Long noncoding RNA NEAT1 aggravates osteosarcoma carcinogenesis via regulating the microRNA-579/MMP13 axis

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Research article

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Abstract

Background: Previous studies have suggested that long non-coding RNAs (lncRNAs) were involved in tumorigenesis in various human carcinomas, including osteosarcoma (OS). However, the expression and specific role of lncRNA NEAT1 in OS remain unknown. The current study aimed at revealing the role of lncRNA NEAT1 and its related mechanism in OS.

Methods: Expression profiles of lncRNAs in OS tissues were constructed, and lncRNA NEAT1 expression was verified with RT-qPCR followed by sub-localization. LncRNA-microRNA (miRNA) and miRNA-mRNA interactions were predicted. Validation was performed using dual luciferase reporter gene assay, and gain- and loss-of-function experiments. The effects of lncRNA NEAT1, miR-579 and MMP13 on the proliferation, migration and invasion, epithelial-mesenchymal transition (EMT) of OS cells were detected using colony formation, cell counting kit-8 (CCK-8), Transwell assays and Western blot analysis.

Results: LncRNA NEAT1 overexpression was observed in OS tissues and cell lines which located in the cytoplasm. Transfection-induced downregulation of lncRNA NEAT1/MMP13 or overexpression of miR-579 blocked the progression of OS cells. LncRNA NEAT1 promotes MMP13 through sponging miR-579.

Conclusion: LncRNA NEAT1 might be beneficial for OS aggravation via sponging miR-579 and facilitating MMP13 expression, which represents a candidate marker and target for OS therapy.

Background

Osteosarcoma (OS) is a kind of bone malignant neoplasm that commonly exists in adolescents and children with an obviously high mortality in cancer-related deaths [1]. The incidence rate of OS is estimated as 5 million each year [2]. Due to improvement in multidisciplinary clinical remedy in the past decades for OS, the outcomes have been ameliorated [3]. However, 25–30% of OS patients showed metastasis which affects the efficacy of treatment negatively, the five-year survival of OS is still poor [4]. A relevant report found that the 5-year survival of patients without symptoms of systematic spread clinically is 60–80%, which is only 20–30% in patients with metastasis at diagnosis [2]. The complicacy of the initiation and progression mechanisms of OS is deemed as the principal obstacle in improving survival rate and prognosis of OS patient [5]. Therefore, it is meaningful to clarify the invisible mechanism underlying tumorigenesis and to develop novel molecular targets of OS.

Long non-coding RNAs (lncRNAs), surpassing over 200 nucleotides in length, are a prominent class of RNAs exerting a crucial regulatory potential in tumor cell behaviors [6]. Increasing evidence has illustrated that aberrantly expressed lncRNAs were highly associated with the occurrence and development of many human malignancies, including OS [7–9]. Nuclear enriched abundant transcript 1 (NEAT1) is a lncRNA transcribed from the diverse endocrinal neoplasia locus [10]. The interaction of lncRNA NEAT1 has been documented in glioma, prostate cancer and lung adenocarcinoma [11–13]. LncRNA NEAT1 was also suggested as a tumor promotor in OS by several studies [14–16].

MicroRNAs (miRNAs) are tiny (~ 22 nucleotides) endogenous non-coding RNA molecules which act at the post-transcriptional level and regulate mRNA expression [17]. They are involved in multiple complicated cellular behaviors [18]. Importantly, emerging evidence has pointed out that miRNAs participate in OS, such as miR-18a-5p, miR-671-5p, miR-1301, and miR-212 [19–22]. MiR-579 was reported to be associated with malignancies, such as breast cancer, ovarian cancer, and human glioblastoma [23–25]. However, its function in OS deserves further studies.

LncRNAs are proved to serve as competing endogenous RNAs (ceRNAs), which sponges certain miRNAs to mediate its target gene, thus changing the post-transcriptional regulation [1]. However, the concrete influence that lncRNA NEAT1 and miR-579 exerts on OS, especially their interaction is still to be elucidated. In this experiment, we investigated whether lncRNA NEAT1 was a ceRNA of miR-579 and the cross-regulation among lncRNA NEAT1, miR-579 and matrix metalloprotease 13 (MMP13) in OS cell progression so as to provide a new molecular target for oncotherapy.

Materials And Methods

Ethics statement

All patients provided written informed consent and this study was approved by the Medical Ethics Committee of Ningbo Sixth Hospital.

Bioinformatics

The distribution of lncRNA NEAT1 in OS and normal cells was analyzed by TCGA and GTEX database (https://xenabrowser.net/heatmap/). The location of lncRNA NEAT1 was predicted by http://lncatlas.crg.eu/. In http://starbase.sysu.edu.cn/, we searched for the potential target miRNA of lncRNA NEAT1 and the potential target gene of the miRNA.

Clinical tissue specimens

A total of 10 cases of primary OS tissues and the adjacent normal muscular tissues were collected from OS patients who underwent surgical resection at Ningbo Sixth Hospital between February 2019 and November 2019. All tissue specimens were immediately frozen in liquid nitrogen after resection and stored at -80 °C. The patients aged from 12 to 33 years with the median age of 23 years. All patients were diagnosed by laboratory tests and imaging examinations with complete imaging data and determinable stage of OS. No patients have been received chemotherapy or radiotherapy or any other surgeries prior to surgery. Besides, patients with other tumors were excluded. According to the common stages of OS, there were 2 cases in stage Ⅱ A (G2T1M0), 3 cases in stage Ⅱ B (G2T2M0), 4 cases in stage Ⅲ A (G2T1M1) and 1 case in stage Ⅲ B (G2T2M1).

Cell lines and transfection

The osteoblastic cell line hFOB and two human OS cell lines MG63 and U2OS were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultivated in RPMI-1640

medium containing 10% fetal bovine serum (FBS) (Gibco, Carlsbad, California, USA) at 37 °C under an atmosphere of 5% CO_2/95% air.

