Yu Z. MicroRNA-411 promoted the osteosarcoma progression by suppressing MTSS1 expression. Environ Sci Pollut Res Int 2018; 25: 12064-12071.

[4] Zhuo W, Ge W, Meng G, Jia S, Zhou X and Liu J. MicroRNA20a promotes the proliferation and cell cycle of human osteosarcoma cells by suppressing early growth response 2 expression. Mol Med Rep 2015; 12: 4989-4994.

[5] Gu R, Sun YF, Wu MF, Liu JB, Jiang JL, Wang SH, Wang XL and Guo Q. Biological roles of microRNA-140 in tumor growth, migration, and metastasis of osteosarcoma in vivo and in vitro. Tumour Biol 2016; 37: 353-360.

[6] Wang N, Li P, Liu W, Wang N, Lu Z, Feng J, Zeng X, Yang J, Wang Y and Zhao W. miR-141-3p suppresses proliferation and promotes apoptosis by targeting GLI2 in osteosarcoma cells. Oncol Rep 2018; 39: 747-754.

[7] Han G, Wang Y, Bi W, Jia J and Wang W. MicroRNA-124 functions as a tumor suppressor and indicates prognosis in human osteosarcoma. Exp Ther Med 2015; 9: 679-684.

[8] Gao J, Zhao P, Chen X, Wang W, Li Y, Xi W, Zhang W, Hu P, Wang T and Shan L. miR-365 inhibits proliferation and promotes apoptosis of SOSP9607 osteosarcoma cells. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2016; 32: 44-48.

[9] Zhang JX, Mai SJ, Huang XX, Wang FW, Liao YJ, Lin MC, Kung HF, Zeng YX and Xie D. MiR-29c mediates epithelial-to-mesenchymal transition in human colorectal carcinoma metastasis via PTP4A and GNA13 regulation of beta-catenin signaling. Ann Oncol 2014; 25: 2196-2204.

[10] Ma J, Li T, Yuan H, Han X, Shui S, Guo D and Yan L. MicroRNA-29a inhibits proliferation and motility of schwannoma cells by targeting CDK6. J Cell Biochem 2018; 119: 2617-2626.

[11] Bae HJ, Noh JH, Kim JK, Eun JW, Jung KH, Kim MG, Chang YG, Shen Q, Kim SJ, Park WS, Lee JY and Nam SW. MicroRNA-29c functions as a tumor suppressor by direct targeting oncogenic SIRT1 in hepatocellular carcinoma. Oncogene 2014; 33: 2557-2567.

[12] Matsuo M, Nakada C, Tsukamoto Y, Noguchi T, Uchida T, Hijiya N, Matsuura K and Moriyama M. MiR-29c is downregulated in gastric carcinomas and regulates cell proliferation by targeting RCC2. Mol Cancer 2013; 12: 15.

[13] Ding DP, Chen ZL, Zhao XH, Wang JW, Sun J, Wang Z, Tan FW, Tan XG, Li BZ, Zhou F, Shao K, Li N, Qiu B and He J. miR-29c induces cell cycle arrest in esophageal squamous cell carcinoma by modulating cyclin E expression. Carcinogenesis 2011; 32: 1025-1032.

[14] Li J, Fu F, Wan X, Huang S, Wu D and Li Y. Up-regulated miR-29c inhibits cell proliferation and glycolysis by inhibiting SLC2A3 expression in prostate cancer. Gene 2018; 665: 26-34.

[15] Wang CM, Wang Y, Fan CG, Xu FF, Sun WS, Liu YG and Jia JH. miR-29c targets TNFAIP3, inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma. Biochem Biophys Res Commun 2011; 411: 586-592.

[16] Arechaga-Ocampo E, Lopez-Camarillo C, Villegas-Sepulveda N, Gonzalez-De LRC, Perez-Anorve IX, Roldan-Perez R, Flores-Perez A, Pena-Curiel O, Angeles-Zaragoza O, Rangel CR, Gonzalez-Barrios JA, Bonilla-Moreno R, Del MO, Herrera LA and Garcia-Carranca A. Tumor suppressor miR-29c regulates radioresistance in lung cancer cells. Tumour Biol 2017; 39: 1393394654.

