Pathology - Research and Practice 215 (2019) 115–120

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

MicroRNA-17 promotes osteosarcoma cells proliferation and migration and inhibits apoptosis by regulating SASH1 expression

Dajiang Wu[1], Hao Zhang[1], Fang Ji*, Wenbin Ding*

Department of Traumatic Orthopedics, Changhai Hospital, Shanghai, 200433, China

ARTICLE INFO

Keywords:
Osteosarcoma
microRNA-17
SASH1
Cell proliferation
Apoptosis

ABSTRACT

MicroRNAs (miRNAs) are abnormally expressed in numerous diseases, which are intimately associated with cell proliferation, migration and invasion. Recent study indicated that miR-17 may be involved in regulating osteosarcoma (OS) occurrence and development, but its function and mechanism have not been reported. In this study, quantitative real-time PCR (qRT-PCR) was used to measure the expression of miR-17, and Western blotting assay was performed to measure the expressions of SAM and SH3 domain containing 1 (SASH1), phosphoinoinositide-3 kinase (PI3K), protein kinase B (AKT), Caspase3, Bcl-2 gene family (Bcl-2, Bax) and matrix metalloprotein (MMP-2, MMP-9) in MG-63 cells. Luciferase reporter assay was conducted to confirm the target of SASH1 by miR-17. Cell proliferation, migration, invasion and apoptosis assay was performed to investigate the role of miR-17 in OS cells. We found that the expression of miR-17 was significantly up-regulated in OS cell lines. MiR-17 inhibitor inhibited the proliferation ability, and induced apoptosis of OS cells. Besides, miR-17 inhibitor prevented the migration and invasion of OS cells. Further, we identified that SASH1 was a target gene of miR-17. In addition, knockdown of miR-17 increased the protein expression of SASH1, and regulate related genes of cell proliferation, invasion and anti-apoptosis in the downstream of OS cells. These findings indicated that miR-17 was over-expressed and promoted cell proliferation, migration and inhibited cell apoptosis by targeting SASH1 in OS cells.

1. Background

Osteosarcoma is the most common primary malignancy tumors of bone in children and young adults [1]. Despite advances in new treatment such as chemotherapy, surgery, and combination of chemotherapy, the rate of 5-year survival rises to 60%–70%, and the rate of metastasis or recurrence is significantly decreased to approximately 30% [2–4]. Previous studies reported a lack of complete understanding with regard to the initiation and development of OS metastasis. The underlying molecular mechanisms of OS tumorigenesis, OS progression and metastasis process remain to be fully elucidated [5]. Therefore, it is an urgent clinical need to investigate innovative therapies target and biomarkers for OS.

MicroRNAs (miRNAs), a kind of non-coding small RNAs, are approximately 19–24 nucleotide in length, which function as genetic modulators [6,7]. MiRNAs regulate the translation from mRNA to protein by targeting the 3′untranslated region (3′UTR) of targets genes [8,9]. Dysregulation of miRNAs was found to contribute to various malignant tumors, including OS [10,11]. Mature miRNAs often function as oncogenes or tumor suppressors with important roles in the pathogenesis of OS, including cell proliferation, migration, invasion and apoptosis [12,13]. MiR-124 was acted as a tumor-suppressive miRNA by inhibiting the expression of Snail2 in osteosarcoma [14]. MiRNA-543 promoted osteosarcoma cell proliferation and glycolysis by partially suppressing protein arginine N-methyltransferase 9 (PRMT9) and stabilizing hypoxia inducible factor-1α (HIF-1α) [15]. MiR-223 was reported as a potential diagnostic and prognostic marker for OS [16].

Recent studies have reported that miR-17 was up-regulated in multiple types of cancer including non-small cell lung cancer [17], breast cancer [18], pancreatic cancer [19] and colorectal cancer [20]. In breast cancer, miR-17 inhibited cell proliferation and invasion by targeting ETV1, signal transducer and activator of transcription 3 (STAT3) was required for sensitization to chemotherapy-induced apoptosis mediated by miR-17, which was used as a diagnostic biomarker for cell proliferation [21,22]. MiR-17 regulates the proliferation and survival by targeting Par4 in colon cancer [23], and miR-17 promotes cancer cell proliferation and tumorigenesis of nasopharyngeal carcinoma by targeting P21 [24]. MiR-17 also play an important roles

* Corresponding authors at: Department of Traumatic Orthopedics, Changhai Hospital, No. 168 of Changhai Road, Shanghai, 200433, China.
E-mail address: dingwenbin201806@163.com (W. Ding).
[1] Contributed equally.

https://doi.org/10.1016/j.prp.2018.10.012
Received 11 July 2018; Received in revised form 17 September 2018; Accepted 17 October 2018

in osteosarcoma via increasing PTEN expression [25]. Previous studies have revealed that SASH1 was a newly discovered candidate tumor suppressor [26]. The loss or inhibition of SASH1 expression might play an important role in tumorigenesis, invasion and metastasis of osteosarcoma [27]. In latest study, expression levels of miR-128 and SASH1 are correlated with the progression of osteosarcoma in patients [28]. These results indicated that SASH1 play an important role in osteosarcoma. However, the relationship between miR-17 and SASH1 in OS progression such as proliferation, metastasis, invasion and apoptosis is still unknown.

