RETRACTED ARTICLE: miR-557 suppressed the malignant behaviours of osteosarcoma cells by reducing HOXB9 and deactivating the EMT process

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ABSTRACT

MicroRNAs (miRNAs) are vital gene regulators, which play a profound role in the process of forming and developing many diseases, especially tumour. The study intends to excavate the potential regulatory mechanisms of miR-557 and its targeting gene Homeobox B9 (HOXB9) in osteosarcoma. GEO dataset on osteosarcoma was applied to detect the expression of miR-557 and HOXB9. Associations between miR-557 and HOXB9 were speculated by prediction software and verified by dual luciferase assay. Cell proliferation, colony formation and mobility were measured by cell counting kit-8, plate clone formation and transwell assays. Expression of mesenchymal transitions (MTs) related proteins was assessed by western blot analysis. Low expression of miR-557 was presented in osteosarcoma tissues and cell lines. Upregulation of miR-557 restrained osteosarcoma cells proliferation, movement and MT process. HOXB9, served as a target gene of miR-557, was highly expressed in osteosarcoma, and its high expression was associated with poor prognosis in patients with osteosarcoma. In addition, overexpression of HOXB9 attenuated the inhibitory effects of miR-557 on tumour progression by MT process. Overexpression of miR-557 suppressed the growth, metastasis and MT process of osteosarcoma cells by targeting HOXB9, affording novel molecular selection for targeted therapy of osteosarcoma.

Introduction

Osteosarcoma is a malignant tumour that usually occurs in the first decade or second decade of life [1]. It is pathologically characterized by spindle cells and abnormal osteoid formation. Extreme malignancy and a strong tendency to metastasize are important properties of osteosarcoma [2]. The occurrence of osteosarcoma is caused by the failure of osteoblasts to differentiate into mature osteoblasts [3]. An unfortunate feature of osteosarcoma is that distant metastasis, especially lung metastasis, which can occur even after chemotherapy and surgery [4,5]. Currently, the treatment of osteosarcoma usually involves a combination of chemotherapy and surgical resection [6]. However, survival rate for osteosarcoma patients has stagnated in recent years, and the prognosis for patients with metastatic or relapsed disease remains poor, with a five-year overall survival rate of 20% [7,8]. Therefore, elucidating novel therapeutic targets and identifying therapeutic strategy are critically important for improving survival rate.

MicroRNAs (miRNAs) are small noncoding RNA that silences genes expression by binding to their 3’ untranslated regions (UTRs) [9]. Due to this property, miRNAs are involved in multiple functions of cells, including proliferation, apoptosis, differentiation and so on [10]. In addition, miRNAs are crucial regulators to the initiation and progression of tumour growth [11]. Dysregulation of miRNAs has been related to the growth, movement and prognosis of osteosarcoma [12,13]. For example, upregulation of miR-145 suppressed invasion and metastasis of osteosarcoma cells through targeting vascular endothelial growth factor [14]. Data from Chen et al. indicated that overexpression of miR-548d-3p reduced osteosarcoma cells growth and migration in vitro by silencing KRAS [15]. Moreover, miR-627-3p and miR-506-3p have been reported to act as tumour suppressors in osteosarcoma by targeting PTN and RAB3D, respectively [16,17].

MiR-557, as a relatively novel miRNA, was screened in xenografts of Ewing’s sarcoma by microarray technology [18]. In 2014, miR-557 was picked out in gastric cancer by Jiang et al. [19]. To data, function of miR-557 in tumour progression has not been clearly clarified excluding that it has been found to serve as a tumour suppressor in lung cancer by downregulating LEF1 [20] whilst, the function of miR-557 and the underlying regulatory mechanism have not been elucidated in osteosarcoma.
In the present study, our outcomes revealed that upregulation of miR-557 limited osteosarcoma cells growth and metastasis. In addition, Homeobox B9 (HOXB9) has been considered to be a target of miR-557 in osteosarcoma and presented a tendency of high expression in patients with osteosarcoma. Tumour suppressor function of miR-557 was performed by reducing HOXB9 and mesenchymal transitions (MTs) process. All these findings afforded novel clues for the clinical diagnosis and treatment for human osteosarcoma patients.

