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Original Article

miR-1297 inhibits osteosarcoma cell proliferation and growth by targeting CCND2

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Abstract: Cyclin D2 (CCND2) is abnormally overexpressed in many tumor types and has been associated with tumor cell proliferation. Although the important role of miR-1297 is well established, the molecular mechanism between CCND2 and miR-1297 in osteosarcoma (OS) has not been determined. In the present study, we found CCND2 was highly expressed in OS cells, and its downregulation suppressed cell proliferation, resulting in G1 phase cell cycle arrest. In contrast, miR-1297 was lowly expressed in OS compared to normal tissue. Several data platforms predicted that CCND2 was a target of miR-1297, which was validated by a dual-luciferase reporter assay that revealed miR-1297 could bind with CCND2-3'UTR. miR-1297 overexpression greatly inhibited CCND2 protein expression and exerted the same phenotypic effect as CCND2 downregulation in OS cells. Furthermore, miR-1297 inhibition could also be rescued by CCND2. Nude mice injected cells stable overexpressing miR-1297 OS cells showed lower size and tumor weight. Moreover, lower fluorescence activity recorded by in vivo imaging system and bone erosion revealed by microCT in the miR-1297 group demonstrated miR-1297 inhibited OS tumor growth via CCND2. Our findings demonstrated that miR-1297 can inhibit proliferation and tumor growth in OS by directly targeting CCND2, which indicates that miR-1297 may represent a novel therapeutic target for OS.

Keywords: Osteosarcoma, microRNA, miR-1297, tumor growth, cell proliferation, cell cycle, cyclin D2

Introduction

Osteosarcoma (OS) is a malignant bone tumor that is primarily treated using chemotherapy and surgery [1]. However, the 5-year survival rate is decreased from 60% to 28% in patients with metastatic disease [2]. To date, the mechanisms underlying OS proliferation remain unclear and further research is necessary to further our understanding.

Cyclin D2 (CCND2) was first reported to participate in testicular and ovarian tumor development [3]. Then, it was discovered that CCND2 is a key regulator of cell proliferation and has since been widely investigated in hematological malignancies [4, 5]. Recent studies have also demonstrated CCND2 plays an essential role in solid cancers and its dysfunction accelerates tumor development. For example, CCND2 regulates and promotes cell cycle progression in colorectal cancer stem cells, and abnormal methylation of CCND2 is closely associated with prostate tumor development [6, 7]. Notably, abnormal CCND2 methylation in pancreatic cancer is closely associated with tumor prognosis [8]. CCND2 overexpression can accelerate the G1/S phase cell cycle transition. Also, studies have demonstrated CCND2 inhibitors can inhibit tumor proliferation and growth [5, 9]. Gene expression analysis from clinical samples confirmed CCND2 is highly expressed in Hodgkin's lymphoma, and subsequent in vitro experiments revealed CCND2 can be activated by aberrant NF-κB pathway activity thereby maintaining the progressive proliferation of Hodgkin's lymphoma [10]. Similarly, abnormal CCND2 expression in colon adenoma tissue revealed that CCND2 may be related to colorectal tumorigenesis [11]. Also, tumor growth in vivo can be inhibited by CCND2 downregulation in melanoma and multiple myeloma

[12, 13]. CCND2 also serves as an important indicator of cell proliferation [14, 15].

Since microRNAs have been identified, a variety of different biological roles have been identified, such as affecting phenotypes through gene regulation and acting as tumor suppressors or oncogenes [16-18]. DNA is transcribed and processed to become pri-miRNA, which finally becomes miRNA in the cytoplasm and plays an important role in regulating target genes [19]. It is well established that microRNAs are dysregulated in various cancers [20].

The main mechanisms of miRNA are to bind to specific fragments of targeted mRNA, which are mainly located in the 3'-UTR region, and regulate gene expression [21, 22]. For instance, miRNA-192 suppresses proliferation via targeting retinoblastoma 1 (RB1) in lung cancer cells [23]. miR-155 promotes cell proliferation by targeting gamma-aminobutyric acid receptor type A subunit alpha 1 (GABRA1) in glioblastoma [24]. Several studies demonstrated that miR-1297 is significantly reduced and functions as a tumor suppressor by regulating its target genes. Astrocyte elevated gene-1 (AEG-1), an oncogene, is also a target of miR-1297 in prostate cancer [25]. Also, miR-1297 can downregulate expression of its target gene high mobility group protein A1 (HMGA1) by pairing with its 3'-UTR, which significantly inhibits glioma cell growth in vitro and in vivo [26]. Chen *et al.* demonstrated that miR-1297 can suppress pancreatic cancer cell proliferation by targeting MTDH [27]. Wang *et al.* revealed miR-1297 can suppress colorectal cancer cell proliferation by modulating CCND2 [28] Conversely, Gao *et al.* demonstrated that miR-1297 is related to gastric cancer prognosis, and may promote tumor growth by targeting CREB1 [29]. miR-1297 is associated with the MALAT1/miR-1297/HMGB2 axis, which plays a crucial regulatory role in the occurrence and development of gastric cancer. MALAT1 is negatively correlated with miR-1297 and acts as a molecular sponge of miR-1297, antagonizing its ability to inhibit HMGB2 expression [30]. It has been reported that microRNAs are involved in CCND2 regulation by targeting its 3'-UTR, thus inhibiting tumor proliferation [13, 31]. CCND2 was identified as a miR-195 target in cervical cancer [32]. miR145 is a key regulator of the epithelial ovarian cancer cell biological behavior by targeting CCND2 [33]. In the present study, we demonstrated that miR-1297 inhibits proliferation and tumor growth in OS via targeting CCND2.

Material and methods

Cell lines and cell culture

OS cell lines (hFOB 1.19, HOS, U2OS, MG63, 143B) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (100 U/mL penicillin and 100 μg/mL streptomycin) at 37°C under 5% CO_2.

Clinical sample

One normal and sixty-four OS samples were collected from patients at the Affiliated Hospital of Jiangsu University (Jiangsu, China). The OS patients did not receive chemotherapy or preoperative anticancer treatments. The histological feature of each specimen was diagnosed by two pathologists using hematoxylin and eosin staining. These samples were maintained at -80°C prior to use. Informed consent was written by all patients. The current study was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Jiangsu University.