In this study, we demonstrated that miR-17 was significantly upregulated in OS cell lines. Knockdown of miR-17 inhibited cell proliferation, migration, invasion and promoted cell apoptosis through suppressing hosphoinoinositide-3 kinase (PI3K)/protein kinase B (AKT) signaling in OS cells by targeting SAM and SH3 domain containing 1 (SASH1). Taken together, our findings suggested that miR-17 was an oncogenes in OS cells.

2. Materials and methods

2.1. Cell culture and transfection

Human osteosarcoma cell lines U2OS and MG-63 were cultured in Dulbecco's Modified Eagle's Medium (DMEM; SH30022.01, Hyclone, GE Health Care, USA), and Saos^{-2} cell lines were cultured in RPMI 1640 mediums (Gibco; SH30096.01, Hyclone, GE Health Care, USA). Culture medium was supplemented with 10% foetal bovine serum (FBS; Gibco; SV30087.02, Thermo Fisher Scientific, Waltham, USA), 100 mg/ml streptomycin, and 100 IU/ml penicillin in a 5% CO2/water-saturated incubator at 37 °C.

2.2. Cell transfection

The MG-63 cells were seeded in six-well plates and transfected with miR-17 inhibitor or the control of miR-17 inhibitor (inhibitor Con) using lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) following the manufacture's protocol. After transfection for 48 h, following experiments were performed.

2.3. RNA isolation and quantitative real-time PCR

The cells samples were lysed in Trizol (Invitrogen, Carlsbad, CA, USA) for total RNA extraction. Hairpin-itTM micoRNA qRT-PCR Kit (GenePharma) was used for the measurement of the relative quantity of miR-17. The primer sequences were as follows: miR-17 forward: 5′-TGCTTACAGTGCAGGTAG-3′ and reverse: 5′-GAACATGTCTGCGTA TCTC-3′; U6 forward: 5′-CTCGCTTCGGCAGCACA-3′ and reverse: 5′-AACGCTTCACGAATTTGCGT-3′. The expression of miR-17 was normalized to the endogenous expression of U6. $2^{-\Delta\Delta Ct}$ method was used in each sample as relative quantification [29].

2.4. Western blot analysis

The Western blot assay was conducted as previously described. In brief, protein lysates were separated using 12% SDS-polyacrylamide gel electrophoresis, which were transferred onto PVDF membranes. The blocked membranes were incubated with primary antibody purchased from Abcam (anti-SASH1, ab200379; anti-PI3K, ab127617; anti-p-PI3K, ab191606; anti-AKT, ab81283; anti-p-AKT, ab38449; anti-Caspase3, ab13847; anti-MMP-2, ab37150; anti-MMP-9, ab38898; anti-Bcl-2, ab32124; anti-Bax, ab32503; anti-GAPDH, ab9485) overnight. Then the membranes were incubated with specific secondary antibodies coupled to horseradish peroxidase at room temperature for 4 h. At the end of the experiment, protein bands were visualized with enhanced chemiluminescence detection system (Super Signal West Dura Extended Duration Substrate; Pierce Chemical). Evaluation of the expression of proteins was performed using ImageJ version 1.38.

2.5. MTT assay

After transfection, the MG-63 cell proliferation was measured using the MTT method. Briefly, MG-63 cells were incubated in 96-well plate for 24 h. MTT reagent (5 mg/ml, 11465007001, Roche) was added to each well, and cells were incubated according to the manufacturer's protocol. The absorbance value at 490 nm was recorded by enzyme immunoassay analyzer.

2.6. Flow cytometry analysis

MG-63 cells were transfected with miR-17 inhibitor or the control of miR-17 inhibitor respectively, and 48 h after the transfection, cells were labeled with annexin V-FITC and propidium iodide (PI) (CST, USA) following with the manufacturer's instructions. Flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) was used to determine cell apoptosis. Tests were repeated at least for three times.

2.7. Cell scratch test

For cell migration ability detection, cell scratch test was performed. 48 h after transfection, MG-63 cells were seeded in a 6-well plate (5×10^5 per well) and incubated in a normal cell culture condition with 5% CO_2 at 37 °C. When cells were in 80% confluence, wounds were formed by the 200 μl pin. After washing with PBS, cells were cultured in media without serum. Pictures were captured using an inverted microscope (Olympus, Japan).

