MiR-486-3p promotes osteogenic differentiation of BMSCs by targeting CTNNBIP1 and activating Wnt/β-catenin pathway

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Research article

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Abstract

Background

Dysfunction in osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) leads to bone loss/osteoporosis. CTNNBIP1 (Catenin beta interacting protein 1) is an inhibitor of Wnt/β-catenin signaling, whose role in osteogenesis remains elusive. This study aims to reveal the effects of miR-486-3p/CTNNBIP1 in osteogenesis.

Methods

Bone marrow samples from control and osteoporosis patients were collected and ovariectomy was performed on mice and levels of CTNNBIP1 and miR-486-3p levels were assessed. Dual-luciferase reporter assay was used to confirm their interactions. MiR-486-3p mimics/inhibitor or CTNNBIP1 overexpression lentivirus were transfected in human BMSCs (hBMSCs) and then osteogenic assay was performed. Alizarin red S (ARS) and Alkaline phosphatase (ALP) intensity with osteogenic gene expression including Runx2, Alp, Bglap and OCN was measured. Key proteins in Wnt/β-catenin pathway including active β-catenin, Bcl-2 and Cyclin D1 were assessed.

Results

CTNNBIP1, an inhibitor of Wnt/β-catenin signaling was upregulated while miR-486-3p was downregulated in ovariectomized (OVX) mice. CTNNBIP1 was confirmed as a target of miR-486-3p. MiR-486-3p overexpression promoted but miR-486-3p knockdown suppressed osteogenic differentiation and Wnt/β-catenin pathway. Rescue experiments further elucidated that the negative effects of CTNNBIP1 overexpression on osteoblastic differentiation and canonical Wnt signaling could be reversed by miR-486-3p mimics.

Conclusion

This study demonstrated that miR-486-3p sponges CTNNBIP1 thus activating Wnt/β-catenin signaling pathway to promote osteogenesis of BMSCs.

Introduction

Osteoporosis is an age-related systemic skeletal disorder characterized by decrease of bone mass and deterioration of bone architecture [1]. It was estimated that pathological bone loss or osteoporosis account for 25–50% of population aged 50 or above, which is becoming an enormous worldwide public health problem [2]. The balance of bone remodeling is maintained by osteoblasts originated from bone marrow mesenchymal stem cells (BMSCs) and osteoclasts differentiated from hemopoietic stem

cells/monocytes [3]. Studies showed cellular senescence and systemic low-grade inflammation would lead to the differentiation function impairment of BMSCs, which is an important cause of osteoporosis especially in older adults [4].

It has been widely recognized that the activation of Wnt/β-catenin pathway is essential for osteogenic differentiation and bone formation [5]. Generally, β-catenin translocated in the nucleus needs to associate with TCF/LEF transcription factors to induce downstream gene expression [6]. CTNNBIP1 (Catenin beta interacting protein 1), also termed as ICAT (Inhibitor of β-catenin and TCF-4), is an 81-amino acid protein which binds to β-catenin and competitively inhibits the interactions between the family members of β-catenin and TCF [7]. Although CTNNBIP1 is a well-known suppressor of Wnt/β-catenin signaling, its underlying regulatory network in osteogenic differentiation of BMSCs remains to be investigated.

MicroRNAs (miRNAs) are single-stranded non-coding RNAs containing 18–25 nucleotides. They participate in regulating multiple biological processes including development, metabolism, aging and carcinogenesis mainly by RNA silencing [8]. Studies have reported series of miRNAs as regulators of osteogenic differentiation, either as promoters including miR-15b, miR-29b and miR-342-3p [9–11] or inhibitors such as miR-103-3p, miR-122 and miR-532-3p [12–14]. Recently, Xu et al. reported that miR-486-3p was significantly downregulated in plasma of ovariectomized rats, which could be reversed by estrogen treatment [15]. In addition, miR-486 increase led to pro-osteogenic phenotype of interstitial cells by upregulate Runx2 and Osx [16]. However, the role of miR-486-3p in osteoblastic differentiation of BMSCs still needs investigate. Our data showed that several databases predicted that miR-486-3p targets CTNNBIP1. Hence, miR-486-3p/CTNNBIP1 axis is likely to regulate osteogenesis.