Subsequently, the lncRNA NEAT1 interference plasmids (sh-NEAT 1,2,3#) and corresponding empty plasmids (sh-negative control [NC]), mimic/inhibitor and mimic/inhibitor control of miR-579 (Thermo Fisher Scientific Inc., Waltham, MA, USA) were transfected into the well-developed MG-63 and U2OS cells. All operations of the transfection were carried out in strict accordance with the manufacturer's protocol of Lipofectamine 2000 Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Forty-eight h after transfection, the cells were collected for follow-up experiments, and the expression level of corresponding genes was detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR) to evaluate the transfection efficiency.

RT-qPCR

Trizol regent (Takara Bio Inc., Otsu, Shiga, Japan) was used for total RNA extraction from cultured cells. Then, the SYBR Green qPCR Mix Kit and the ABI 7500 Fast Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) were used to determine mRNA levels. All primers were purchased from Invitrogen (Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were used for endogenous control as appropriate. Primer sequences are listed below.

miR-579 Forward 5'-CGTGCCGTTCATTTGGTATAAAC-3',

Reverse 5'-GAGCAGGGTCCGAGGT-3';

LncRNA NEAT1 Forward 5'-TGAGTAGTGGAAGCAGGAGGA-3',

Reverse 5'-GGAGGCAAGGACGAGACAGA-3';

MMP13 Forward 5'-GACTTCCCAGGAATTGGTGA-3',

Reverse 5'-TGACGCGAACAATACGGTTA-3';

GAPDH Forward 5'-GAGTCCACTGGCGTCTTCAC-3',

Reverse 5'-ATCTTGAGGCTGTTGTCATACTTCT-3';

U6 Forward 5'-CTCGCTTCGGCAGCACA-3',

Reverse 5'-AACGCTTCACGAATTTGCGT-3'.

The relative quantitative expression of interest genes was expressed as fold change (2-ΔΔCt method). $\Delta\Delta Ct = [Ct\ (target\ gene) - Ct\ (GAPDH/U6)]_{experimental\ group} - [Ct\ (target\ gene) - Ct\ (GAPDH/U6)]_{control\ group}$. Each sample was examined in triplicate.

Transwell assay

Cell invasion was detected in a Nunc™ polycarbonate inserted cell culture device (Thermo Fisher Scientific Inc., Waltham, MA, USA) pre-positioned with 8-μm perforated plate. In brief, cell suspension was seeded in the apical chamber, and the chemokine was added to the basolateral chamber. After 24 h of routine culturing, cells were stained with crystal violet and photographed under an optical microscope. The indirect counting method was used to count cells.

For migration detection, we only need to remove the matrix gel, the procedure is the same as the invasion experiment.

Subcellular localization

First, we used Lncatlas (http://lncatlas.crg.eu/) to predict the position of lncRNA NEAT1. Fluorescence in situ hybridization (FISH) experiment was used to further determine the location of lncRNA NEAT1 in MG-63 and U2OS cells, and the cell solution was treated with trypsin. After denaturing, the cells were hybridized with probes, and the nuclei were stained and photographed under a fluorescence microscope. Finally, the nucleus was isolated from the cytoplasm by the PARIS kit (cat. No. am1921; Thermo Fisher Scientific Inc., Waltham, MA, USA), and the expression level of lncRNA NEAT1 was detected in the nucleus and cytoplasm.

Western blot analysis

The expression level of epithelial-mesenchymal transition (EMT) related markers in OS cells was detected by Western blot analysis. The cell lysate "Western and IP cell lysate" (No.: p0013) purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China) was thawed and mixed up. The equivalent cracking solution was added to phenylmethyl sulfonylfluoride until the concentration was 1 mM (No.: ST506, Beyotime Biotechnology Co., Ltd., Shanghai, China). The transfected cells were lysed with Western and IP cell lysates. After full cracking, the cells were centrifuged at 10000–14000 g for 3–5 min with the supernatant collected. Bicinchoninic acid kit (No.: p0009, Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to determine the protein concentration. Electrophoresis was then performed in polyacrylamide gel (5% concentrate and 12% separation gel). Tris-buffered saline Tween-20 (TBST) containing 5% bovine serum albumin (BSA) was used to seal the membrane in a decolorizing shaker at room temperature for 1 h. The sealing solution was discarded, and the membrane was put into the plastic groove added with 5% BSA to prepare the primary antibody solution of corresponding concentration overnight at 4 °C. The membrane was washed with TBST three times, 10 min each time. Then the membrane was incubated with the secondary antibody solution at 4 °C for 4 h. The membrane was washed with TBST 3 times, 10 min each time. The membrane was immersed in electrochemiluminescence developer (wbkls0100, Merck Millipore, Billerica, MA, USA) for visualization. The relative optical density (OD) of all immunoblotting bands was analyzed. The antibodies (Abcam Inc., Cambridge, MA, USA) included primary antibodies E-cadherin (1:30000, ab40772), N-cadherin antibody (1:100, ab18203), Vimentin (1:3000, ab92547), GAPDH (1:2500, ab9485) and corresponding horseradish peroxidase labeled secondary antibody (1:50000, ab205718).

Dual luciferase reporter gene assay

The relationship among lncRNA NEAT1, miR-579 and MMP13 was detected by dual luciferase reporter gene assay. The wild type (WT)/mutant type (MUT)-lncRNA NEAT1 and WT/MUT-MMP13 3'UTR including the binding site regarding miR-579 were synthetized by GenePharma (Shanghai, China) and subcloned to pMIR-REPORTTM vector (Thermo Fisher Scientific Inc., Waltham, MA, USA) [26]. The activity intensity of luciferase was detected by Dual-Luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA).