2.8. Cell invasion assay

Transwell assay was used to detect cell invasion ability. MG-63 cells were transfected with miR-17 inhibitor or the control of miR-17 inhibitor respectively until cells reached approximately 60% confluence. After 48 h, the cells were harvested and then re-suspended in serum-free medium, subsequently, the cells were seeded into the upper chamber with matrigel-coated membrane matrix. The cell culture medium containing 20% FBS was added to the lower chamber as a chemoattractant. The cells were incubated for 48 h at 37 °C with 5% CO_2. Finally, the non-invading cells on the upper surface were cleared, and the invasive cells on the underside surface were fixed and then stained with hematine. A microscope was used to observe the stained cells. Tests were repeated for three times.

2.9. Dual luciferase reporter assay

Bioinformatics software (http://www.targetscan.org) was carried out to predict the potential targets of miR-17, and the findings showed that SASH1 was a potential target of miR-17 [30]. To confirm our prediction, MG-63 cells were seeded into each well of a 24-well plate (5×10^4 cells), 24 h later, the cells were then co-transfected with SASH1 3′UTR pmirGLO plasmid (containing mutant SASH1 3′ UTR or Wild type SASH1 3′ UTR) and a miR-17 mimic or mimic control (NC) vector using Lipofectamine 2000 reagent according to the manufacturer's instructions. Two days after incubation, the luciferase activity was detected using the dual-luciferase reporter assay system (Promega, USA).

2.10. Statistical analysis

All statistical analysis were performed using GraphPad Prism software version 5.0 (GraphPad, Software, Inc., La Jolla, CA, USA). The significant differences between two groups were analyzed by t-test, and the significant differences among more than two groups were analyzed by one-way ANOVA. $P < 0.05$ was considered to indicate a statistically

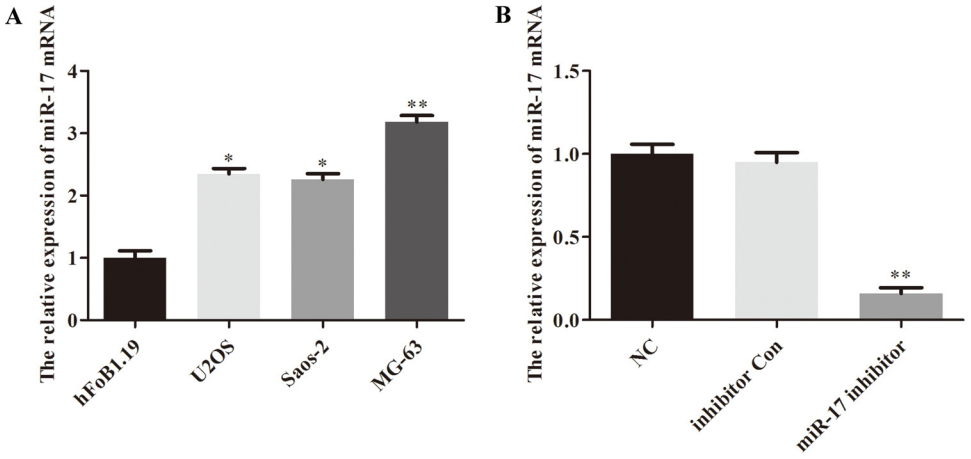

Fig. 1. MiR-17 expression was upregulated in human OS cell lines. (A) The expression levels of miR-17 were determined in human OS cell lines (MG63, Saos^{-2} and U2OS) and the normal cell line (hFOB1.19) by qRT-PCR assay. Data are expressed as mean ± standard error. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. hFOB1.19 cell. (B) MG-63 cells were transfected with miR-17 inhibitors for 48 h. The levels of miR-17 were determined by qRT-PCR assay. Data are expressed as mean ± standard error. $^{**}P < 0.01$ vs. negative control.