Based on the biological features of interactions among miR-486-3p, CTNNBIP1 and canonical Wnt signaling, we hypothesized that miR-486-3p targets CTNNBIP1 to upregulate Wnt/β-catenin signaling, in the end promote osteoblastic differentiation of BMSCs. The miR-486-3p/CTNNBIP1 may serve as part of the regulatory network of osteogenesis and potential therapeutic target for osteoporosis.

Materials And Methods

Isolation and culture of human BMSCs

The procedures in this study were approved by the Medical Ethics Committee of Changzheng Hospital, Second Military Medical University. Informed consent was obtained from every participant. Femoral bone marrow samples were collected from 8 patients with low BMD (T<-2.5) and 10 patients with normal BMD (T≥-2.5). Primary human BMSCs were isolated as previously described [19]. Normal medium consisting of DMEM (Cytiva Life Sciences) with 10% FBS (Gibco) and 1% Penicillin/ Streptomycin (Shanghai Yuanye Bio-Technology) was used to culture the cells placed in 25-cm2 flasks at the condition of 37C, 5% CO2, and in a humidified incubator.

Animals and ovariectomy model establishment

Female C57BL/6 mice (8 weeks; weight 18-22g) were purchased from Shanghai Jihui Laboratory Animal Care Co. Ltd (Shanghai, China) and housed at specific pathogen free (SPF) conditions in Animal Experimental Center of second military medical university. All procedures in this study complied with the recommendations of the Guide for the Care and Use of Laboratory Animals provided by the National Institutes of Health.

Twenty mice were randomly allocated into sham surgery (Control, Con) and ovariectomy (OVX) group according to their weight and random numbers were generated by Excel software. The procedure of OVX has been described previously [17]. Briefly, shave hair off the flank area of mice after its anesthesia with pentobarbital. Then make a longitudinal incision in the skin and separate the musculature. Locate the ovary, pull the ovarian fat pad out carefully and remove it. After stopping bleeding, close he muscle layer and skin with absorbable sutures. Six weeks after surgery, femur of mice from Con and OVX groups were collected.

Micro-computed tomography (μCT) analysis

Femora collected from mice were fixed in 4% paraformaldehyde for two days. Then distal femoral structure was assessed by micro-computed tomography (SkyScan 1076, Bruker) at the condition of 80 kV and 124 μA, with resolution of 8 μm. The parameters of trabecular bone were analyzed including bone volume/total volume (BV/TV, %), trabecular bone mineral density (BMD, g/cc), trabecular number (Tb.N, 1/mm) and trabecular space (Tb.Sp, mm).

Isolation and culture of mouse primary BMSCs

Isolation and culture of BMSCs were described previously [18]. Briefly, after mice were euthanized, the lower limbs were dissected and femur and tibias were harvested. Small cuts were made in the proximal and distal ends of the bones and put them in the sectioned tips within the centrifuge tubes. Bone marrow flushed by centrifuge at 10000g for 15 s were suspended using Normal Medium. Then the cells were plated in 25-cm2 flasks and cultured at condition of 37C and 5% CO2. Second passage of BMSCs were harvested and used to extract mRNA and microRNA.

Cell culture of human BMSCs (hBMSCs)

Human BMSCs for transfection and osteogenic differentiation were purchased from National Collection of Authenticated Cell Cultures. Normal medium was used to culture the cells placed in 25-cm2 flasks at the condition of 37C, 5% CO2, and in a humidified incubator (Thermo Fisher Scientific).