Materials and methods

Data collection

The Gene Expression Omnibus (GEO) dataset on osteosarcoma (numbered GSE28423 and GSE65071) was used to screen for important miRNA as miR-557 and to quantify miR-557 expression in osteosarcoma. Expression level of HOXB9 in osteosarcoma was acquired from GEO dataset named GSE24824. GEO dataset numbered GSE16091 was used to evaluate the relationship between HOXB9 expression and patients’ prognosis.

Cell lines and cell culture

All cell lines including three osteosarcoma cell lines (MG-63, U2OS and Saos-2) and normal cell line hFOB 1.19 were purchased from Chinese Academy of Science Cell Bank (Shanghai, China), cultured in DMEM medium (Gibco, Grand Island, NY) accompanied by 10% foetal bovine serum (Gibco, Grand Island, NY), 100 U/mL penicillin and 0.1 mg/mL streptomycin at 37°C in an atmosphere with 5% CO2.

Gene overexpression and silencing

All small fragments including miR-557 mimic, miR-557 inhibitor, their negative control (NC), si-HOXB9#1 (5′-GGCCGTCGCTGCTATACAAAG-3′), si-HOXB9#2 (5′-CAAAGAAGGAGCCGATCAAC-3′) and si-con (5′-CGACUCACUGGUCUGACC-3′) were synthesized by GenePharma (Shanghai, China). These fragments were respectively transfected into cells by Lipofectamine2000 (Invitrogen, Carlsbad, CA) to regulate the expression of miR-557 and HOXB9. After 24 h, we detected the transfection efficiency and performed the subsequent experiments.

RNA extraction and quantitative real-time PCR (qRT-PCR)

The whole RNA was separated from the treated cells utilizing Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the directions. Quantitative analysis of miR-557 was executed with miScript II RT Kit and miScript SYBR Green PCR Kit (Qiagen, Hilden, Germany) whilst, quantitative analysis of mRNA was performed by PrimeScript RT Reagent Kit and SYBR Premix Ex Taq II (Takara, Osaka, Japan). The relative expression levels of miRNA and mRNA were assessed by 2−ΔΔCt method and normalized to U6 and GAPDH, respectively.

The primers sequences were presented as below:

| Primers          | sequences                          |
|------------------|------------------------------------|
| miR-557 F        | 5′-GTTTGACGGGTTGGGC-3′             |
| miR-557 R        | 5′-GAACATGTCGCTATATCTC-3′          |
| U6 F             | 5′-GAAACATGTCGCTATATCTC-3′         |
| si-HOXB9#1 F     | 5′-CTCGCTCAGCGACACATATACT-3′       |
| si-HOXB9#1 R     | 5′-ACGCTTCACAGAATTTCGCT-3′         |
| si-HOXB9#2 F     | 5′-TGGCAGGAAAGCCGGAGAAAG-3′        |
| si-HOXB9#2 R     | 5′-TCTCTCTTCTAGTCCAGGCTC-3′        |
| GAPDH F          | 5′-TGTCCTCCTGGATGTCTGA-3′          |
| GAPDH R          | 5′-CCTGCTCACCCACCTTCTGA-3′         |