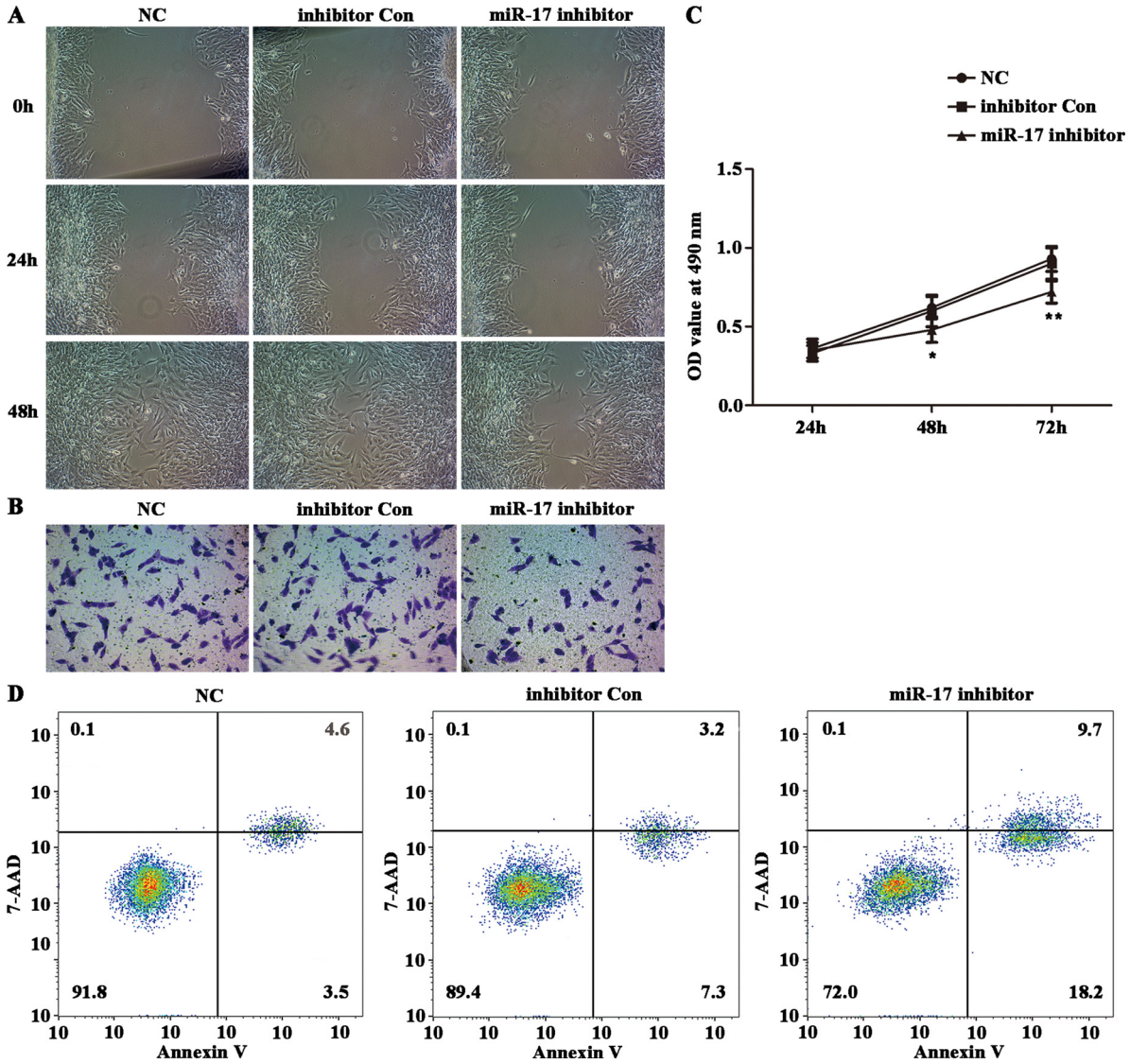

Fig. 2. Knockdown of miR-17 inhibited cell proliferation, migration and invasion in OS cells. (A) Cell scratch test after cell transfection with miR-17 inhibitor or the control of miR-17 inhibitor. (B) Cell migration was detected using Transwell assay. (C) MTT assay results showed that knockdown of miR-17 suppressed OS cell proliferation. Data are expressed as mean ± standard error. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. negative control. (D) Flow cytometry demonstrated that knockdown of miR-17 induced cell apoptosis.

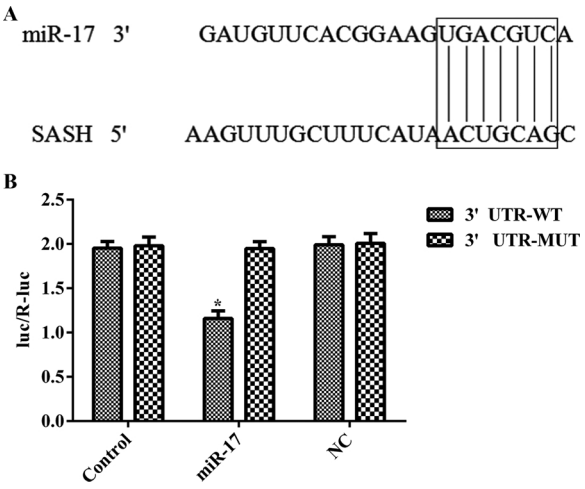

Fig. 3. SASH1 is the target gene of miR-17. (A) Predicted miR-17 target sequence in the 3′-UTR of SASH1. (B) Analysis of relative luciferase activities of SASH1-WT and SASH1-MUT. Data are expressed as mean ± standard error. $^{*}P < 0.05$ vs. 3′ UTR-WT group.

significant difference. All data are shown as the mean ± standard deviation.