Osteogenic differentiation

For osteogenic differentiation, hBMSCs were seeded in 12-well plates at the density of 70–80%. Then change the NM to osteogenic induced medium (DMEM with 10% FBS, 1% Penicillin/ Streptomycin, 5mM β-glycerophosphate (Sigma-Aldrich), 50μM ascorbic acid (Sigma-Aldrich) and 100nM dexamethasone (Sigma-Aldrich)) for 21 days.

Alizarin red S (ARS) staining and Alkaline phosphatase (ALP) staining

ARS staining was used to measure the mineralization of BMSCs induced by osteogenic medium, as described previously [13]. Briefly, differentiated BMSCs were rinsed by PBS twice, fixed in 4% PFA for 15min and stained by 1% ARS solution for 10min. As for quantitative analysis, cells were incubated with cetylpyridinium chloride (Sigma-Aldrich) and then measured by spectrophotometer at 570nm.

ALP staining was performed to analyze osteogenesis degree of induced BMSCs, which followed the instructions of ALP test kit (Wako Pure Chemical). The absorbance value at 420nm was measured by spectrophotometer.

MiRNA Transfection

Oligonucleotides used in this study were synthesized by GenePharma (Shanghai, China). The sequences were as follows. Hsa-miR-486-3p mimic: primary chain, 5’- CGGGGCAGCUCAGUACAGGAU – 3’; passenger chain, 5’- CCUGUACUGAGCUGCCCGUU – 3’; has- miR-486-3p inhibitor: 5’- GCCCCGUCGAGUCAUGUCCUA – 3’; mimic-NC: 5’-UUCUCCGAACGUGUCACGUTT-3’ and 5’-ACGUGAACAGUUCGGAGAATT-3’; inhibitor-NC: 5’-CAGUACUUUUGUGUAGUACAA-3’. When 30–40% confluent, hBMSCs were transfected with oligonucleotides using Lipofectamine 3000 (Invitrogen) reagent following the manufacturer’s instructions.

Construction of CTNNBIP1 Overexpression Vector and cell transfection

The CTNNBIP1-overexpression lentivirus vector and NC lentivirus vector were synthesized by Genechem (Shanghai, China). HBMSCs were seeded into 6-well plate at a density of 60–80% and then incubated with lentivirus vectors for 24 hours, according to manufacturer’ instructions. Then 1ug/ml puromycin was used to screen the transfected hBMSCs for 3 days. After the medium was refreshed, transfection efficiency of the lentivirus was verified by qRT-PCR and western blot.

Dual-Luciferase Reporter Gene Assay

The putative binding site of miR-486-3p and 3′ UTR of CTNNBIP1 was predicted by DIANA database 5th (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=site/index). The wild type (WT) and mutant type (MUT) of CTNNBIP1 3′ UTR vectors were constructed. 293T cells were seeded and cultured in 24-plate until they reached the confluence of 50%-60%, then cotransfected with control or miR-486-3p mimics using Lipofectamine 2000 (Invitrogen). Forty-eight hours later, cells were collected to measure the luciferase activities according to the instructions of Dual Luciferase Reporter assay.

Real time quantitative polymerase chain reaction (RT-qPCR)

The total RNAs of induced hBMSCs were extracted by RNA-quick purification kit (ES Science, YiShan Biotech) following the manufacturer’s instructions. Then mRNAs were transcribed into cDNAs using cDNA Reverse Transcription Kit (thermo fisher scientific). For miRNAs, cDNA was synthesized using a

miRNA First Strand Synthesis Kit (Agilent Technologies). Then cDNA was assessed by real-time PCR using SYBR Green reagent. Relative expression was calculated using the 2-ΔΔCT normalized by β-actin. Primers used in this study were listed here: Runx2: forward 5'- GCTCTTCCCAAAGCCAGAGT-3'; reverse 5'-GGATCCTGACGAAGTGCCAT-3'; Alp: forward 5'- GAATCTTCCCCAAGGGCCAA-3'; reverse 5'-CAGAATGTTCCACGGAGGCT-3'; Bglap: forward 5'-TTCTGCTCACTCTGCTGACC-3'; reverse 5'-TTTGTAGGCGGTCTTCAAGC-3'; OCN: forward 5'- ATGGCGTCCTCTCTGCTTG-3'; reverse 5'-TGAAAGGTCAGCGTATGGCTT-3'.