RNA extraction and qRT-PCR

Total RNA from tissue samples and cell lines was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). RNA quality and quantity were determined by spectrophotometric analysis using Nanodrop 1000 (Nanodrop; Thermos Fisher Scientific, Waltham, MA). Expression of gene cDNA and mature miRNA were separately analyzed using a PrimeScript RT Master Mix Kit (TaKaRa Bio, Japan) and using Taqman MiR Assay (Ribobio, China) in a Stem-Loop RT-PCR assay. β-actin and U6 snRNA were set as internal references to normalize the RNA expression. qRT-PCR was performed using an Applied Biosystems 7900 Real-time PCR System (Applied Biosystems, Foster City, USA). All the gene and miR Taqman qRT-PCR specific probes are commercially available from Ribobio. The primer sequences used for all experiments are listed: β-actin-F: CATGTACGTTG-

CTATCCAGGC, β-actin-R: CTCCTTAATGTCACGCACGAT; CCND2-F: TTTGCCATGTACCCACCGTC, CCND2-R: AGGGCATCACAAGTGAGCG.

Cell transfection and plasmid construction

All of the plasmid transfections were performed using Lipofectamine TM 3000 reagent (Invitrogen, Carlsbad, CA), and all RNAs were transfected with Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's introductions. The CCND2 expression vector was constructed by cloning the PCR product into a pcDNA3.1 vector (GenePharma, Suzhou, China). We investigated the transfection efficiency 48 hours after transfection by western blotting and qRT-PCR.

Luciferase reporter assay

The mutant or wild-type of 3'-UTR of CCND2 was constructed into a GP-miRGLO luciferase reporter vector (GenePharma, Suzhou, China). The vectors were cotransfected with miR-1297 or an inhibitor and control into HOS and U2OS. Luciferase activity was detected by the Dual-Luciferase Reporter Assay System (Promega, USA) 48 hours after cotransfection.

Western blotting assays

Protein obtained from cells or tissue was extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with a protease inhibitor cocktail (1 ml each of PMSF, leupeptin, aprotinin, pepstatin, and Aphosphatase inhibitors). (KangCheng, Shanghai, China). Protein concentration was quantified using BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Proteins were separated by electrophoresis on SDS-PAGE gels (10%) and electrotransferred onto PVDF membranes (Millipore, Bedford, MA). The membranes were blocked in 5% skimmed milk, then incubated with primary antibodies against CCND2 (67048; Proteintech), and β-actin (66009; Proteintech) used as the internal control at 4°C overnight. Then, the membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated (HRP) secondary antibody IgG at room temperature for an hour. Finally, images were captured by AFP-Imaging System (Tanon, China) and analyzed using Tanon software (Tanon, China).

Animal experiments

To investigate the effects of miR-1297 in vivo, HOS cells stably overexpressing miR-1297 or a control and pcDNA3.1-CCND2 or a control were subcutaneously and locally injected into nude mice, and tumor growth was evaluated (volume = (longest diameter × shortest diameter^2)/2). To evaluate subcutaneous tumor growth, 2 × 10^6 cells in 100 μl of PBS were subcutaneously injected into 4-week-old male nude mice (eight mice per group). Mice were weighed and tumor sizes were recorded every three days by vernier caliper measurement and the mice were sacrificed with more than 3 times the anesthetic dose of pentobarbital sodium after 4 weeks. To evaluate limb local tumor growth, we injected 2 × 10^6 cells in 100 μl PBS into the cavity of tibia marrow of 4-week-old male nude mice (eight mice per group). Similarly, mice were weighed and tumor sizes in the largest perimeter of the limb was recorded every three days. Notably, we counted the subcutaneous and local tumor weight and volume after 4 weeks. In vivo imaging system (IVIS) (Bruker, USA) was used to detect fluorescence activity. Then, local tumors were collected and microCT was performed to detect bone erosion. All animals were obtained from Gempharmatech (Nanjing, China) and received humane care in compliance with the Animal Experiment protocol of the Animal Research Committee of Jiangsu University.

Histopathologic assessment

First, 4% buffered paraformaldehyde was used to fix the tissue species for 36 to 48 hours, and then decalcification was performed with buffered EDTA (20% EDTA [pH 7.4]). Next, tissue sections were treated with trypsin (0.05%) for 8 min and then treated with 3% (vol/vol) H_2O_2 for 15 min. The sections were blocked with 10% goat serum at room temperature for at least an hour. After washing with PBS for 30 min, the sections were incubated with anti-CCND2 antibody (1:100 dilution) at 4°C overnight. Following a PBS wash, the sections were incubated with biotinylated secondary antibody using a Histostain Plus kit (Invitrogen, Carlsbad, CA, USA). Lastly, the sections were washed and incubated with 3,30-diaminobenzidine (DAB) substrate for 2 min.

Flow cytometry analysis and EdU assay

Flow cytometry analysis was performed 48 hours after transfection. Following trypsin detachment, the cells were washed with PBS and fixed in 70% ethanol at 4°C for 24 h, and then conducted according to the manufacturer's instructions (BD Biosciences, MA, USA). The final mixture was analyzed using a FACS Calibur flow cytometer (BD Biosciences, MA, USA). The results were analyzed by ModFit software (BD Biosciences). All OS cells were treated with 50 μM EdU in 96-well plates according to the manufacturer's protocol (Riobio). Hoechst was used to counterstain the cell nuclei, then epifluorescence microscopy was applied to record results. ImageJ was used for analysis.

Statistical analysis

The data of multiple experiments are presented as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; ns: non-significance. Statistical analyses were performed using SPSS 19.0 software to analyze significant differences between treatment and control groups. *P*-values were calculated using student's t-test and one-way analysis of variance (ANOVA). *P*-value < 0.05 was considered to be statistically significant.