3. Results

3.1. MiR-17 was significantly up-regulated in OS cell lines

The expression levels of miR-17 in human OS cell lines (MG63, Saos^{-2}, U2OS) and the normal human osteoblast cell line hFoB1.19 were analyzed using qRT-PCR analysis. As shown in Fig. 1A, the expression of miR-17 in MG-63, Saos^{-2} and U2OS were significantly increased compared with hFoB1.19, and MG-63 cells had the highest expression of miR-17. These results indicated that miR-17 was significantly up-regulated in OS cell lines. And we used MG-63 cells to perform further analysis.

3.2. MiR-17 promoted cell proliferation, migration and inhibited apoptosis in OS cells

To explore the potential biological role of miR-17 in the development of OS cells, the MG-63 cell lines were transfected with miR-17 inhibitor and the control of miR-17 inhibitor, and transfection efficiency was detected using qRT-PCR analysis. As shown in Fig. 1B, the expression of miR-17 was significantly inhibited in MG-63 by miR-17 inhibitor. Since migration and invasion are the key stages in malignant progression and metastasis. We studied the effects of miR-17 on cell migration and invasion in MG-63 OS cells. The results indicated that down-regulation of miR-17 significantly inhibited migration (Fig. 2A) and invasion (Fig. 2B) in the MG-63 cells, compared with the negative control. Thus, our results suggested that knockdown of miR-17 inhibited cell proliferation, migration and invasion, and promoted cell apoptosis in OS cells. Then cell proliferation ability was detected by using MTT assay. Compared with the negative control, the proliferation of cells transfected with miR-17 inhibitor were significantly decreased (Fig. 2C). Thus, knockdown of miR-17 inhibited MG-63 cell proliferation. Finally, we also performed the flow cytometry assay to evaluate the effect of miR-17 on the cell apoptosis. As shown in Fig. 2D, Flow cytometry demonstrated that knockdown of miR-17 significantly induced cell apoptosis in MG-63 cells.

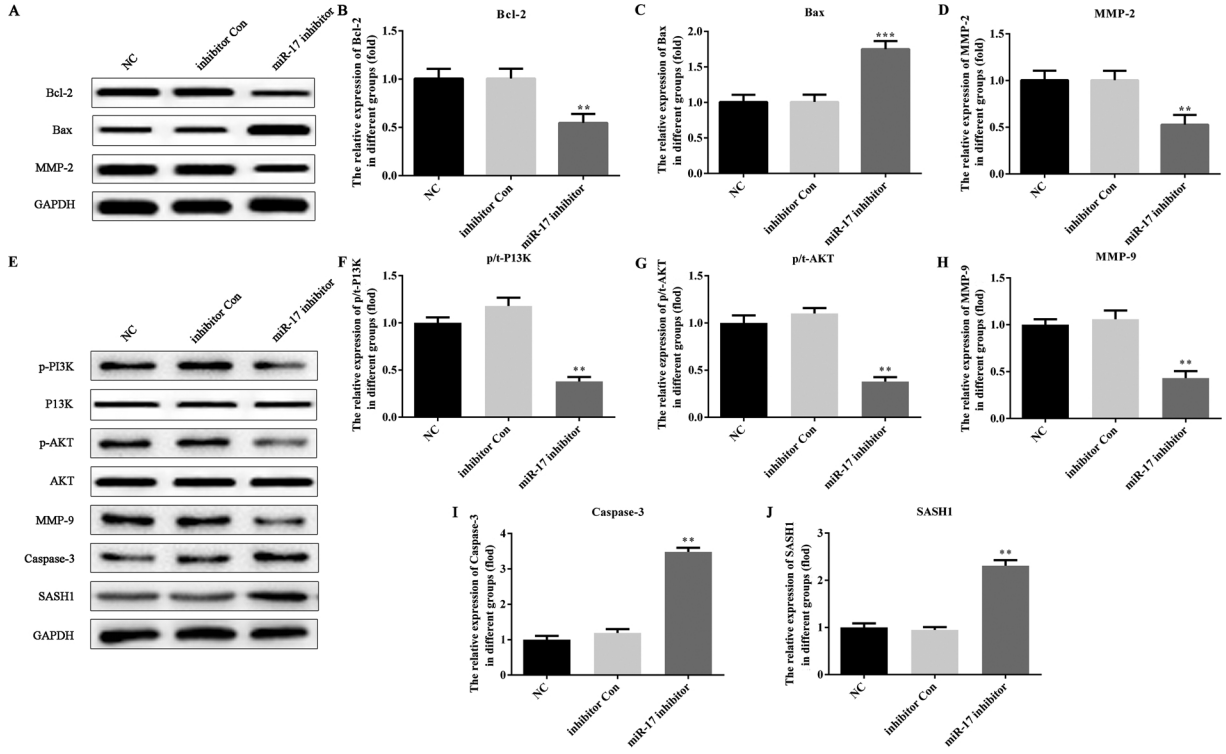

Fig. 4. MiR-17 regulated the gene expression related in cell proliferation, migration and apoptosis of OS. The protein levels of Bcl-2/Bax/MMP-2 (A) and p-PI3K/p-AKT/MMP-9/caspase3/SASH1 (E) were measured by Western blotting at 48 h post-transfection and normalized to that GAPDH. Bcl-2 (B), p-PI3K (F), p-AKT (G), MMP-2 (D) and MMP-9 (H) were decreased in MG63 cells transfected with miR-17 inhibitor respectively. The levels of Bax (C) caspase-3 (I) and SASH1 (J) were significantly increased in MG63 cells transfected with miR-17 inhibitor respectively. Data are expressed as mean ± standard error. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. negative control.