Cell counting kit-8 (CCK-8) assay

After the cells were treated with 10 μL/well CCK-8 solution (Beyotime Biotechnology Co., Ltd., Shanghai, China), the cells were cultured at 37℃ for 2 h, and then the OD at 490 nm was measured with BioTek instruments.

Colony formation assay

The transfected cells were treated with trypsin and then collected and added to the 6-well plate (200 cells/well). The cells were cultured in Dulbecco's modified Eagles Medium containing 10% FBS for 2 weeks under the external environment of 37℃ and 5% CO_2. The medium was changed regularly once every 3 days. Cells were fixed with methanol for 15 min at room temperature, stained with 1% crystal violet for 30 min at room temperature, and images (including > 50 cells/colony) were captured with an optical microscope (magnification, × 100; Olympus Optical Co., Ltd, Tokyo, Japan). Finally, the number of visible colonies was counted manually.

Statistical analysis

Data were analyzed using SPSS 21.0 (IBM Corp. Armonk, NY, USA). Data were in normal distribution according to Kolmogorov-Smirnov method and described as mean ± standard deviation. Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA) or two-way ANOVA. Tukey's multiple comparisons test was used for the pairwise comparison after ANOVA analysis. p was obtained by two-tailed test and $p < 0.05$ was considered statistically significant.

Results

LncRNA NEAT1 is highly expressed in OS cells and acts as an oncogenic factor

In TCGA (https://www.cancer.gov/) and GTEX database (https://www.gtexport.org/home/index.html), we obtained the expression of lncRNA NEAT1 in OS and normal muscle tissues. We found that the content of lncRNA NEAT1 in OS was much higher than that in normal tissues (Fig. 1A). Compared with osteoblastic cell line (hFOB), the expression of lncRNA NEAT1 in OS cell line (MG-63, U2OS) was significantly increased detected by RT-qPCR (Fig. 1B).

In order to verify the role of lncRNA NEAT1 in OS, we determined its role through a control experiment. First, sh-lncRNA NEAT1 was transfected into MG-63 and U2OS cells. The expression of lncRNA NEAT1 was measured by RT-qPCR, and the transfection efficiency of lncRNA NEAT1 was obtained (Fig. 1C). The migrating and invading rates were measured by Transwell assay, and the expression of proteins (E-cadherin, N-cadherin, vimentin) related to EMT process was detected by Western blot analysis. We found that cells transfected with sh-lncRNA NEAT1 showed decreased migrating and invading rates, declined expression of N-cadherin and vimentin, and increased E-cadherin expression. These results suggested that lncRNA NEAT1 promoted the OS cell migration, invasion and EMT.

LncRNA NEAT1 is sub-localized in the cytoplasm

In http://lncatlas.crg.eu/, it is predicted that LncRNA NEAT1 was located in the cytoplasm (Fig. 2A). In order to further verify whether LncRNA NEAT1 was located in the cytoplasm, we conducted FISH experiments and nuclei isolation experiments in MG-63 and U2OS cells (Fig. 2B-C). The results showed that LncRNA NEAT1 was located in the cytoplasm of OS cells.

LncRNA NEAT1 works as a molecular sponge for miR-579 in OS

Through the analysis of bioinformatics website (http://starbase.sysu.edu.cn/), it was found that there was a potential target relationship between lncRNA NEAT1 and miR-579 (Fig. 3A).

RT-qPCR was used to measure the expression of miR-579 in OS and adjacent normal tissues. We found that miR-579 significantly decreased in OS tissues. Compared with human osteoblastic cell line (hFOB), OS cells (MG-63 and U2OS) showed lower miR-579 expression (Fig. 3B).

The expression of miR-579 was detected in MG-63 and U2OS cells transfected with miR-579 inhibitor/mimic by RT-qPCR. The results showed that miR-579 was effectively transfected (Fig. 3C), and inhibited cell migration, invasion and EMT of OS (Fig. 3D-E).

We found that in MG-63 and U2OS cells, the expression of miR-579 increased significantly when the expression of lncRNA NEAT1 decreased (Fig. 3F). At the same time, miR-579 mimic had obvious effect on WT-lncRNA NEAT1 but not on MUT-lncRNA NEAT1 (Fig. 3G). The results demonstrated that there was a negative regulation between lncRNA NEAT1 and miR-579.

miR-579 negatively targets MMP13

Through the analysis of bioinformatics website (http://starbase.sysu.edu.cn/), it was found that there was a potential target relationship between MMP13 and miR-579 (Fig. 4A).

RT-qPCR was used to measure the expression of MMP13 in OS and adjacent normal tissues. We found that MMP13 significantly increased in OS tissues. Compared with human osteoblastic cell line (hFOB), OS cells (MG-63 and U2OS) showed higher MMP13 expression (Fig. 4B).

The expression of miR-579 and MMP13 was detected in MG-63 and U2OS cells transfected with sh-lncRNA NEAT1 1#/sh-lncRNA NEAT1 2# by RT-qPCR. The results showed that miR-579 was upregulated while MMP13 was downregulated (Fig. 4C). miR-579 mimic had obvious effect on WT- MMP13 but not on

MUT- MMP13 (Fig. 4D). The results demonstrated that there was a negative regulation between miR-579 and MMP13.