Western blot assay

Total protein from the treated cells was separated by radioimmunoprecipitation buffer (Thermo Fisher Scientific, Rockford, IL). Then, the concentration of protein was measured by bicinchoninic acid assay. Subsequently, the proteins were isolated by 12% sodium dodecyl sulphate-PAGE, and transferred to polyvinylidene difluoride membranes through electroblotting. Membranes were blocked in 5% skimmed milk for 1 h at about 25°C, and subsequently incubated with primary antibodies (Abcam, Cambridge, UK) including HOXB9 (ab133701, 1:2000, 28 kDa), TRIP13 (ab128153, 1:5000, 49 kDa), CAT1 (ab232918, 1:1000, 43 kDa), MLLT11 (ab109016, 1:5000, 10 kDa), Slug (ab183760, 1:5000, 37 kDa), Snail (ab216347, 1:1000, 29 kDa), Twist (ab49254, 1:1000, 21 kDa) and GAPDH (ab181602, 1:1000, 36 kDa) at 4°C overnight. Then membranes were incubated with a horseradish peroxidase-conjugated secondary antibody at about 25°C for 1 h and visualized by enhanced chemiluminescence (Thermo Fisher Scientific, Rockford, IL). GAPDH was used as the internal parameter. Image J software (Bethesda, MD) was applied to measure the densitometry of the protein bands.

Cell proliferation assay

Cell proliferation ability was evaluated by cell counting kit-8 (CCK-8). All treated cells were implanted onto 96-well plate at the density of 1000 cells per well and cultured under above condition. The cells viability was measured every 24 h. Before detection, 10 μL of CCK-8 reagent was put into each well and incubated for 1.5 h. The optical density was detected at 450 nm wavelength using microplate reader, and the proliferation curve was drawn by GraphPad Prism5.0 (GraphPad Software, La Jolla, CA).

Plate clone formation assay

Treated cells were seeded on 6 mm dish with the density of 500 cells, and incubated at 37°C in a 5% CO2 incubator for 1–2 weeks. Cells were constantly observed and cultures were terminated when clones visibly appeared in the dish. We discarded the medium and washed the cells with PBS. Subsequently, cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The number of colonies was counted and analysed.


**Cell invasion and migration assays**

For the invasion and migration assays, 1 × 10^5 treated cells suspended in 100 μL of serum-free DMEM medium were implanted onto the upper part of a transwell chamber including or excluding a matrigel. Five hundred microlitres of complete culture medium was filled into the lower chamber. After incubation for 24 h, the non-invaded/non-migrated cells were removed using a cotton swab. Invaded/migrated cells were fixed using 4% paraformaldehyde for 30 min, and then were stained with 0.1% crystal violet for 20 min. The wells were rinsed with running water and naturally dried. The number of invaded or migrated cells was counted and analysed.

**Dual luciferase assay**

Associations between miR-557 and HOXB9 were predicted by bioinformatics analysis. Dual luciferase assay was used to confirm this assumption. First, the cDNA encoding HOXB9 was prepared using PCR fragments and subcloned into pLG3 vector, which was named wild type (wt) HOXB9. The mutant (mut) HOXB9 was amplified by overlap PCR and cloned into the pLG3 vector, named mut HOXB9 then, cotransfected pLG3-wt-HOXB9 or pLG3-mut-HOXB9 and miR-557 mimic or NC into cells. Cells were implanted onto 96-well plates at the density of 1 × 10^4 cells per well and cultured for 48 h. Finally, the cells were lysed and the luciferase activity was measured by the dual luciferase assay kit (Promega, Madison, WI) to reflect the relationship between miR-557 and HOXB9.

**Statistical analysis**

All statistical analyses were carried out by SPSS software 16.0 (SPSS, Chicago, IL), and graphics were created by GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). Comparison among multiple groups was processed with one-way analysis of variance followed by Bonferroni’s post hoc test. Student’s t-test or Mann–Whitney’s test was used to analyse the difference between two groups. The survival rate was calculated by using Kaplan–Meier’s method with the log-rank test applied for comparison. All experiments were independently repeated three times. p Value less than .05 means that difference is statistically significant.