Western blot Analysis

Total protein was extracted by radioimmunoprecipitation assay (RIPA), quantified by bicinchoninic acid (BCA) protein assay kit, separated by SDS-PAGE electrophoresis and then transferred to a polyvinylidene fluoride (PVDF) membrane. Then the membranes were blocked by 5% skim milk and incubated with anti-CTNNBIP1 (Abcam), anti-active β-catenin (Cell Signaling Technology), anti-Bcl2 (Abcam) and anti-CyclinD1 (Abcam) and anti-β-tubulin (Transgene).

Statistical Analysis

Data analysis was performed by GraphPad prism software (GraphPad Software, USA). Kolmogorov-Smirnov method was performed to check if the data were in normal distribution. F test were used to compare variances of different groups. Student's unpaired t test was used for the comparisons between two groups, while two-way ANOVA for the comparisons among multiple groups. Differences were considered significant at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

Results

High CTNNBIP1 and low miR-486-3p expression is correlated with decreased BMD

To study the relationship between CTNNBIP1 and osteoporosis, we measured its expression level in BMSCs isolated from control people and osteoporosis patients. Compared to control group, CTNNBIP1 were significantly upregulated in osteoporosis population (Fig. 1A). In addition, OVX model in mice was constructed. Compared to control group, mice in OVX group showed great bone loss manifested as decreased BV/TV, BMD, Tb.N and increased Tb.Sp (Figure S1). Then we isolated primary BMSCs from mice and measured the expression of CTNNBIP1. The transcription levels of CTNNBIP1 were higher in OVX mice (Fig. 1B).

To further explore the molecular mechanism by which CTNNBIP1 is implicated in bone metabolism, CTNNBIP1 targeted miRNAs were predicted using Targetscan, miRWalk and DIANA databases. Venn diagram showed that 35 miRNAs were predicted in all three databases (Fig. 1C), in which miR-145-5p, miR-204-5p and miR-486-3p were evolutionarily conserved between human and mice (Fig. 1D). In addition, it was reported that miR-145-5p and miR-204-5p negatively regulate osteoblastic differentiation [19–21], while on the opposite, miR-486-3p positively related to BMD [15]. Hence, we speculated that miR-

486-3p participated in osteogenesis by targeting CTNNBIP1. As expected, miR-486-3p were significantly lower in osteoporosis patients and OVX group (Fig. 1E, F). Besides, negative association was found between miR-486-3p and CTNNBIP1 (Fig. 1G). These results demonstrated that miR-486-3p were likely to interact with CTNNBIP1, further impacting bone metabolism.

CTNNBIP1 was downregulated while miR-486-3p upregulated during osteogenic differentiation

To evaluate the relationship between CTNNBIP/miR-486-3p and osteogenesis, we induced hBMSCs osteogenic differentiation and collected total RNA and protein on day 0, 4, 8, 12. The mRNA levels of osteogenic related genes including Runx2, Alp, Bglap and OCN were notably enhanced along osteoblastic differentiation (Fig. 2A-D). The transcriptional and translational level of CTNNBIP1 gradually decreased after osteogenic differentiation (Fig. 2E-G). On the opposite, the expression level of miR-486-3p were upregulated during the process of osteogenesis (Fig. 2H). These data suggested CTNNBIP1 negatively and miR-486-3p positively associated with osteogenesis of hBMSCs.

CTNNBIP1 is a target gene of miR-486-3p

The DIANA database was used to aid in the detection of the binding sites of miR-486-3p on CTNNBIP1 3’ untranslated region (3’UTR) (Fig. 3A). Luciferase reporter plasmids containing wild type (WT) or mutated (MUT) miR-486-3p binding sequence were constructed. The dual luciferase reporter gene assay showed that miR-486-3p mimics suppressed WT CTNNBIP1 3'UTR luciferase reporter activity but not MUT CTNNBIP1 3'UTR reporter activity (Fig. 3B).