LncRNA NEAT1 functions as a ceRNA to up-regulate MMP13 expression via miR-579 in OS

In order to verify the mechanism of lncRNA NEAT1-miR-579-MMP13 axis in OS, MG-63 and U2OS cells were transfected with sh-NC, sh-lncRNA NEAT1 1#, sh-lncRNA NEAT1 1# + miR-control, sh-lncRNA NEAT1 1# + miR-579 inhibitor, respectively. Analysis was carried out by colony formation assay and Transwell assay, Western blot analysis and RT-qPCR (Fig. 5A-D). The inhibition effect of lncRNA NEAT1 alone was the strongest, the number of tumor cell colony and cell proliferation was the least, the inhibition of EMT process was the strongest, and the expression level of MMP13 was the lowest. The inhibition of lncRNA NEAT1 and miR-579 increased the number of tumor cell colony and cell proliferation, the met process also improved, and the expression level of MMP13 increased. To sum up, we determined the regulation relationship among the LncRNA NEAT1-miR-579-MMP13 axis. LncRNA NEAT1 promotes the development of OS by sponging miR-579 and upregulating MMP13.

Discussion

OS is a common aggressive mesenchyme-derived bone tumor ranked among the top causes of cancer-related death in the pediatric populations [27]. The cancer's large tumor heterogeneity and its low prevalence make it tough to gain meaningful progress in patient's survival [28]. Dysregulated lncRNAs have been confirmed to be relevant to the disease development [29]. Recent studies suggested that lncRNAs might serve as effective therapeutic targets for OS treatment [15, 18, 30]. In this study, we found that lncRNA NEAT1 was highly expressed in OS in related to the counterparts. Knockdown of lncRNA NEAT1 inhibited OS cell migration, invasion and EMT. We also revealed that lncRNA NEAT1 accelerated OS development through the up-regulation of MMP13 via sponging miR-579. Briefly, the findings of this study indicated that lncRNA NEAT1 was a vital regulator in the OS progression by targeting the miR-579/MMP13 axis (Fig. 6–7).

Our study presented overexpression of lncRNA NEAT1 in OS cells when compared to the normal osteoblasts. In addition, downregulation of lncRNA NEAT1 expression evidently reduced proliferative, invasion and EMT capacities, suggesting that lncRNA NEAT1 was a considerable regulator in the OS cell growth as an oncogene. NEAT1 is a newly acknowledged nuclear-restricted lncRNA, which has obtained a reputation as a regulator transcriptionally for numerous genes [15]. Recently, different studies indicated that lncRNA NEAT1 dysregulation boosted tumorigenesis in a variety of human malignancies, including OS. For example, Ji et al found that lncRNA NEAT1 participated in the development of OS as a ceRNA sponging to miR-34a-5p and thus regulate HOXA13 expression [29]. Hu et al demonstrated that knockdown of lncRNA NEAT1 sensitized the OS cells to cisplatin-induced tumor progression, delayed the tumor growth, suggesting that lncRNA NEAT1 is an oncogene and chemotherapy resistant factor in OS

[31]. The results showed similarity with that in other studies. Therefore, explorations on the influence of lncRNA NEAT1 on accelerating OS cell growth are of great significance for in-depth studies of OS.

Through isolation of cytoplasm and nucleus, we confirmed that lncRNA NEAT1 was dominantly distributed in the cell cytoplasm, indicating that lncRNA NEAT1 might serve as a ceRNA. It is well-known that abnormal expression of lncRNAs could work as ceRNAs for miRNA so as to modulate tumor progression [29]. Subsequently, bioinformatics and dual luciferase reporter gene assay clarified the binding relation between lncRNA NEAT1 and miR-579. The role of miR-579 in cancer cells is gradually but not fully investigated yet. A study indicated that interrupting miR-579 binding affinity may increase the risk of breast cancer [23]. miR-579 was obviously associated with overall survival and tumor-specific survival of patients with locally advanced rectal cancer [32]. Upregulation of miR-579 could block proliferation, cell cycle, migration and also trigger the apoptosis of glioblastoma multiform cells [24]. Our study demonstrated that miR-579 was downregulated in OS cells, and transfection with miR-579 mimic inhibited the proliferative, invasion, and EMT capabilities of OS cells, which could be reversed by lncRNA NEAT1 overexpression. We believed that both lncRNA NEAT1 and miR-579 may participate in the occurrence and progression of OS.

Then, bioinformatics tools were adopted to predict the candidate targets of miR-579, and found that MMP13 was a downstream target of miR-579. MMP13 is a crucial MMP family member, and plays a vital role in invasion and metastasis of tumor cells through degrading the extracellular matrix [33]. Previous studies showed that MMP13 could act as a tumor oncogene in OS progression [34–36]. Thus, in the present study, we detected whether MMP13 could act as a target of miR-579 in OS. Consequently, the results revealed that miR-579 could directly target MMP13 and negatively modulate the expression of MMP13 in OS cells. Furthermore, lncRNA NEAT1 can modulate MMP13 expression by sponging miR-579 in vitro. Interesting, a relevant reported that lncRNA NEAT1 knockdown could reduce the MMP13 expression in osteoarthritis [37]. Accordingly, our study testified that lncRNA NEAT1 upregulated MMP13, the target gene of miR-579, further leading to abnormal proliferation, invasion and EMT of OS cells.

Conclusion

To sum up, lncRNA NEAT1 functioned as a ceRNA to regulate MMP13 expression by sponging miR-579, thus regulating the development of OS. Further studies are suggested to clear the exact effect of lncRNA NEAT1-miR-579-MMP13 axis on OS in animal models.

Abbreviations

lncRNAs, long non-coding RNAs; miRNAs, microRNAs; OS, osteosarcoma; MMP13, matrix metallopeptidase 13; NEAT1, Nuclear enriched abundant transcript 1; NC, negative control; RT-qPCR, reverse transcription quantitative polymerase chain reaction; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; FISH, Fluorescence in situ hybridization; EMT, epithelial-mesenchymal transition; TBST, Tris-buffered saline Tween-20; BSA, bovine serum albumin; OD, optical density; WT, wild type; MUT,

mutant type; CCK-8, Cell counting kit-8; ATCC, American Type Culture Collection; FBS, fetal bovine serum; ANOVA, analysis of variance.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

LLW conceived the study and participated in its design and coordination. JZZ performed all experiments. YZ and TH collected tissue samples and clinical data. YNS analyzed and interpreted the data. The draft was improved through discussion and editing by all the authors who read and approved the final manuscript.