**Results**

**Identification of miRNAs differentially expressed in osteosarcoma**

By performing microarray analysis based on the GSE28423 dataset from GEO database, a total of 225 miRNAs were obtained including 109 upregulated miRNAs and 116 downregulated miRNAs. Then, a total of 265 miRNAs were acquired by microarray analysis based on the GSE65071 dataset, among which 205 miRNAs were upregulated and 60 miRNAs were downregulated. The downregulated miRNAs in the two datasets were intersected to obtain three miRNAs, namely miR-338-3p, miR-142-5p and miR-557 (Figure 1(A)). According to literature analysis [21–23], we observed that the function of miR-338-3p and miR-142-5p has been reported in osteosarcoma. Therefore, miR-557 was selected for further study and expression level of miR-557 in osteosarcoma was assessed. Data from GSE28423 including 19 tumour tissues and four normal tissues revealed that miR-557 was lower expressed in osteosarcoma tissues in contrast with the normal (Figure 1(B), p = .0002). Similarly, data from GSE65071 with 20 tumour tissues and 15 normal tissues also found that miR-557 was lower expressed in osteosarcoma (Figure 1(C), p < .0001). Subsequently, expression of miR-557 in osteosarcoma cell lines (U2OS, Saos-2 and MG-63) was determined by qPCR. As expected, miR-557 expression was significantly reduced in osteosarcoma cell lines compared with hFOB1.19 (Figure 1(D), p < .01). These data underlined the importance of miR-557 in osteosarcoma.

**Mir-557 inhibited the proliferation of osteosarcoma cells**

Next, CCK-8 assay and plate cloning experiment were performed to evaluate the influence of miR-557 on osteosarcoma cells growth. Due to the low expression of miR-557 in U2OS cells, miR-557 mimic was used in U2OS cells to detect the impacts of miR-557 on osteosarcoma cells behaviour. As displayed in Figure 2(A), we observed that the expression of miR-557 was highly increased after miR-557 mimic treatment. Data from Figure 2(B,E,F) showed that overexpression of miR-557 reduced U2OS cells viability and cloning ability. Whilst, the relative high expression of miR-557 in MG-63 cells, miR-557 inhibitor was applied to detect the effects of miR-557 on osteosarcoma cells growth. The data from Figure 2(C) showed that miR-557 inhibitor treatment significantly reduced miR-557 expression. Moreover, the results indicated that silencing miR-557 promoted MG-63 cells proliferation and clone formation (Figure 2(D,G–H), p < .01). These findings indicated that upregulation of miR-557 suppressed osteosarcoma cells growing ability, and vice versa.

**HOXB9, which was a direct target of miR-557, was highly expressed in osteosarcoma and linked to patients outcome**

Then, we used bioinformatics analysis to explore the downstream target of miR-557. By performing microarray analysis based on the GSE28424 dataset, a total of 359 mRNAs were obtained including 48 upregulated mRNAs and 311 downregulated mRNAs. The target genes of miR-557 were predicted by miRand, miRDB, miRWalk and Targetscan website and a total of 1200 genes were found in all the four databases. By overlapping the predicted genes of miR-557 and the upregulated genes in GSE24824, four genes were obtained, namely HOXB9, TRIP13, BCA1 and MLLT11 (Figure 3(A)). Then, we analysed the mRNA and protein expression of HOXB9, TRIP13, BCA1 and MLLT11 in osteosarcoma cell lines (U2OS, Saos-2 and MG-63) and hFOB1.19 cells. The data from Figure 3(B–J) indicated that mRNA and protein expression of HOXB9, TRIP13, BCA1 and MLLT11 were highly increased in osteosarcoma cell lines (U2OS, Saos-2 and MG-63) compared with hFOB1.19 cells. An