Results

Relative expression of miR-1297 and CCND2 in clinical samples and cell lines

To investigate the relative expression of miR-1297, qRT-PCR revealed that miR-1297 expression was significantly lower in OS cell lines U2OS, HOS, and MG63 compared to osteoblast cell line hFOB1.19 cells (P < 0.05, **Figure 1A**). Thus, these results suggest miR-1297 expression levels are relatively low in OS. To further explore the mechanisms of miR-1297 in OS, we utilized several databases (miRDB, miRbase, mirDIP) to predict targets of miR-1297. We identified CCND2, which is an established gene in cell cycle regulation and its dysregulation facilitates tumor growth, as one of the potential target genes. Using qRT-PCR, western blotting, and immunohistochemical staining (IHC) we revealed that the relative expression of CCND2 in OS was highly expressed (P < 0.05), as shown in **Figure 1B-E**. Also, IHC was performed to assess CCND2 expression in different lesions in OS patients, and the results demonstrated that CCND2 was highly expressed in other lesions beyond the femur/tibia (**Table 1**).

miR-1297 inhibited osteosarcoma cell proliferation

Because of their lower CCND2 expression, HOS and U2OS cell lines were selected to perform the following experiments. To determine the effect of miR-1297 on cell proliferation, we performed an EdU assay and found proliferation cells transfected miR-1297 mimic was obviously suppressed, whereas the miR-1297 inhibitor showed opposite effects (**Figure 2A** and **2B**). Besides, we performed cell cycle analysis to analyze the possible mechanism of miR-1297 on OS cell proliferation. The percentages of HOS and U2OS cells in the G1 phase obviously increased compared with the controls when miR-1297 was upregulated (**Figure 3A** and **3B**), indicating that overexpression of miR-1297 led to G1 phase arrest in OS cells.

miR-1297 regulated osteosarcoma cell proliferation by targeting CCND2

To reveal the mechanism between miR-1297 and CCND2, we performed dual-luciferase reporter assays and showed that there was a direct binding site between CCND2 and miR-1297. The predicted binding site and mutant sequence of the target gene, CCND2, were constructed separately downstream of the luciferase reporter gene in the PMIRGLO-basic vector. The results indicated that miR-1297overexpression greatly inhibits luciferase reporter activity with wild-type CCND2 3'UTR, whereas the mutant miR-1297 binding site exhibited no effect (P < .05) (**Figures 4** and S1).

When we regulated miR-1297 expression by transfection mimic and control, we found that CCND2 protein showed a significant reduction in both cell lines, but mRNA levels were unchanged. Treatment with the miR-1297 inhibitor increased CCND2 protein levels (**Figure 5**). To further investigate the relationship between miR-1297 and CCND2, miR-1297 and CCND2 were co-transfected, which revealed miR-1297-mediated OS cell proliferation inhibition and CCND2 protein abundance were rescued by CCND2 overexpression (**Figure 6A-D**). Furthermore, flow cytometry analysis also

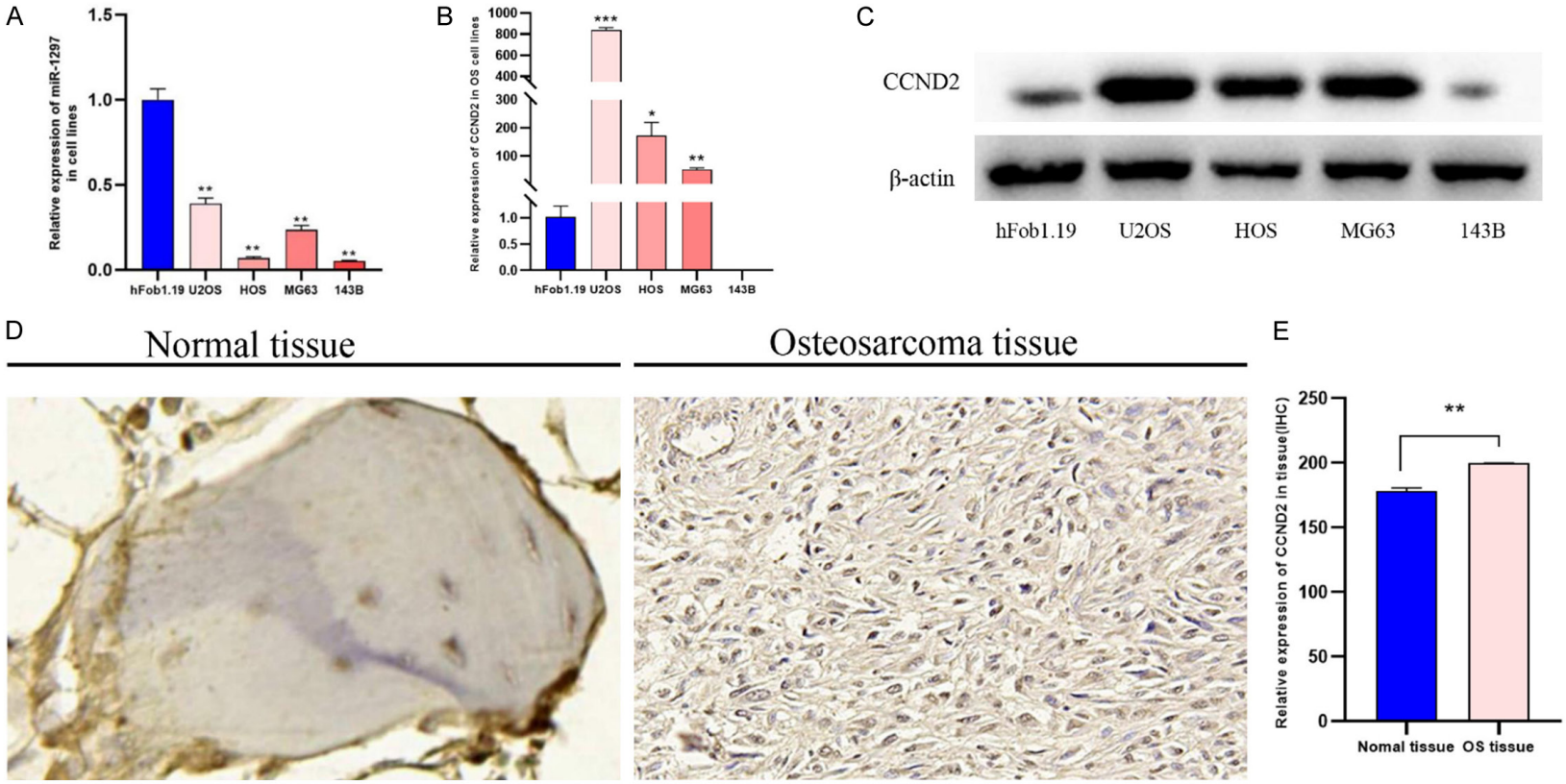

Figure 1. Relative expression of miR-1297 and CCND2 in OS. A. miR-1297 was downregulated in four OS cell lines detected by qRT-PCR compared to hFOB1.19 osteoblast cells. B, C. qRT-PCR and western blotting showed that CCND2 is highly expressed in three OS cell lines, except for 143B. D, E. Immunohistochemical assays showed CCND2 was upregulated in OS clinical samples. All the data were presented as the mean ± SD from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ns: non-significance.