To further confirm the interaction between miR-486-3p and CTNNBIP1 in osteogenesis, we transfected hBMSCs with miR-486-3p mimics, miR-486-3p inhibitors (anti-miRNA oligonucleotides) and their controls. Twenty-four hours after transfection, miR-486-3p levels were about 6 times higher in mimics group, while anti-miRNA oligonucleotides inhibited miR-486-3p expression by approximately 65% (Fig. 3C). Meanwhile, the transcription and translation levels of CTNNBIP1 was significantly decreased by miR-486-3p overexpression and enhanced by miR-486-3p knockdown (Fig. 3D-F). Taken together, these results illuminated that CTNNBIP1 is a target gene of miR-486-3p in hBMSCs.

MiR-486-3p promotes osteogenic differentiation of hBMSCs

To study whether miR-486-3p participate in osteogenic differentiation of hBMSCs, the MiR-486-3p mimics, inhibitors and their controls were transfected into hBMSCs which were then cultured in osteogenic medium for 3 weeks. MiR-486-3p mimics significantly increased but miR-486-3p inhibitors decreased mineralized matrix formation showed by ARS staining in osteogenic induced hBMSCs (Fig. 4A-D). Similarly, alkaline phosphatase (ALP) staining showed ALP activity was upregulated in miR-486-3p mimics group and downregulated in miR-486-3p inhibitor group (Fig. 4A-D). Besides, the expression of osteoblast-specific genes including Runx2, Alp, Bglap and OCN were promoted after miR-486-3p overexpression while impeded after miR-486-3p knockdown (Fig. 4E). Therefore, miR-664a-5p is a positive regulator in osteogenesis.

CTNNBIP1 overexpression impairs osteogenesis, which could be rescued by miR-486-3p

To further elucidate the involvement of CTNNBIP1 in the influence of miR-486-3p during osteoblastic differentiation of hBMSCs, we constructed CTNNBIP1-overexpressing hBMSCs using lentivirus transduction and then miR-486-3p mimics were cotransfected (Figure S2). Measured by RT-qPCR and western blot, the expression level of CTNNBIP1 was approximately 2 times higher in overexpression group compared to hBMSCs transfected with negative control (NC) lentivirus (Fig. 5A-C). However, when cotransfected with miR-486-3p mimics, the level of CTNNBIP1 was decreased to normal (Fig. 5A-C).

Then hBMSCs of different groups were induce by osteogenic medium for 21 days. ARS and ALP staining revealed that hBMSCs with overexpressed CTNNBIP1 exhibited reduced mineralized matrix and ALP activity (Fig. 5D-G). Osteogenic markers including Runx2, Alp, Bglap and OCN were also downregulated in CTNNBIP1 overexpression group (Fig. 5H). In contrast, when cotransfected with miR-486-3p, the staining intensity of ARS and ALP staining as well as the expression of osteogenic markers were reversed to control levels (Fig. 5D-H). These results further elucidated that miR-486-3p/CTNNBIP1 participates in osteogenic differentiation.

CTNNBIP1 blocks but miR-486-3p enhances the Wnt/β-catenin signaling

CTNNBIP1 is well-known as an interacting protein of β-catenin to inhibit Wnt/β-catenin signaling driving osteogenic differentiation of BMSCs. To further reveal the molecular mechanism of miR-486-3p/CTNNBIP1 in osteogenesis, western blot analysis was performed to measure changes of proteins in Wnt/β-catenin pathway. It was shown that miR-486-3p mimics inhibited but miR-486-3p inhibitor increased the levels of β-catenin, Bcl-2 and Cyclin D (Fig. 6A, B). In addition, the overexpression of CTNNBIP1 also blocked the expression of β-catenin, Bcl-2 and Cyclin D, which was reversed by miR-486-3p mimics (Fig. 6C, D). Taken together, miR-486-3p suppressed the CTNNBIP1 expression to activate Wnt/β-catenin signaling, in the end promote osteogenesis (Fig. 6E).