3.3. SASH is a target gene of miR-17

As shown in Fig. 3, the potential target genes of miR-17 were analyzed by TargetScan databases. Bioinformatic analysis indicated that 3′-UTR of SASH1 contains a predicted binding site for miR-17 (Fig. 3A). Luciferase reporter assay was used to determine whether SASH1 was targeted to miR-17. As shown in Fig. 3B, the relative activity of luciferase in the reporter containing a wild-type SASH1 3′-UTR was markedly reduced upon miR-17 mimic co-transfection. The data indicated that SASH was a target of miR-17.

3.4. Knockdown of miR-17 inhibited the expression of p-PI3K/p-AKT/MMP-9 and increased the expression of SASH1 and Caspase3 in OS cell

In this study, we showed that knockdown of miR-17 inhibited the expression of p-PI3K/p-AKT in OS cell (Fig. 4E-G). Thus, miR-17 silencing inhibited cell proliferation, migration, invasion and promoted cell apoptosis by suppressing p-PI3K/p-AKT signaling in OS cells. We also found that knockdown of miR-17 significantly inhibited the expressions of Bcl-2, MMP-2 and MMP-9 using western blotting assay (Fig. 4A, B, E, D, H). In addition, we found that knockdown of miR-17 promoted the apoptosis of OS cell and increased the expression of caspase3 and Bax in OS cells (Fig. 4A, C, E, I). The western blot assay also showed that knockdown of miR-17 significantly enhanced the level of SASH1 in MG-63 cells (Fig. 4E, J), indicating that miR-17 negatively regulated SASH1 expression in OS cells.

4. Discussion

OS are aggressive primary tumor of the bone. The etiology and molecular pathogenesis of OS remain unclear. Thus, it is urgent to explore the molecular mechanisms governing rapidly growth of OS [15]. MiRNAs are important epigenetic regulators of gene expression at the posttranscriptional level. Several miRNAs have recently been found to be involved in basic biological processes, including cell proliferation, differentiation and apoptosis. Several miRNAs have been linked to OS, however, their roles in the regulation of OS remain to be fully elucidated.

MiR-17, which belongs to the miR-17～92 cluster (including other two members miR-92a and miR-20a), was upregulated in human cancer [31]. The miR-17～92 cluster acted as a potential biomarker for the early diagnosis of gastric cancer [32]. However, Downregulation of miR-17～92a cluster was reported to promote autophagy induction in response to celastrol treatment in prostate cancer cells. Zhang suggested that miR-17 downregulation contributed to erlotinib resistance in non-small cell lung cancer cells [33]. In gastric cancer, miR-17 regulated cell proliferation and migration by targeting transforming growth factor-β receptor 2 [34]. In our studies, we demonstrated that human OS cells (MG63, Saos^{-2} and U2OS) have higher expression of miR-17 compared with normal controls (hFOB). Next, downregulation of miR-17 inhibited cell proliferation and promoted cell apoptosis by MTT assay and flow cytometry assay in MG63 cells. Downregulation of miR-17 significantly inhibited migration and invasion by using Transwell assay in the MG-63 cell lines compared with the negative controls. Taken together, we indicated that knockdown of miR-17 inhibited cell proliferation, migration and promoted cell apoptosis in OS cell.

PI3K/AKT signaling is frequently dysregulated in multiple cancer types, including breast cancer, prostate cancer, non-small cell lung cancer and ovarian cancer [35,36]. Akt is capable of phosphorylating substrates involved in a number of processes, including cell proliferation, apoptosis and migration [37]. Knockdown of miR-17 inhibited cell proliferation, migration and promoted cell apoptosis by suppressing PI3K/AKT signaling in OS cells. MMPs, a family of zinc-binding proteins including MMP-2 and MMP-9, have been shown to play a central role in tumor cell migration, invasion and metastasis due to their ability to degrade the extracellular matrix [38,39]. Furthermore, various moderators of apoptosis exerted their effects through the caspase enzyme system. Among them, caspase-3 has been demonstrated to be one of the most markedly associated with apoptosis [40]. Bcl-2 and Bax are mutual antagonists that play important roles in the regulation of tumor cell apoptosis [41].