Table 1. Correlation between the clinicopathologic characteristisc and CCND2 expression in osteosarcoma (N=64)

Characteristics		CCND expression			
		N	Low N=15	High N=54	P
Sex	Male	41	10	31	0.4804
	Female	23	5	18	
Age, years	<18	25	8	17	0.6453
	≥18	39	7	32	
Tumor stage	I-II	58	13	45	0.3090
	III	6	2	4	
Location	Femur/tibia	45	13	32	0.0400*
	Elsewhere	19	2	17	

*Statistically Significant.

showed that the suppression of miR-1297 on OS cell cycle was rescued by CCND2 overexpression (**Figure 6E** and **6F**).

Additionally, CCND2 knockdown resulted in the same effect as miR-1297 overexpression (**Figure 7**). Taken together, our results demonstrated that miR-1297 repressed cell proliferation by targeting CCND2 in OS cells.

miR-1297 inhibits tumor growth by targeting CCND2 in vivo

To assess the effect of miR-1297 on OS growth in vivo, HOS cells were transfected with either a lentiviral expression vector to overexpress miR-1297, miR-1297, and CCND2, or negative control. OS cells were injected subcutaneously or into the local tibia and the weight and size of the resulting tumors formed were greatly reduced by miR-1297 overexpression (**Figure 8A** and **8B**). Additionally, we performed anatomic and histologic analysis to evaluate the relative expression of CCND2 in these tumors. The tumors formed by miR-1297-overexpressing HOS cells had lower CCND2 expression, as demonstrated by IHC (**Figure 8C** and **8D**). Notably, the group upregulated both miR-1297 and CCND2 indicating that the effect of miR-1297 could be also rescued by overexpression of CCND2 in vivo (**Figure 8**). Also, we estimated the effect of miR-1297 on local OS with IVIS and microCT. The data showed that the miR-1297-overexpressing group displayed lower tumor fluorescence in the limb compared to the control group and the miR-1297 plus CCND2 group (**Figure 8E**). Moreover, microCT revealed the miR-1297-overexpressing group had less bone erosion than the control (**Figure 8F**). Taken together, these findings strongly suggest that miR-1297 targets oncogene CCND2 and functions as an important tumor suppressor to restrain OS tumor growth.

Discussion

Emerging research has revealed that microRNAs are dysregulated in various tumor types and play a vital role in regulating tumor biology [34, 35]. microRNAs function as a regulator by targeting and downregulating genes, which may be transcription factors or key regulators in signal pathways, thereby regulating cell invasion, migration, and proliferation [36, 37]. For instance, miR-152 can inhibit gastric tumor metastasis and improve poor prognosis by targeting the transcription factor E2F3 [38]. Additionally, miR-125b can suppress the PI3K/Akt pathway, which can promote Ewing's sarcoma progression [39]. miR-1297 has been shown to target different genes to either suppress or promote tumor growth. For example, miR-1297 inhibits the growth of several tumor types, including colorectal cancer, pancreatic carcinoma, prostatic cancer, and lung adenocarcinoma, by targeting respective genes [25, 27, 28, 40]. Inversely, miR-1297 can promote cell proliferation in esophageal carcinoma by targeting PTEN and has been related to the prognosis of gastric cancer by downregulating CREB1, which is an antioncogene [29, 41].

In the present study, we investigated the role of miR-1297 in OS proliferation and tumor growth. First, we analyzed miR-1297 expression in OS, and the results showed that miR-1297 was lowly expressed. Then, we searched for target genes of miR-1297 in several data platforms and found that CCND2, a cell cycle regulator, was predicted to be a target of miR-1297. We confirmed that CCND2 was highly expressed in three OS cell lines and tissue. We demonstrated that CCND2 knockdown in OS cells was remarkably inhibited and the cells were arrested in the G1 phase. When miR-1297 was overexpressed in OS cells, the resulting phenotype was observed after CCND2 downregulation. Notably, when we upregulated both CCND2 and