Discussion

The regulatory role of microRNAs in the differentiation of BMSCs were of great interest nowadays, for it provides new targets for clinical screening, diagnosis, and therapy of bone diseases. In this study, we reported miR-486-3p were downregulated and CTNNBIP1 upregulated in osteoporosis patients and ovariectomized mice. Then we elucidated that miR-486-3p mimics promoted while CTNNBIP1 overexpression inhibited osteogenic differentiation of hBMSCs. The underly mechanisms were revealed that miR-486-3p binds to the 3'UTR of CTNNBIP1 mRNA to inhibit CTNNBIP1 expression level, in the end activating Wnt/β-catenin pathway to promote osteogenesis.

CTNNBIP1 is an inhibitor of Wnt/β-catenin signaling, which is widely expressed in multiple tissues and some type of tumors. CTNNBIP1 repression was highly associated with gene activation induced by progastrin and Tcf-4 in human intestinal tumors [22]. Downregulation of CTNNBIP1 was also observed in psoriasis lesion skin tissue samples compared with control samples [23]. In addition, Phospholipase D1

suppression led to CTNNBIP1 increase, which impaired colorectal cancer growth [24]. Mechanically, CTNNBIP1 binds to β-catenin to competitively inhibit the interactions between β-catenin and TCF, while not affecting β-catenin/cadherin binding [25]. Considering the important role of canonical signaling in osteogenesis, CTNNBIP1 is likely to participate in bone metabolism. Notably, a previous article reported the overexpression of CTNNBIP1 impedes while knockdown of CTNNBIP1 promotes proliferation and osteogenic differentiation of human adipose tissue-derived mesenchymal stem cell [26]. However, the alteration of canonical Wnt pathway were not assessed in that study. Our research demonstrated that overexpression of CTNNBIP1 suppressed the expression of key proteins in Wnt/β-catenin pathway including active β-catenin, Bcl-2 and Cyclin D. In addition, the accumulation of CTNNBIP1 significantly reduces the capacity of osteogenic differentiation of BMSCs.

Series of microRNAs were reported to participate in the regulation of bone formation [27, 28]. In this study, we focused on the functions of miR-486 on osteogenesis. Previously, miR-486 have been known as a tumor suppressor and were found decreased in several cancers such as lung cancer, gastric adenocarcinoma and so on [29–31]. Apart from the effects in carcinogenesis, miR-486 is an important content in extracellular vehicles (EVs). MiR-486 in EVs from tonsil-derived mesenchymal cells attenuated liver fibrosis [32]. Sertoli cell-derived exosomal MicroRNA-486 promotes differentiation of spermatogonial stem cell by targeting PTEN [33]. Besides, circulating miR-486 were associated with sarcopenia in the old [34]. Results of some studies also hinted that miR-486 participated in bone homeostasis. The levels of miR-486 were reduced in plasma of ovariectomized rats compared to controls, which was rescued by estrogen supplement [15]. Besides, miR-486 enhancement resulted in pro-osteogenic phenotype of interstitial cells [16]. Our data showed that miR-486-3p significantly decreased in osteoporosis patients and mice of OVX group. The overexpression of miR-486-3p in hBMSCs resulted in increased mineralization, ALP activity and osteogenic markers including Runx2, Alp, Bglap and OCN. On the contrary, miR-486-3p knockdown inhibited osteogenic phenotype. Hence, miR-486-3p is a positive regulator of osteogenesis.