SASH1 is a novel candidate tumor suppressor. Overexpression of SASH1 inhibited TGF-β1-induced EMT in gastric cancer cells through the PI3K/Akt signaling pathway [42]. The expression levels of SASH1, Bax and caspase-3 were upregulated by miR-128 inhibition in osteosarcoma, whereas Bcl-2 was downregulated in the same group [28]. Moreover, the expression of cyclin D1, MMP-9 displayed a down-regulation in MG-63 cells from pcDNA3.1-SASH1 group compared to the empty vector group and blank control group [27]. In present study, we found that SASH1 was a target gene of miR-17, and miR-17 negative regulated the expression of SASH1 in OS cell. Further, we revealed that knockdown of miR-17 promoted the apoptosis of OS cell, and regulated the expression of caspase3, Bcl-2 and Bax in OS cells. We showed that miR-17 exerted oncogenic function on OS via activating PI3K/AKT signaling and decreasing the expression of MMP-2 and MMP-9. Taking together, these results indicated that miR-17 could regulate related genes of cell proliferation, invasion and anti-apoptosis by regulating SASH1 expression in OS.

5. Conclusion

In summary, our study detected a high expression level of miR-17 in OS cell, which was functioned as an oncogenic miRNA by promoting OS cell proliferation, migration and invasion. This study provided important evidences in OS development. And this study suggested that targeting miR-17/SASH1 axis might represent a potential therapeutic strategy to eradicate OS cells.

Authors' contributions

DW and HZ was responsible for the study design and the acquisition of data, undertook data analysis and performed the functional experiments. FJ helped to design the experiments and interpret the data. WD undertook project design and manuscript revisions.

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (No: 81272942, No: 81702666 and No: 81502328)

Competing interests

The authors declare that they no financial conflicts of interest

Availability of data and materials

The datasets used/or analyzed during the current study are available from the corresponding author on reasonable request