[17] Zhang S, Jin J, Tian X and Wu L. hsa-miR-29c-3p regulates biological function of colorectal cancer by targeting SPARC. Oncotarget 2017; 8: 104508-104524.

[18] Chen G, Zhou T, Li Y, Yu Z and Sun L. p53 target miR-29c-3p suppresses colon cancer cell invasion and migration through inhibition of PHLDB2. Biochem Biophys Res Commun 2017; 487: 90-95.

[19] Hong Q, Fang J, Pang Y and Zheng J. Prognostic value of the microRNA-29 family in patients with primary osteosarcomas. Med Oncol 2014; 31: 37.

[20] Gao S, Cheng C, Chen H, Li M, Liu K and Wang G. IGF1 3'UTR functions as a ceRNA in promoting angiogenesis by sponging miR-29 family in osteosarcoma. J Mol Histol 2016; 47: 135-143.

[21] Bao RF, Shu YJ, Hu YP, Wang XA, Zhang F, Liang HB, Ye YY, Li HF, Xiang SS, Weng H, Cao Y, Wu XS, Li ML, Wu WG, Zhang YJ, Jiang L, Dong Q and Liu YB. miR-101 targeting ZFX suppresses

tumor proliferation and metastasis by regulating the MAPK/Erk and Smad pathways in gallbladder carcinoma. Oncotarget 2016; 7: 22339-22354.

[22] Zhao Y, Lu G, Ke X, Lu X, Wang X, Li H, Ren M and He S. miR-488 acts as a tumor suppressor gene in gastric cancer. Tumour Biol 2016; 37: 8691-8698.

[23] Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S and Allgayer H. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 2008; 27: 2128-2136.

[24] Chen HY, Lin YM, Chung HC, Lang YD, Lin CJ, Huang J, Wang WC, Lin FM, Chen Z, Huang HD, Shyy JY, Liang JT and Chen RH. miR-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors DAPK and KLF4. Cancer Res 2012; 72: 3631-3641.

[25] Fang L, Li H, Wang L, Hu J, Jin T, Wang J and Yang BB. MicroRNA-17-5p promotes chemotherapeutic drug resistance and tumour metastasis of colorectal cancer by repressing PTEN expression. Oncotarget 2014; 5: 2974-2987.

[26] Liu ZL, Wang H, Liu J and Wang ZX. MicroRNA-21 (miR-21) expression promotes growth, metastasis, and chemo- or radioresistance in non-small cell lung cancer cells by targeting PTEN. Mol Cell Biochem 2013; 372: 35-45.

[27] Arechaga-Ocampo E, Lopez-Camarillo C, Villegas-Sepulveda N, Gonzalez-De LRC, Perez-Anorve IX, Roldan-Perez R, Flores-Perez A, Pena-Curiel O, Angeles-Zaragoza O, Rangel CR, Gonzalez-Barrios JA, Bonilla-Moreno R, Del MO, Herrera LA and Garcia-Carranca A. Tumor suppressor miR-29c regulates radioresistance in lung cancer cells. Tumour Biol 2017; 39: 1393394654.

[28] Zhang W, Qian JX, Yi HL, Yang ZD, Wang CF, Chen JY, Wei XZ, Fu Q and Ma H. The microRNA-29 plays a central role in osteosarcoma pathogenesis and progression. Mol Biol (Mosk) 2012; 46: 622-627.

[29] Lu Y, Hu J, Sun W, Li S, Deng S and Li M. MiR-29c inhibits cell growth, invasion, and migration of pancreatic cancer by targeting ITGB1. Onco Targets Ther 2015; 9: 99-109.

[30] Hong Q, Fang J, Pang Y and Zheng J. Prognostic value of the microRNA-29 family in patients with primary osteosarcomas. Med Oncol 2014; 31: 37.