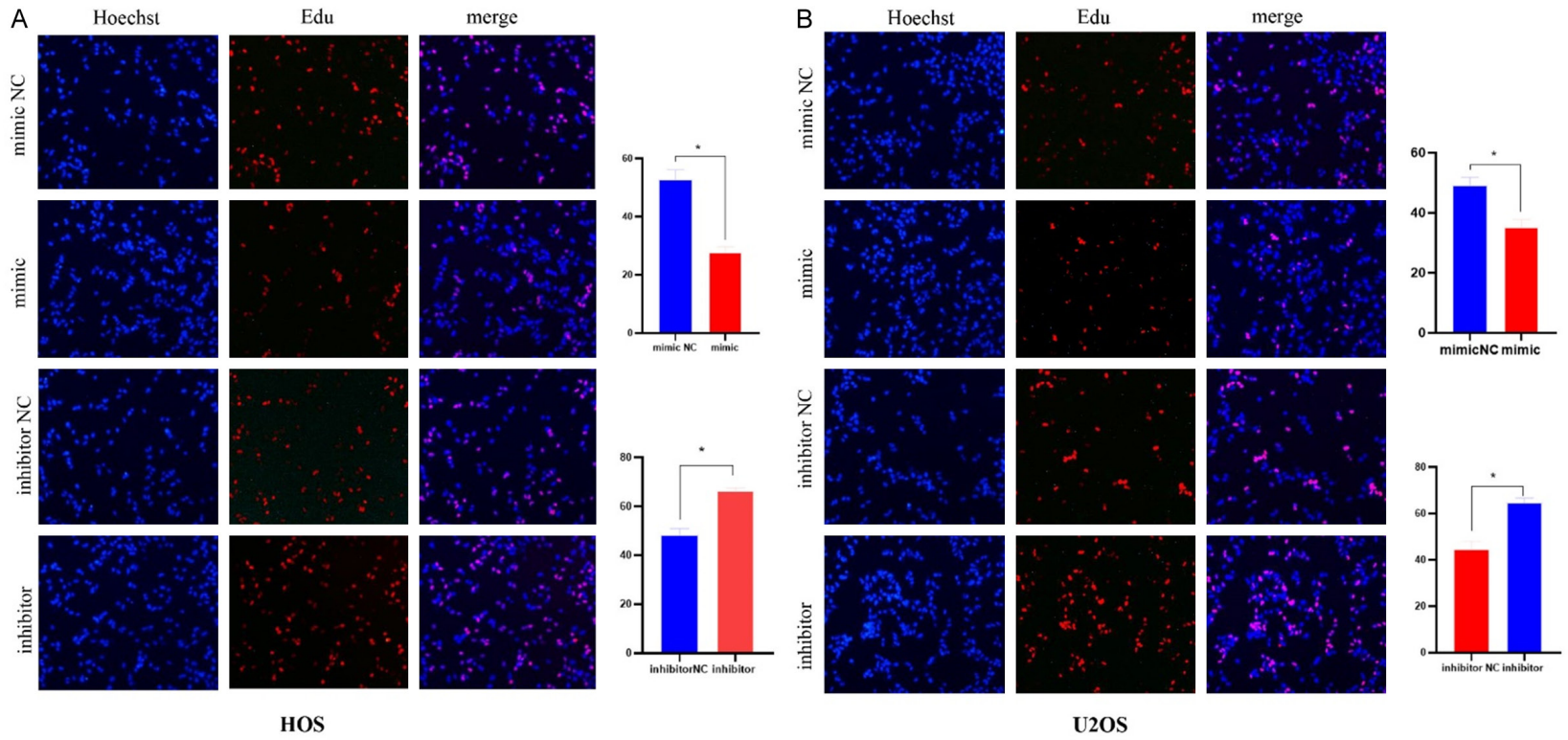

Figure 2. miR-1297 inhibits proliferation of HOS (A) and U2OS (B) cells assessed by EdU labeling (× 20). The EdU assay is shown in percentiles. All the data were presented as the mean ± SD from three independent experiments. *P < 0.05.

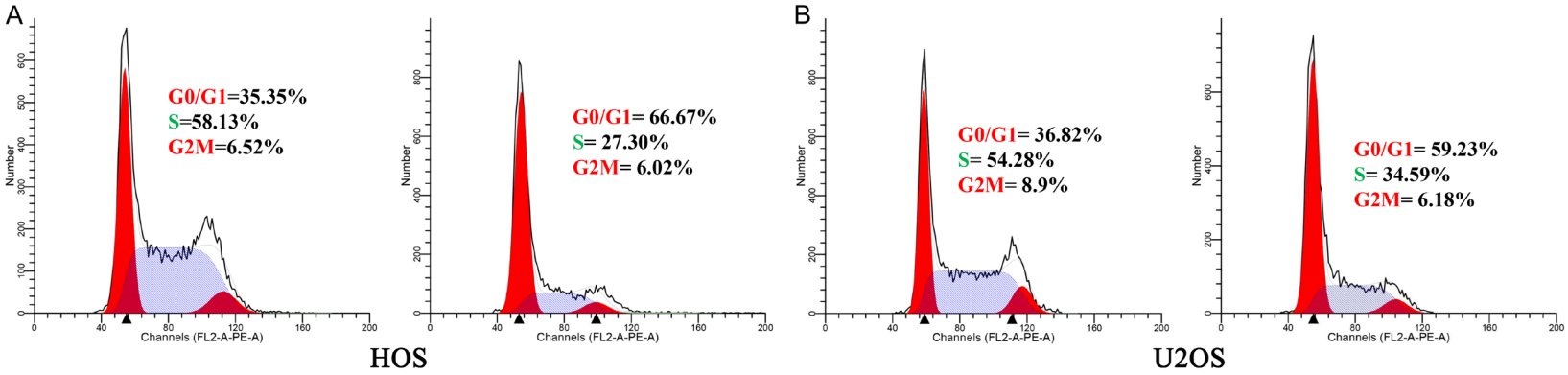

Figure 3. Cell cycle was arrested in G1 phase in cells with miR-1297 overexpression as determined by flow cytometry in HOS (A) and U2OS (B).

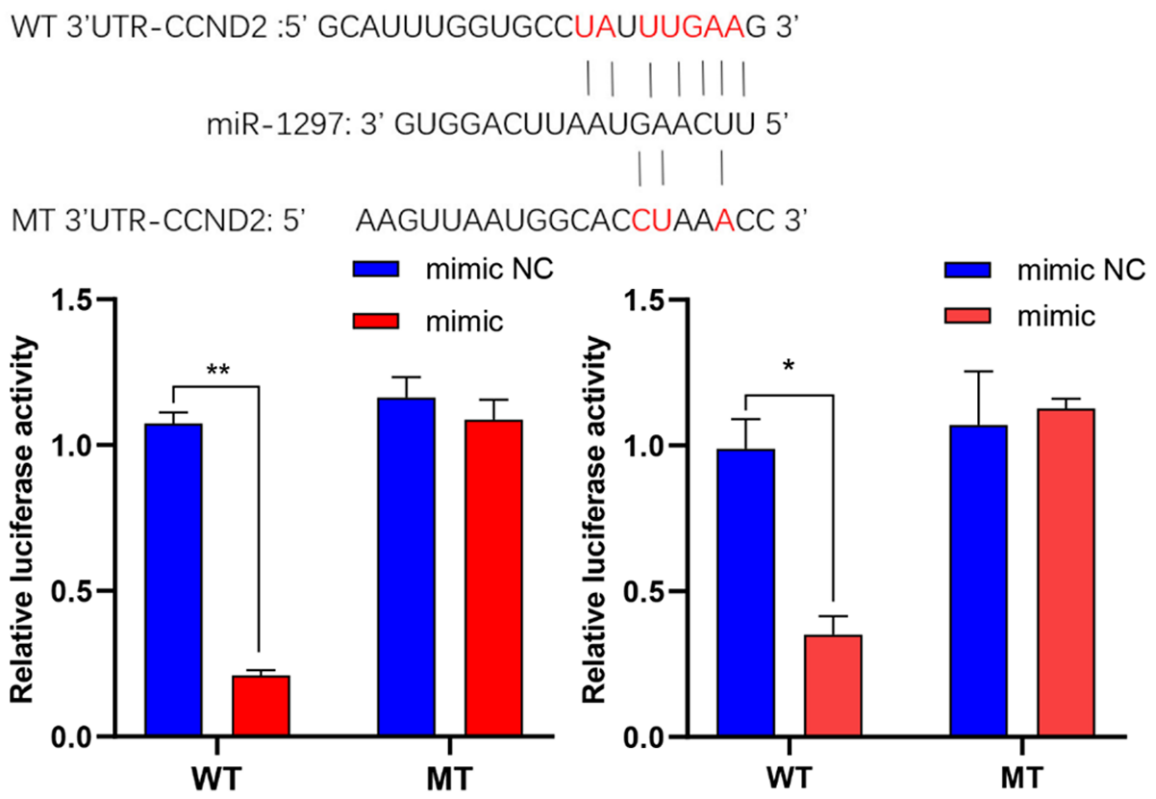

Figure 4. Data platforms predicted that miR-1297 may target CCND2. Comparing to non-significant mutant-type reporters, miR-1297 overexpression could significantly suppress fluorescence reporter activity in wild-type cells, whereas the inhibitors had opposite effect. All the data were presented as the mean ± SD from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****p < 0.0001; ns: non-significance.