osteosarcoma dataset GSE16091 with clinical information was selected to analyse the effect of the expression of HOXB9, TRIP13, BCAT1 and MLLT11 on the prognosis of patients. The results showed that only low expression of HOXB9 had significantly better survival than the high expression group. The differential expression of the other three genes had no significant effect on patients' survival. Therefore, HOXB9 was picked up for further experimental examination (Figure 3(K–N)). Data from GEO database including 19 osteosarcoma tissues and four normal tissues showed that HOXB9 was significantly upregulated in osteosarcoma (Figure 3(O), \( p = 0.0002 \)). The binding sites between miR-557 and HOXB9 are presented in Figure 4(A). Subsequently, luciferase assay was carried out to confirm this target relationship between miR-557 and HOXB9. As presented in U2OS and MG-63 cells, in wt-HOXB9 group, the luciferase activity was reduced or increased after transfected with miR-557 mimic or inhibitor (Figure 4(B,C), \( p < 0.01 \)). However, the luciferase activity barely changed in mut-HOXB9 group. Next, the effects of miR-557 on HOXB9 were performed in U2OS and MG-63 cells. In U2OS cells, the results showed that HOXB9 expression was obviously reduced after miR-557 mimic treatment; however, HOXB9-OE treatment alleviated the inhibitory effect of miR-557 mimic on HOXB9 expression (Figure 4(D,E)). Whilst in MG-63 cells, knockdown of miR-557 increased HOXB9 expression, but si-HOXB9 treatment suppressed the promoting effect of miR-557 inhibitor on HOXB9 expression (Figure 4(F,G)). All these results suggested that HOXB9 expression was negatively regulated by miR-557.

Inhibitory effects of miR-557 on osteosarcoma cells
behaviours were performed by reducing HOXB9 expression

In U2OS cells, overexpression of miR-557 inhibited cells activity, decreased the number of colonies from 622 ± 58 to 280 ± 47 (\( p = 0.001 \)), reduced the number of invaded/migrated cells from 53 ± 13/101 ± 14 to 23 ± 8 (\( p = 0.032 \))/54 ± 14 (\( p = 0.012 \)). However, overexpression of HOXB9 and miR-557 promoted cells vitality, increased the number of colonies from 280 ± 47 to 630 ± 73 (\( p = 0.001 \)), and elevated the number of invaded/migrated cells from 23 ± 8/54 ± 14 to 57 ± 9 (\( p = 0.017 \))/104 ± 12 (\( p = 0.008 \)) compared to only upregulation of miR-557, indicating that overexpression of HOXB9 relieved the inhibitory effects of miR-557 mimic on U2OS cells growth and metastasis (Figure 5(A,C,D,G,H), \( p < 0.01 \)).

In MG-63 cells, silencing miR-557 increased the viability of cells, elevated the number of colonies from 306 ± 67 to 715 ± 77 (\( p = 0.001 \)), and aggrandized the number of invaded/migrated cells from 34 ± 9/62 ± 10 to 75 ± 13 (\( p = 0.006 \))/117 ± 8 (\( p = 0.002 \)). Whilst, knockdown of HOXB9 and
miR-557 suppressed cells vitality, declined the number of colonies from $715 \pm 77$ to $297 \pm 50$ ($p = .001$), and lowered the number of invaded/migrated cells from $75 \pm 13/117 \pm 8$ to $29 \pm 8$ ($p = .003$)/$56 \pm 12$ ($p = .001$) in contrast with just exhaustion of miR-557, suggesting that knockdown of HOXB9 restricted the positive effect of miR-557 inhibitor on MG-63 cells proliferation and movement (Figure 5(B,E,F,I,J), $p < .01$).

**Regulation of miR-557/HOXB9 on osteosarcoma cells was partly mediated by MT process**

According to the current level of knowledge, the occurrence of osteosarcoma is considered to be derived from the transformation of mesenchymal cells. To investigate whether miR-557/HOXB9 affected the biological behaviour of osteosarcoma cells through the MT process, we detected the expression of markers related to the MT process. In U2OS cells, overexpression of miR-557 reduced the expression of Slug, Twist and Snail. However, upregulation of miR-557 and HOXB9 elevated the expression of Slug, Twist and Snail compared to only miR-557 mimic transfection (Figure 6(A,B), $p < .01$). In MG-63 cells, downregulation of miR-557 increased the expression of Slug, Twist and Snail. But silencing miR-557 and HOXB9 decreased the expression of Slug, Twist and Snail in contrast with just miR-557 inhibitor transfection (Figure 6(C,D), $p < .01$). These findings indicated that function of miR-557/HOXB9 on osteosarcoma cells cancer-related behaviours was modulated partially through MT process.