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Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

All patients provided written informed consent and this study was approved by the Medical Ethics Committee of Ningbo Sixth Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

LncRNA NEAT1 is highly expressed in OS with tumor-promoting effects. A, the expression of lncRNA NEAT1 in 396 normal muscle tissues from the GTEX database and 265 OS tissues from the TCGA database (unpaired t-Test, *p < 0.05). B, lncRNA NEAT1 expression in human osteoblastic cell line (hFOB) and OS cell lines (MG-63, U2OS) detected by RT-qPCR (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). C, MG-63 and U2OS were co-transfected with sh-lncRNA NEAT1 1#, sh-lncRNA NEAT1 2#, and sh-lncRNA NEAT1 3#, respectively. The expression of lncRNA NEAT1 was measured by RT-qPCR to measure the transfection efficiency. D, MG-63 and U2OS were co-transfected with sh-lncRNA NEAT1 1#, and sh-lncRNA NEAT1 2#, respectively. Migrated and invaded cells were counted by Transwell assay (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). E, MG-63 and U2OS were co-transfected with sh-lncRNA NEAT1 1#, and sh-lncRNA NEAT1 2#, respectively. The expression of E-cadherin, N-cadherin and Vimentin was detected by Western blot analysis (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05).

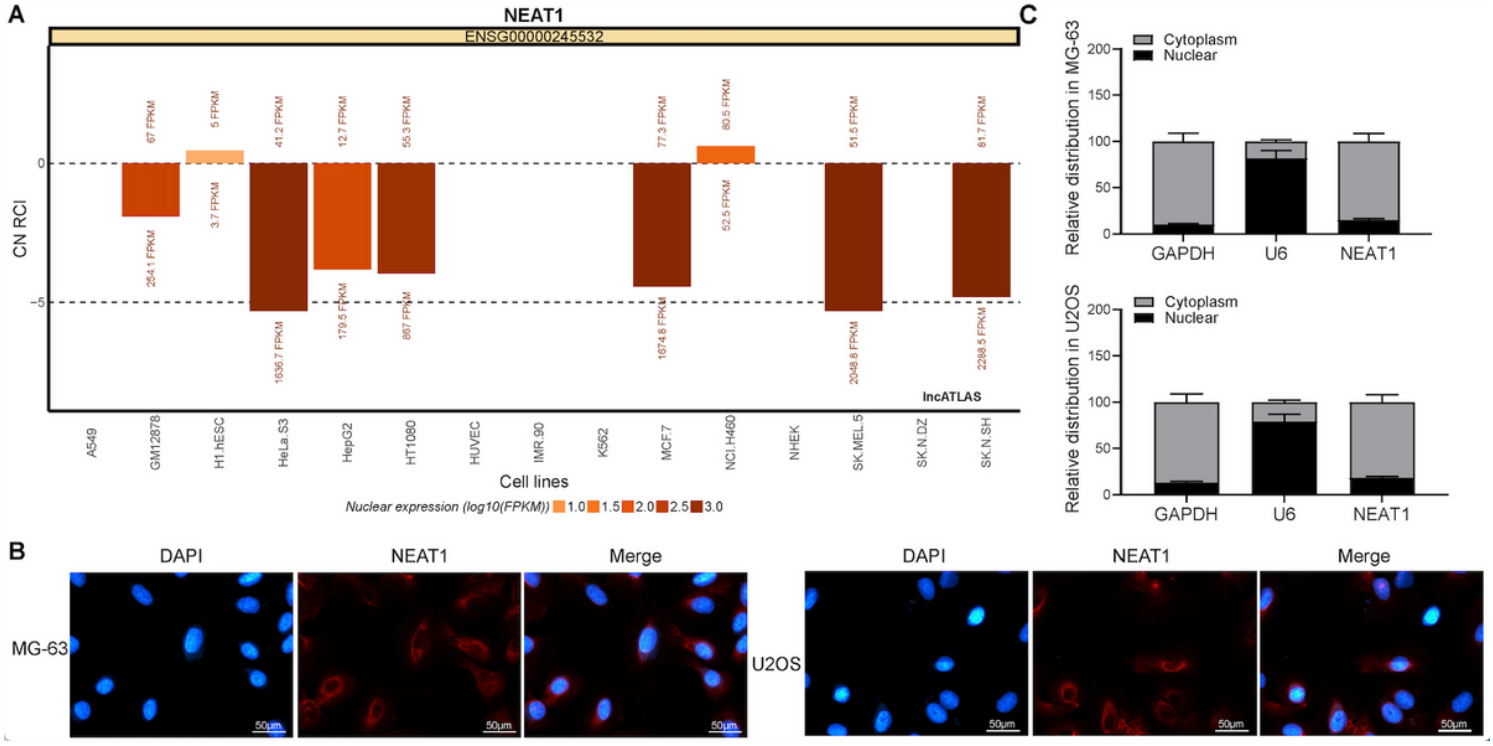

Figure 2

LncRNA NEAT1 is located in the cytoplasm of OS cells. A, lncRNA NEAT1 was predicted located in the cytoplasm by http://lncatlas.crg.eu/. B, FISH assay in MG-63 and U2OS cells. C, cytoplasmic and nuclear isolation in MG-63 and U2OS cells (the data were analyzed by two-way ANOVA, n = 3).