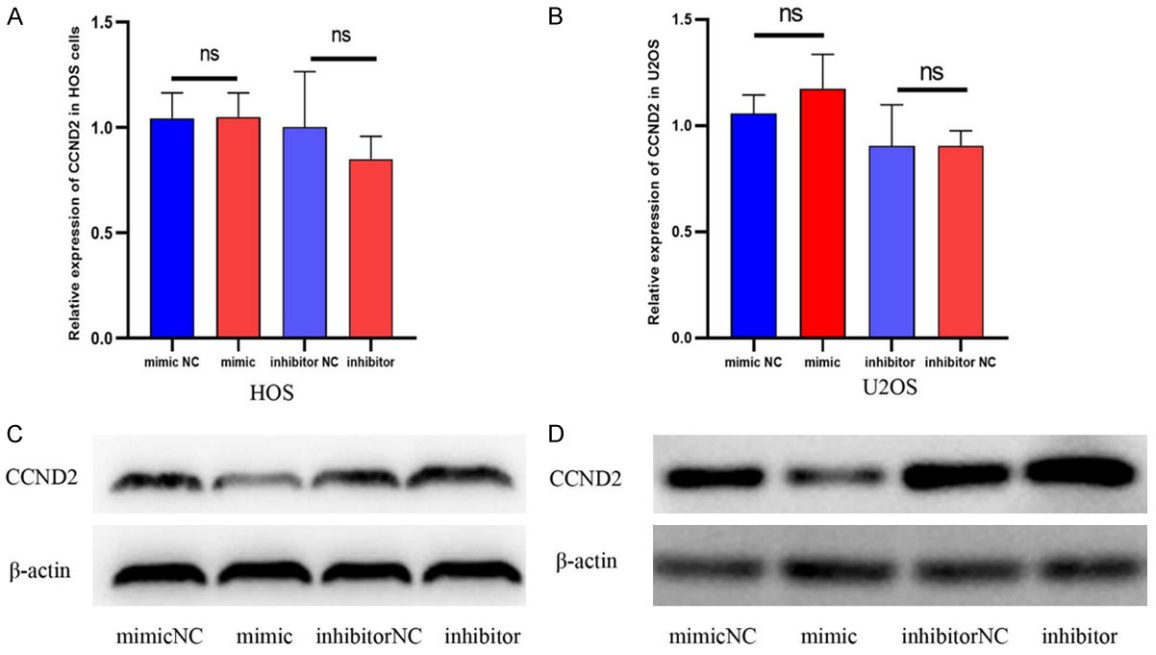

Figure 5. miR-1297 suppressed CCND2 protein abundance, but mRNA levels were not downregulated. Both HOS (A) and U2OS (B) showed non-significant change in CCND2 mRNA after transfecting mimic, inhibitor, or respective control. CCND2 protein abundance was inhibited by miR-1297 overexpression in HOS (C) and U2OS (D). All the data were presented as the mean ± SD from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns: non-significance.