**Discussion**

The present study showed that miR-557 was lower expressed in osteosarcoma samples and inhibited osteosarcoma cells cancer-related behaviours. Moreover, a downstream direct target of miR-557 was identified as HOXB9, which attenuated
(A) Prediction of target genes of miR-557. (B–E) The mRNA expression of HOXB9, TRIPI3, BCAT1 and MLLT11 was increased in these osteosarcoma cell lines including U2OS, Saos-2 and MG-63, ANOVA with Bonferroni’s post hoc test, data presented as mean ± SD, **p<.01 represented vs. hFOB1.19. (F–J) The protein expression of HOXB9, TRIPI3, BCAT1, and MLLT11 was increased in these osteosarcoma cell lines including U2OS, Saos-2 and MG-63, ANOVA with Bonferroni’s post hoc test, data presented as mean ± SD, **p<.01 represented vs. hFOB1.19. (K–N) Survival analysis of BCAT1, HOXB9, MLLT11 and TRIPI3 in osteosarcoma patients, log-rank test. (O) HOXB9 was significantly upregulated in osteosarcoma tissues compared to normal, Mann–Whitney’s test, p=.0002.
the inhibitory effects of miR-557 on osteosarcoma cell growth and metastasis partly through MT process.

Multiple miRNAs have been found to be involved in the initiation and development of osteosarcoma. For example, depletion of miR-183 accelerated invasion and migration of osteosarcoma by targeting Ezrin [24]. Upregulation of miR-34a accelerated cell cycle arrest and apoptosis and inhibited cell adhesion by reducing DUSP1 in osteosarcoma [25]. In addition, overexpression of miR-665 inhibited invasion and metastasis of osteosarcoma through directly suppressing RAB23 [26]. Given this, a continued study for such miRNAs is indispensable to afford novel targets and therapeutic methods for this disease. Based on the microarray analysis from GSE28423 and GSE65071, three miRNAs were obtained including miR-338-3p, miR-142-5p and miR-557. Since miR-557 is a neoteric RNA, and the function of miR-557 is not elucidated in the tumorigenesis of human osteosarcoma, we focussed our attention on miR-557. Our study stated that miR-557 was lower expressed in osteosarcoma tissues and cell lines, and overexpression of miR-557 inhibited the cancer-related behaviours of osteosarcoma cells, suggesting that miR-557 functioned as a tumour suppressor gene in osteosarcoma.