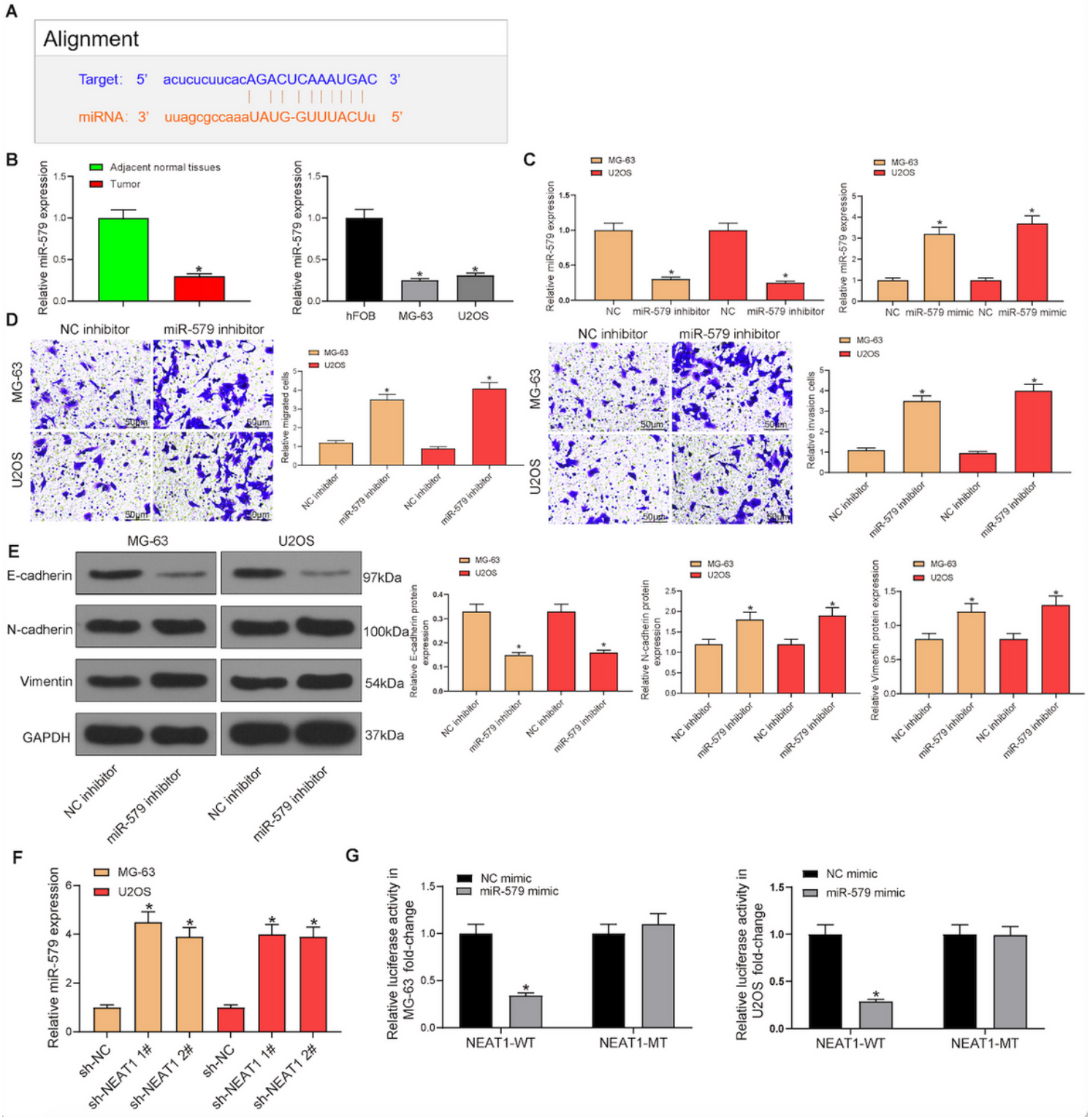

Figure 3

LncRNA NEAT1 negatively regulates miR-579. A, potential binding sequence of miR-579 and lncRNA NEAT1. B, miR-579 expression in OS and normal tissues (unpaired t test, n = 3, *p < 0.05), human osteoblastic cell line (hFOB), OS cells (MG-63 and U2OS) (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05) detected by RT-qPCR. C, miR-579 expression in MG-63 and U2OS cells transfected with miR-579 inhibitor/mimic detected by RT-qPCR (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). D, cell migration and invasion in MG-63 and U2OS cells transfected with miR-579 inhibitor detected by Transwell assay (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). E, expression of E-cadherin, N-cadherin and Vimentin in MG-63 and U2OS cells transfected with miR-579 inhibitor detected by Western blot analysis (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). F, miR-579 expression in MG-63 and U2OS cells transfected with sh-lncRNA NEAT1 1# and sh-lncRNA NEAT1 2# detected by RT-qPCR (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). G, MG-63 and U2OS cells transfected with WT-lncRNA NEAT1/MUT-lncRNA NEAT1 and miR-579 mimic, respectively. The fluorescent enzyme activity was detected by dual luciferase reporter gene assay (the data were analyzed by two-way ANOVA, n = 3, *p < 0.05).