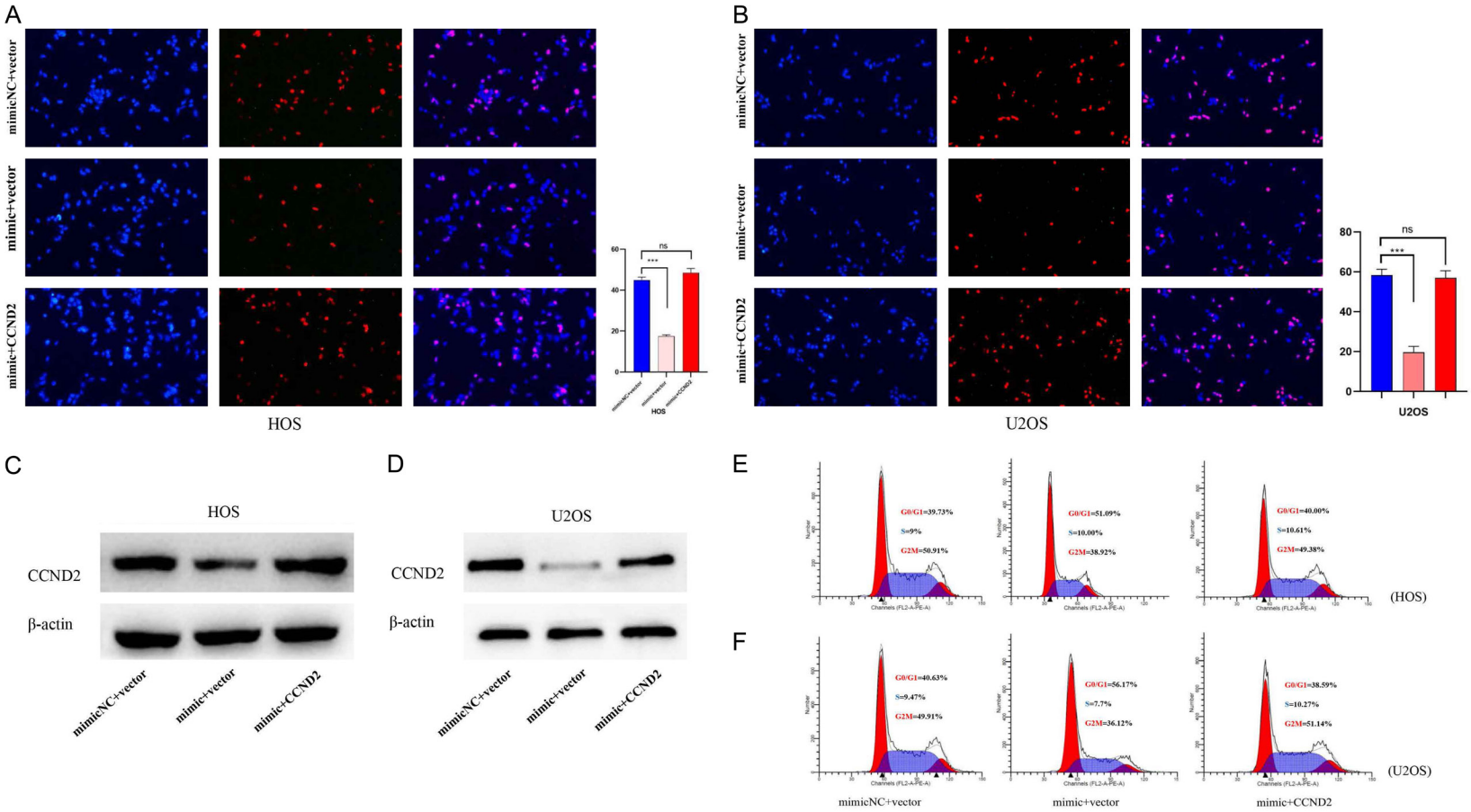

Figure 6. The inhibition of miR-1297 could be rescued by CCND2. (A, B) CCND2 overexpression rescues the inhibition of miR-1297 detected by EdU assay. (C, D) Western blotting showed the downregulation of CCND2 protein abundance by miR-1297 is rescued by CCND2. The flow cytometry analysis (E, F) indicated miR-1297 overexpression could reverse the inhibition of miR-1297. EdU assay is shown in percentiles. All the data were presented as the mean ± SD from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns: non-significance.

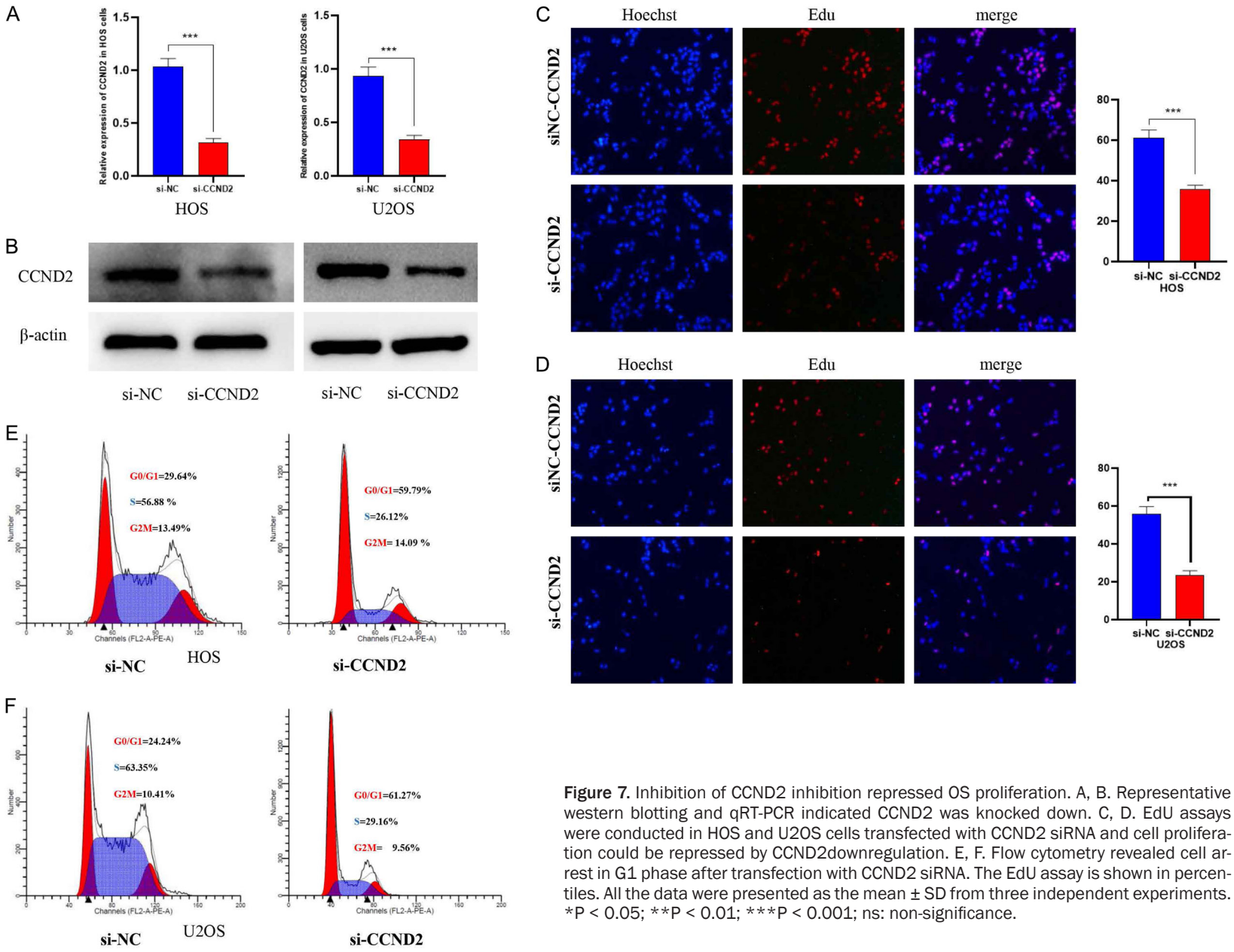

Figure 7. Inhibition of CCND2 inhibition repressed OS proliferation. A, B. Representative western blotting and qRT-PCR indicated CCND2 was knocked down. C, D. EdU assays were conducted in HOS and U2OS cells transfected with CCND2 siRNA and cell proliferation could be repressed by CCND2downregulation. E, F. Flow cytometry revealed cell arrest in G1 phase after transfection with CCND2 siRNA. The EdU assay is shown in percentiles. All the data were presented as the mean ± SD from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ns: non-significance.