In order to detect the downstream regulation mechanism of miR-557, bioinformatics analysis was employed. Interestingly, the results by miRand, miRDB, miRWalk, Targetscan analysis indicated that miR-557 might bind to 3'UTR of HOXB9. HOXB9 is a member of the family of homeobox-containing (HOX) transcriptional factor, which contains 39 genes in humans and are divided into four distinct clusters: HOX A, B, C and D, all of which play crucial roles in embryonic development [27,28]. In addition to their vital roles in development, accumulating evidence has illustrated that HOX family genes are correlated with tumour progression [29,30]. For example, HOXB9 expression promoted tumour cells growth and angiogenesis and was related to clinical outcomes in breast cancer patients [31]. In addition, HOXB9 was significantly upregulated in oral squamous cell carcinoma and hepatocellular carcinoma and promoted tumour cells invasion and migration [32,33]. HOXB9 was considered as a crucial prognostic factor for head and neck squamous cell carcinoma [34] and ovarian cancer [35]. These findings strongly indicated that HOXB9 is implicated in tumour occurrence and development. In the current study, by dual luciferase assay, targeted relationship between miR-557 and HOXB9 was confirmed. In addition, microarray analysis revealed that HOXB9 was significantly upregulated in osteosarcoma tissues and its high expression was associated with poor prognosis in patients with osteosarcoma. Moreover, HOXB9 promoted the proliferation and migration
Figure 5. Function of miR-557/HOXB9 on osteosarcoma cells growth and metastasis. (A) U2OS cells viability was measured by CCK-8 reagent after miR-557 mimic or miR-557 mimic + HOXB9-OE transfection, **p<.01 represented vs. NC, ***p<.01 represented vs. miR-557 mimic. (B) MG-63 cells viability was measured by CCK-8 reagent after miR-557 inhibitor or miR-557 inhibitor + si-HOXB9 transfection, **p<.01 represented vs. NC, ***p<.01 represented vs. miR-557 inhibitor. (C, D) The number of U2OS cells colonies was reduced after miR-557 mimic transfection, whilst upregulation of miR-557 and HOXB9 inhibited the downward trend of cell colonies, **p<.01 represented vs. NC, ***p<.01 represented vs. miR-557 mimic. (E, F) Downregulation of miR-557 increased the number of MG-63 cells colonies, but the increasing number of MG-63 cells colonies was suppressed after silencing miR-557 and HOXB9, **p<.01 represented vs. NC, ***p<.01 represented vs. miR-557 inhibitor. (G, H) The number of invaded and migrated U2OS cells was reduced after overexpression of miR-557, but this phenomenon was reversed by miR-557 mimic or miR-557 mimic + HOXB9-OE transfection, *p<.05 represented vs. NC, **p<.01 represented vs. miR-557 mimic. (I–J) Silencing miR-557 elevated the number of MG-63 cells migration and invasion, and this phenomenon was suppressed after knockdown of miR-557 and HOXB9, **p<.01 represented vs. NC, ***p<.01 represented vs. miR-557 inhibitor. ANOVA with Bonferroni's post hoc test, data presented as mean ± SD.
of osteosarcoma cells, and weakened the inhibitory effects of miR-557 on the biological behaviours of osteosarcoma cells. These results will be great helpful for developing potential clinical therapeutic schedule.

In addition to the research in terms of cell growth, migration and invasion abilities, our study concentrated on the impacts of miR-557/HOXB9 on MT. Increasing evidence suggested that EMT accelerated aggressive tumour phenotypes, leading to local invasion and distant metastasis by lymphatic and blood circulation [36]. Multiple studies indicated that targeting EMT regulatory factors may inhibit tumour invasion and metastasis [37,38]. Therefore, targeting EMT process is considered as a promising strategy to limit metastasis and raise living quality of cancer patients. To our knowledge, osteosarcoma is derived from the mesenchymal cells, so function of miR-557/HOXB9 on osteosarcoma cells MTs process was examined. In the current study, overexpression of miR-557 reduced the expression of snail, slug and twist, but HOXB9 abolished the inhibition of MT process by miR-557. These results insinuated that the inhibitory effects of miR-557 on osteosarcoma cells were performed by reducing HOXB9 and inactivating MT process.

Finally, the limitation of this study should be pointed out. Our research is mainly carried out in osteosarcoma cells in vitro, and lack of in vivo experiments to support these results. Next, we will further demonstrate these findings in vivo.

Conclusions

In general, our outcomes suggested that lower expression of miR-557 was presented in osteosarcoma tissues and cells. Downstream target of miR-557 in osteosarcoma was identified as HOXB9. Moreover, miR-557 inhibited the proliferation and metastasis of osteosarcoma cells by targeting HOXB9 and mediating the MT process.
Disclosure statement
No potential conflict of interest was reported by the author(s).

Data availability statement
Data are available from corresponding author with the proper reason.

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