The upstream regulators of CTNNBIP1 have not been investigated in detail. Some studies predicted that CTNNBIP1 may be the target for several osteogenic microRNAs. MiR-29b enhanced osteogenic differentiation of MC3T3-E1 cells by down-regulating suppressors of osteogenesis including HDAC4, TGF3, CTNNBIP1 [9]. MiR-133b and MiR-211 exacerbated the calcification of Vascular Smooth Muscle, which was also correlated with CTNNBIP1 inhibition [35]. In this study, three databases including Targetscan, miRWalk and DIANA were used to predict potential microRNAs that interacts with 3’UTR of CTNNBIP1. It was found that miR-486-3p, a potential osteogenic microRNA, is predicted in all databases and conserved in human, rat and mouse. We further revealed that miR-486-3p significantly inhibited CTNNBIP1 expression by binding to its mRNA (sites: 232–250), then leading to the upregulation of osteogenesis of BMSCs.

Conclusion

In conclusion, this study demonstrated that miR-486-3p is a positive regulator of osteogenic differentiation of BMSCs by inhibiting the expression of CTNNIBIP1 and activating Wnt/β-catenin pathway, which might contribute to new tactics for the screening, diagnosis and therapy of osteoporosis.

Abbreviations

Bone marrow mesenchymal stem cells (BMSCs); Catenin beta interacting protein 1 (CTNNBIP1); Ovariectomized (OVX); Specific pathogen free (SPF); Micro-computed tomography (μCT); Bone volume/total volume (BV/TV), Trabecular bone mineral density (BMD); Trabecular number (Tb.N); Trabecular space (Tb.Sp); Human BMSCs (hBMSCs); Alizarin red S (ARS) staining ;Alkaline phosphatase (ALP); Real time quantitative polymerase chain reaction (RT-qPCR); 3' untranslated region (3’ UTR)

Declarations

Declarations:

None

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None

Disclosure statement

The authors report no conflict of interest

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Author contributions:

Study design: XH, JM, RG and ZZ. Study conduct: ZZ, WJ, MH, YM and LS. Data collection: ZZ, WJ and MH. Technical support: YM and LS. Data analysis: ZZ, WJ and MH. Data interpretation: ZZ, WJ, MH, YM and LS. Drafting manuscript: ZZ, WJ and MH. Revising manuscript content: XH, JM and RG. Approving final version of manuscript: ZZ, WJ, MH, YM, LS, RG, JM and XH. XH takes responsibility for the integrity of the data analysis.

Data availability statement

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

References

1. Compston JE, McClung MR,Leslie WD. Osteoporosis. Lancet (London, England) 2019; 393(10169):364-376.
2. Yang L, Li Y, Gong R, Gao M, Feng C, Liu T *et al*. The Long Non-coding RNA-ORLNC1 Regulates Bone Mass by Directing Mesenchymal Stem Cell Fate. Molecular therapy : the journal of the American Society of Gene Therapy 2019; 27(2):394-410.
3. Feng X,McDonald JM. Disorders of bone remodeling. Annual review of pathology 2011; 6:121-145.
4. Qadir A, Liang S, Wu Z, Chen Z, Hu L,Qian A. Senile Osteoporosis: The Involvement of Differentiation and Senescence of Bone Marrow Stromal Cells. International journal of molecular sciences 2020; 21(1).
5. de Winter TJJ,Nusse R. Running Against the Wnt: How Wnt/β-Catenin Suppresses Adipogenesis. Frontiers in cell and developmental biology 2021; 9:627429.
6. Baron R,Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nature medicine 2013; 19(2):179-192.
7. Stow JL. ICAT is a multipotent inhibitor of beta-catenin. Focus on "role for ICAT in beta-catenin-dependent nuclear signaling and cadherin functions". American journal of physiology Cell physiology 2004; 286(4):C745-746.
8. Gebert LFR,MacRae IJ. Regulation of microRNA function in animals. Nature reviews Molecular cell biology 2019; 20(1):21-37.
9. Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM *et al*. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. The Journal of biological chemistry 2009; 284(23):15676-15684.
10. Han Y, Zhang K, Hong Y, Wang J, Liu Q, Zhang Z *et al*. miR-342-3p promotes osteogenic differentiation via targeting ATF3. FEBS letters 2018; 592(24):4051-4065.
11. Lu X, Zhang Y, Zheng Y,Chen B. The miRNA-15b/USP7/KDM6B axis engages in the initiation of osteoporosis by modulating osteoblast differentiation and autophagy. Journal of cellular and molecular medicine 2021; 25(4):2069-2081.
12. Sun Z, Wang H, Wang Y, Yuan G, Yu X, Jiang H *et al*. MiR-103-3p targets the m(6) A methyltransferase METTL14 to inhibit osteoblastic bone formation. Aging cell 2021; 20(2):e13298.
13. Meng YC, Lin T, Jiang H, Zhang Z, Shu L, Yin J *et al*. miR-122 Exerts Inhibitory Effects on Osteoblast Proliferation/Differentiation in Osteoporosis by Activating the PCP4-Mediated JNK Pathway. Molecular therapy Nucleic acids 2020; 20:345-358.
14. Fan Q, Li Y, Sun Q, Jia Y, He C,Sun T. miR-532-3p inhibits osteogenic differentiation in MC3T3-E1 cells by downregulating ETS1. Biochemical and biophysical research communications 2020; 525(2):498-504.
15. Xu X, Zhang P, Li X, Liang Y, Ouyang K, Xiong J *et al*. MicroRNA expression profiling in an ovariectomized rat model of postmenopausal osteoporosis before and after estrogen treatment.