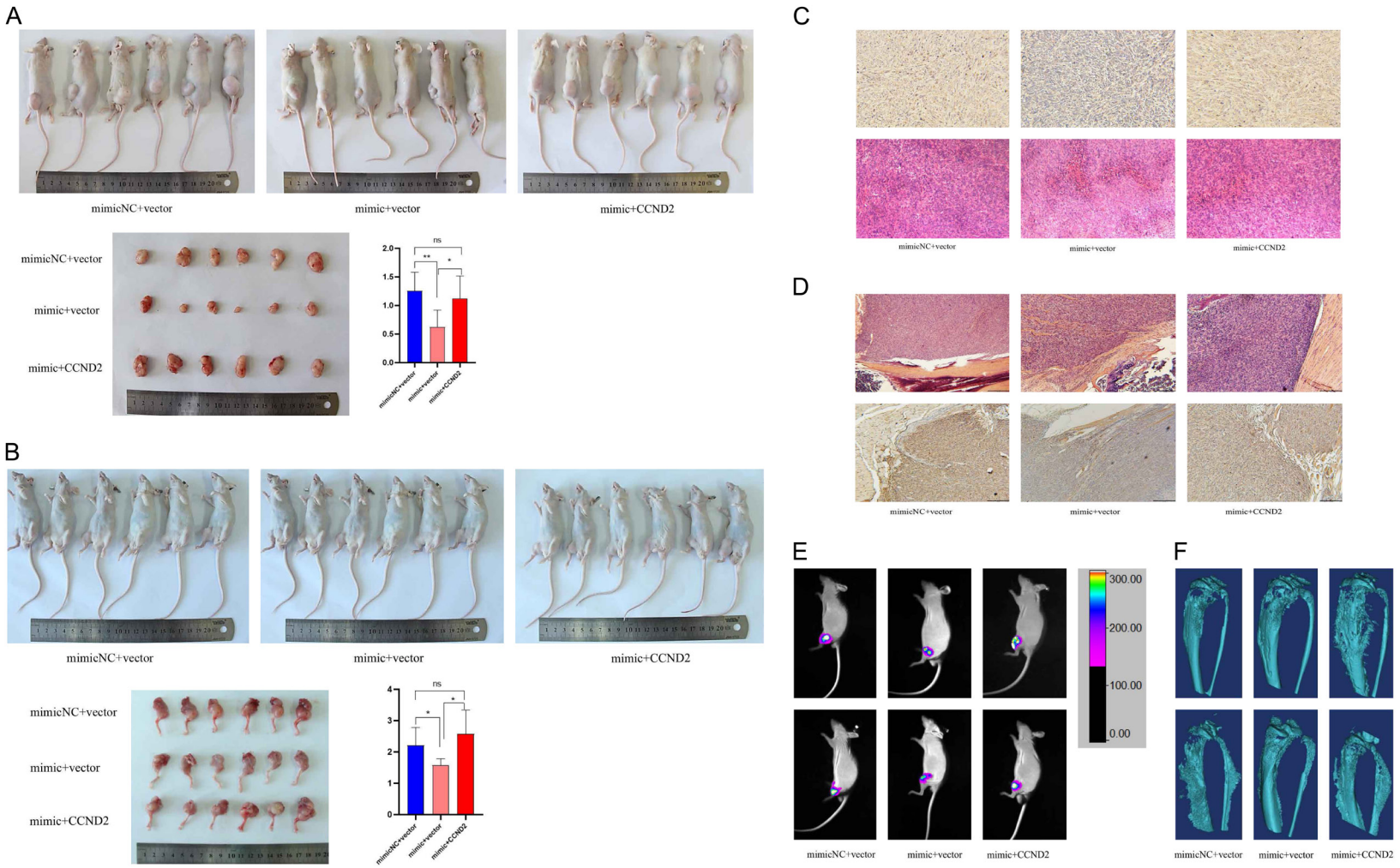

Figure 8. miR-1297 repressed OS tumor growth in vivo. Stable expression of miR-1297 or miR-1297 and CCND2 or control HOS cells were injected into nude mice. The sizes and weight were reduced by miR-1297 in subcutaneous (A) and local (B) limb tumors (mean ± SD; n=6). Tumor tissues from subcutaneous (C) and local (D) HOS xenograft model were harvested to perform immunohistochemical analysis and results showed lower CCND2 expression in stably-expressed miR-1297 HOS mice, and the inhibition of miR-1297 was rescued by CCND2. (E) In vivo imaging system was applied in local mice 28 days after local limb injection of cells infected with PC-control, PC-miR-1297, or PC-miR-1297 and CCND2 and results revealed lower fluorescence in PC-miR-1297. (F) microCT showed less bone erosion in PC-miR-1297 mice.

miR-1297, the inhibition of miR-1297 was rescued by CCND2 in cell proliferation. These results suggest that CCND2 acted as an oncogene in OS cells by promoting cell cycle progression.

The primary mechanism by which microRNAs regulate their target genes is by binding and degrading their 3'-UTR [42]. Therefore, to elucidate the detailed mechanism between CCND2 and miR-1297, we performed dual-luciferase reporter assays and the cells transfected both miR-1297 and wild-type of 3'UTR of CCND2 luciferase reporter vector recorded lower luciferase activity, which demonstrated that miR-1297 may target the 3'UTR of CCND2. However, a previous study demonstrated binding between CCND2 and miR-1297 at different predicted sites, suggesting a complex regulation mechanism of CCND2 by microRNA by targeting multiple sites [28]. Furthermore, we overexpressed miR-1297 and found the protein abundance of CCND2 was greatly downregulated, but mRNA levels were unchanged. These results differed from a previous study and implied that process miR-1297 may post-transcriptionally regulate CCND2 in OS [28]. The present study also demonstrated that miR-1297 can suppress tumor growth in vivo. The tumors formed by stably overexpressing miR-1297 cells showed significantly lower weight and volume than the control, and CCND2 expression in these tumors was also downregulated. The tumors had lower fluorescence activity, as detected with IVIS, and less bone erosion by microCT in miR-1297 stably-expressed mice. The results showed that miR-1297 may decrease cell number promoting cell cycle arrest, which may reduce bone erosion. Therefore, miR-1297 may inhibit tumor growth by inducing cell cycle arrest in vivo. However, the present study mainly focused on the effect of miR-1297 suppression on cell proliferation and tumor growth. The effect of miR-1297 on migration, invasion, and lung metastasis in OS still needs further investigation.

In summary, our findings demonstrated that CCND2 was highly expressed in OS. In contrast, miR-1297 was lowly expressed in OS and could inhibit OS cell proliferation and tumor growth by directly targeting CCND2. Thus, miR-1297 might represent a potential therapeutic target for OS patients.

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Disclosure of conflict of interest

None.

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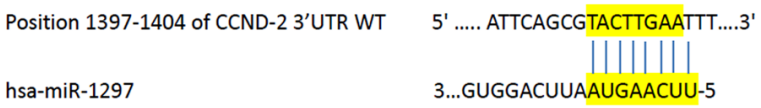

Figure S1. The pair sequence of CCND2 and miR-1297.