Acknowledgements

Not applicable

References

[1] G. Ottaviani, N. Jaffe, The epidemiology of osteosarcoma, Cancer Treat. Res. 152 (2009) 3–13.
[2] M.E. Anderson, Update on survival in Osteosarcoma, Orthop. Clin. North Am. 47 (1) (2016) 283–292.
[3] L. Mirabello, R.J. Troisi, S.A. Savage, Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program, Cancer 115 (7) (2009) 1531–1543.
[4] R.A. Durfee, M. Mohammed, H.H. Luu, Review of osteosarcoma and current management, Rheumatol. Ther. 3 (2) (2016) 221–243.
[5] S. Pant, et al., Osteosarcoma: a diagnostic dilemma, J. Exp. Ther. Oncol. 12 (1) (2017) 61–65.
[6] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2) (2004) 281–297.
[7] R.A. Irwandi, A. Vacharaksa, The role of microRNA in periodontal tissue: a review of the literature, Arch. Oral Biol. 72 (2016) 66–74.
[8] L.A. Macfarlane, P.R. Murphy, MicroRNA: biogenesis, function and role in cancer, Curr. Genomics 11 (7) (2010) 537–561.
[9] Y. Huang, et al., Biological functions of microRNAs: a review, J. Physiol. Biochem. 67 (1) (2011) 129–139.
[10] R. Di Fiore, et al., Let-7d miRNA shows both antioncogenic and oncogenic functions in osteosarcoma-derived 3AB-OS Cancer stem cells, J. Cell. Physiol. 231 (8) (2016) 1832–1841.
[11] X. Tian, et al., MiRNA-15a inhibits proliferation, migration and invasion by targeting TNFAIP1 in human osteosarcoma cells, Int. J. Clin. Exp. Pathol. 8 (6) (2015) 6442–6449.
[12] X. Qiang, Y. Yu, Q. An, et al., MicroRNA-100 suppresses human osteosarcoma cell proliferation and chemo-resistance via ZNRF2, Oncotarget 8 (21) (2017) 34678–34686.
[13] Y.Z. Song, J.F. Li, Circular RNA hsa_circ_0001564 regulates osteosarcoma proliferation and apoptosis by acting miRNA sponge, Biochem. Biophys. Res. Commun. 495 (3) (2018) 2369–2375.
[14] J. Huang, et al., MicroRNA-124 acts as a tumor-suppressive miRNA by inhibiting the expression of Snail2 in osteosarcoma, Oncol. Lett. 15 (4) (2018) 4979–4987.
[15] H. Zhang, et al., MiRNA-543 promotes osteosarcoma cell proliferation and glycolysis by partially suppressing PRMT9 and stabilizing HIF-1alpha protein, Oncotarget 8 (2) (2017) 2342–2355.
[16] J. Dong, et al., miRNA-223 is a potential diagnostic and prognostic marker for osteosarcoma, J. Bone Oncol. 5 (2) (2016) 74–79.
[17] G. Zhang, et al., Long non-coding RNA HNF1A-AS1 promotes cell proliferation and invasion via regulating miR-17-5p in non-small cell lung cancer, Biomed. Pharmacother. 98 (2018) 594–599.
[18] J. Li, et al., miR-17-5p suppresses cell proliferation and invasion by targeting ETV1 in triple-negative breast cancer, BMC Cancer 17 (1) (2017) 745.
[19] Y. Zhu, et al., MiR-17-5p enhances pancreatic cancer proliferation by altering cell cycle profiles via disruption of RBL2/E2F4-repressing complexes, Cancer Lett. 412 (2018) 59–68.
[20] F. Fu, et al., Circulating exosomal miR-17-5p and miR-92a-3p predict pathologic stage and grade of colorectal Cancer, Transl. Oncol. 11 (2) (2018) 221–232.
[21] F. Yang, et al., miR-17 as a diagnostic biomarker regulates cell proliferation in breast cancer, Onco. Ther. 10 (2017) 543–550.
[22] X.H. Liao, et al., STAT3 is required for MiR-17-5p-mediated sensitization to chemotherapy-induced apoptosis in breast cancer cells, Oncotarget 8 (9) (2017) 15763–15774.
[23] D. Lu, et al., miR-17-3P regulates the proliferation and survival of colon cancer cells by targeting Par4, Mol. Med. Rep. 17 (1) (2018) 618–623.
[24] C. Chen, et al., MiR-17-5p promotes cancer cell proliferation and tumorigenesis in nasopharyngeal carcinoma by targeting p21, Cancer Med. 5 (12) (2016) 3489–3499.
[25] Y. Gao, et al., miR-17 inhibitor suppressed osteosarcoma tumor growth and metastasis via increasing PTEN expression, Biochem. Biophys. Res. Commun. 444 (2) (2014) 230–234.
[26] M. Martini, et al., The candidate tumor suppressor SASH1 interacts with the actin cytoskeleton and stimulates cell-matrix adhesion, Int. J. Biochem. Cell Biol. 43 (11) (2011) 1630–1640.
[27] Q. Meng, et al., SASH1 regulates proliferation, apoptosis, and invasion of osteosarcoma cell, Mol. Cell. Biochem. 373 (1-2) (2013) 201–210.
[28] Z. Li, et al., Regulatory mechanism of microRNA-128 in osteosarcoma tumorigenesis and evolution through targeting SASH1, Oncol. Lett. 15 (6) (2018) 8687–8694.
[29] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (4) (2001) 402–408.
[30] V. Agarwal, et al., Predicting effective microRNA target sites in mammalian mRNAs, Elife 4 (2015).
[31] M. Abasi, et al., Differential maturation of miR-17 ~ 92 cluster members in human Cancer cell lines, Appl. Biochem. Biotechnol. 182 (4) (2017) 1540–1547.
[32] H. Li, et al., The miR-17-92 cluster as a potential biomarker for the early diagnosis of gastric cancer: evidence and literature review, Oncotarget 8 (28) (2017) 45060–45071.
[33] W. Zhang, et al., miR-17-5p down-regulation contributes to erlotinib resistance in non-small cell lung cancer cells, J. Drug Target. 25 (2) (2017) 125–131.
[34] Y. Qu, et al., MiR-17-5p regulates cell proliferation and migration by targeting transforming growth factor-beta receptor 2 in gastric cancer, Oncotarget 7 (22) (2016) 33286–33296.
[35] C. Bartholomeusz, A.M. Gonzalez-Angulo, Targeting the PI3K signaling pathway in cancer therapy, Expert Opin. Ther. Targets 16 (1) (2012) 121–130.
[36] C. Kandoth, et al., Mutational landscape and significance across 12 major cancer types, Nature 502 (7471) (2013) 333–339.
[37] M. Moral, J.M. Paramio, Akt pathway as a target for therapeutic intervention in HNSCC, Histol. Histopathol. 23 (10) (2008) 1269–1278.
[38] N.E. Deakin, M.A. Chaplain, Mathematical modeling of cancer invasion: the role of membrane-bound matrix metalloproteinases, Front. Oncol. 3 (2013) 70.
[39] H.D. Foda, S. Zucker, Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis, Drug Discov. Today 6 (9) (2001) 478–482.
[40] U. Tormanen-Napankangas, et al., Expression of caspases-3, -6 and -8 and their relation to apoptosis in non-small cell lung carcinoma, Int. J. Cancer 93 (2) (2001) 192–198.
[41] Y. Tang, et al., Icarisid II inhibits the proliferation of human osteosarcoma cells by inducing apoptosis and cell cycle arrest, Tumour Biol. 39 (6) (2017) 1010428317705745.
[42] W. Zong, et al., Overexpression of SASH1 inhibits TGF-beta1-Induced EMT in gastric Cancer cells, Oncol. Res. 24 (1) (2016) 17–23.