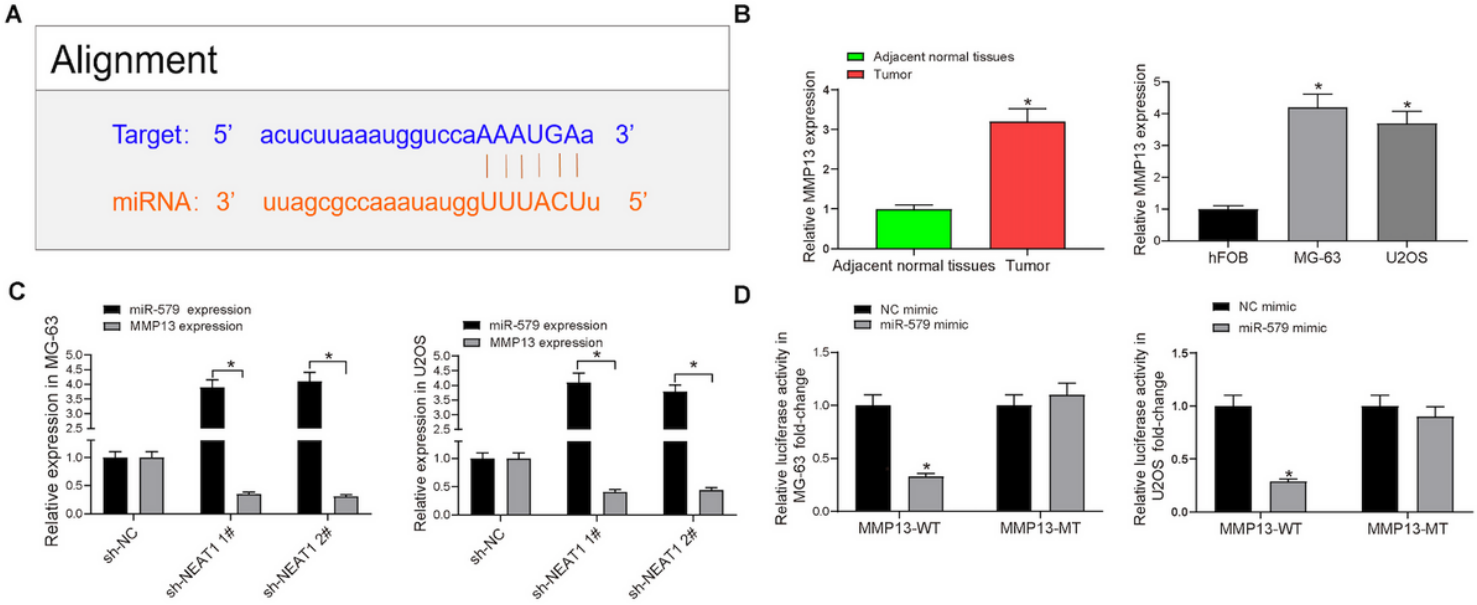

Figure 4

MMP13 is a target gene of miR-579. A, potential binding sequence of miR-579 and MMP13. B, MMP13 expression in OS and normal tissues (the data were analyzed by unpaired t test, n = 3, *p < 0.05), human osteoblastic cell line (hFOB) and OS cells (MG-63 and U2OS) (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05) detected by RT-qPCR. C, miR-579 and MMP13 expression in MG-63 and U2OS cells transfected with sh-lncRNA NEAT1 1#/sh-lncRNA NEAT1 2# detected by RT-qPCR (the data were analyzed by two-way ANOVA, n = 3, *p < 0.05). D, MG-63 and U2OS cells transfected with MMP13-WT/MMP13–MUT and miR-579 mimic, respectively. The fluorescent enzyme activity was detected by dual luciferase reporter gene assay (the data were analyzed by two-way ANOVA, n = 3, *p < 0.05).

Figure 5

LncRNA NEAT1-miR-579-MMP13 axis regulates OS progression. MG-63 and U2OS cells were transfected with sh-NC, sh-lncRNA NEAT1 1#, sh-lncRNA NEAT1 1# + miR-control, sh-lncRNA NEAT1 1# + miR-579 inhibitor, respectively. A, Colony number (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05) and proliferating cell number (the data were analyzed by two-way ANOVA, n = 3, *p < 0.05) were determined by colony formation and CCK-8 assays. B, migrated and invaded cell number determined by Transwell assay (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). C, the expression of E-cadherin, N-cadherin and Vimentin detected by Western blot analysis (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05). D, MMP13 expression detected by RT-qPCR (the data were analyzed by one-way ANOVA, n = 3, *p < 0.05).

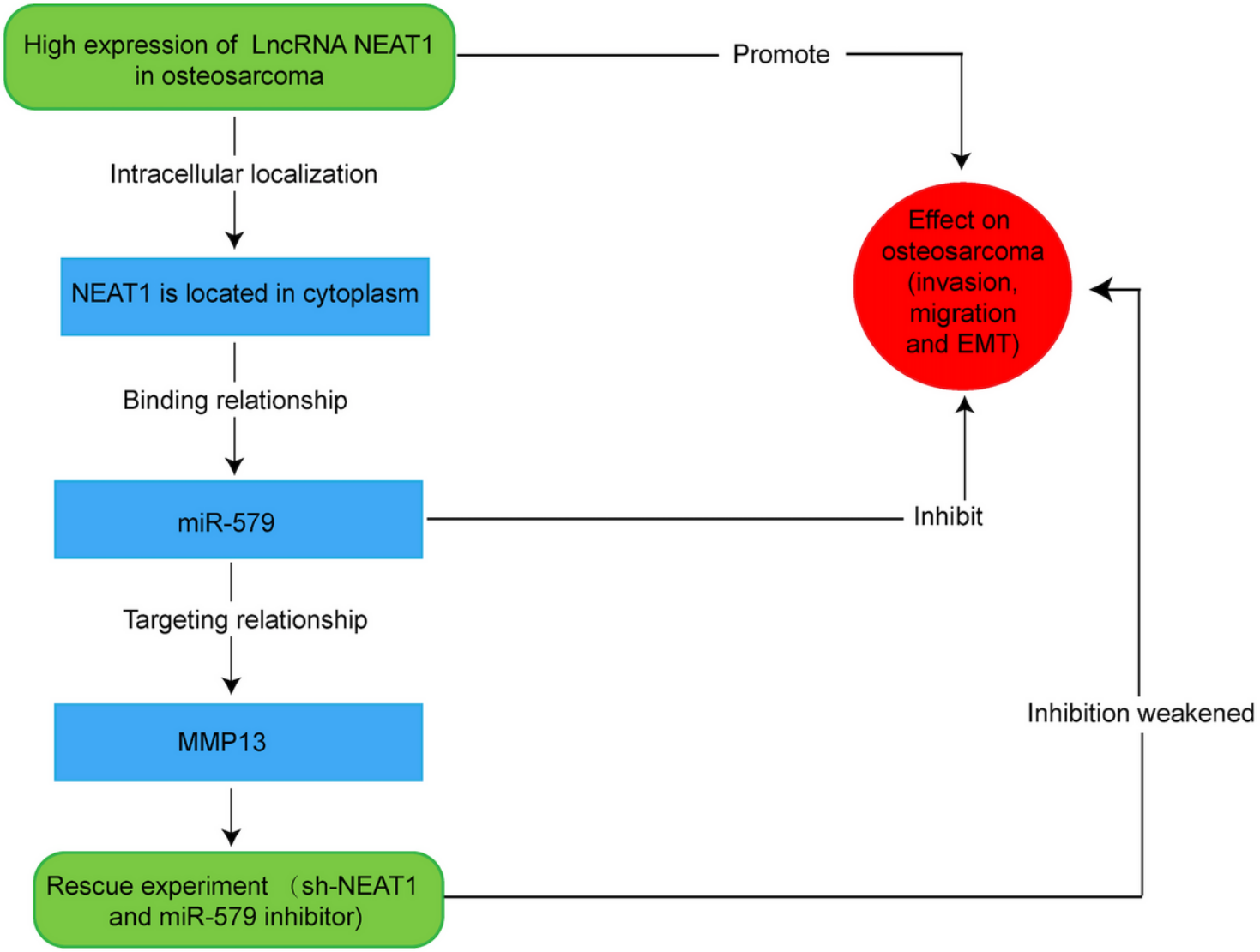

Figure 6

Flow chart of the experiment design.

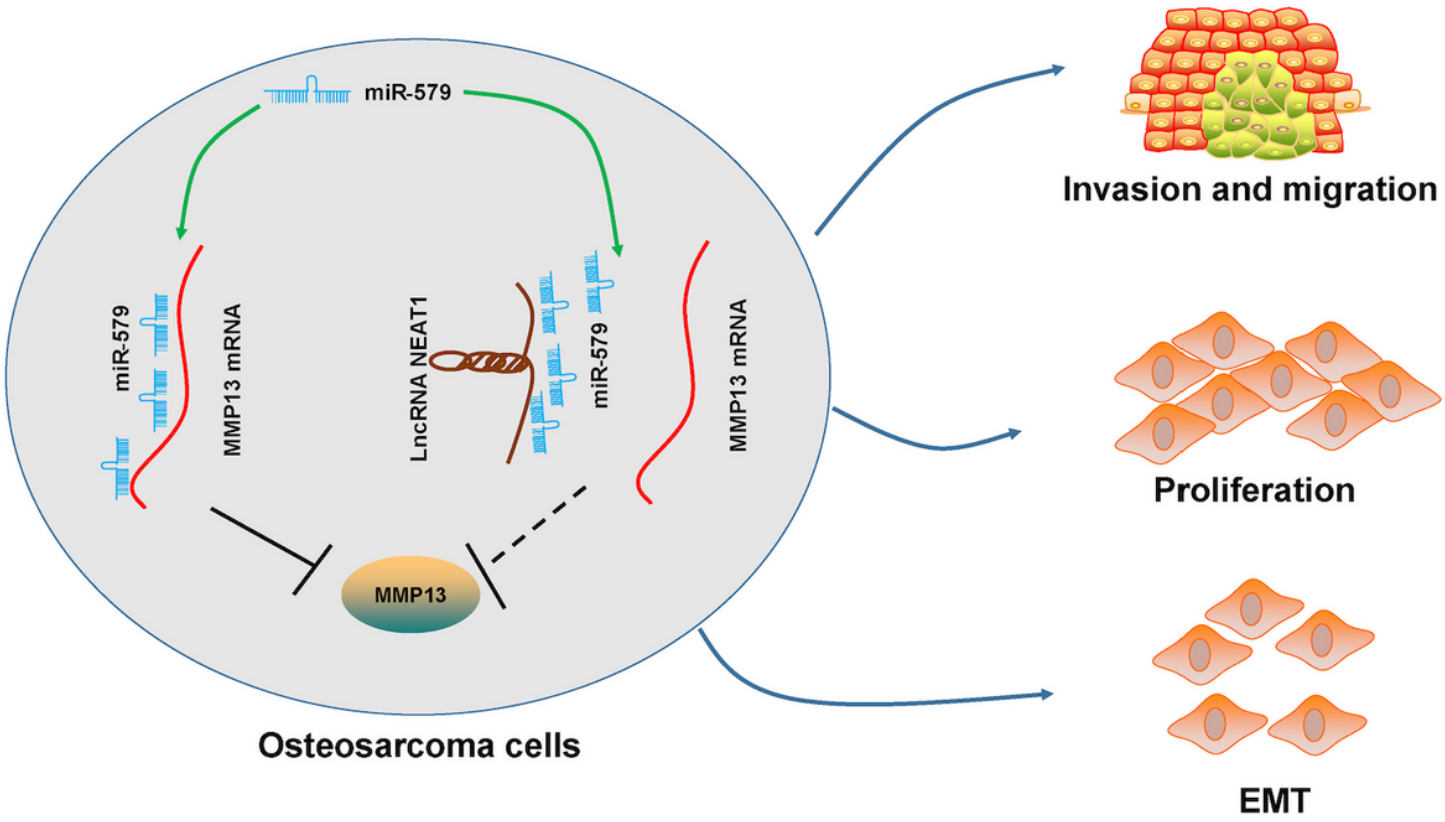

Figure 7

The possible mechanism of lncRNA NEAT1 regulates OS progression.