American journal of translational research 2020; 12(8):4251-4263.

16. Song R, Fullerton DA, Ao L, Zhao KS, Reece TB, Cleveland JC, Jr. *et al*. Altered MicroRNA Expression Is Responsible for the Pro-Osteogenic Phenotype of Interstitial Cells in Calcified Human Aortic Valves. Journal of the American Heart Association 2017; 6(4).
17. Chen X, Zhang Z, Hu Y, Cui J, Zhi X, Li X *et al*. Lactulose Suppresses Osteoclastogenesis and Ameliorates Estrogen Deficiency-Induced Bone Loss in Mice. Aging and disease 2020; 11(3):629-641.
18. Maridas DE, Rendina-Ruedy E, Le PT,Rosen CJ. Isolation, Culture, and Differentiation of Bone Marrow Stromal Cells and Osteoclast Progenitors from Mice. Journal of visualized experiments : JoVE 2018(131).
19. Jin Y, Hong F, Bao Q, Xu Q, Duan R, Zhu Z *et al*. MicroRNA-145 suppresses osteogenic differentiation of human jaw bone marrow mesenchymal stem cells partially via targeting semaphorin 3A. Connective tissue research 2020; 61(6):577-585.
20. Liu X, Zhu W, Wang L, Wu J, Ding F,Song Y. miR-145-5p suppresses osteogenic differentiation of adipose-derived stem cells by targeting semaphorin 3A. In vitro cellular & developmental biology Animal 2019; 55(3):189-202.
21. Zhou Y, Liu S, Wang W, Sun Q, Lv M, Yang S *et al*. The miR-204-5p/FOXC1/GDF7 axis regulates the osteogenic differentiation of human adipose-derived stem cells via the AKT and p38 signalling pathways. Stem cell research & therapy 2021; 12(1):64.
22. Pannequin J, Delaunay N, Buchert M, Surrel F, Bourgaux JF, Ryan J *et al*. Beta-catenin/Tcf-4 inhibition after progastrin targeting reduces growth and drives differentiation of intestinal tumors. Gastroenterology 2007; 133(5):1554-1568.
23. Wang C, Wang H, Peng Y, Zeng B, Zhang Y, Tang X *et al*. CTNNBIP1 modulates keratinocyte proliferation through promoting the transcription of β-catenin/TCF complex downstream genes. Journal of the European Academy of Dermatology and Venereology : JEADV 2021; 35(2):368-379.
24. Kang DW, Lee BH, Suh YA, Choi YS, Jang SJ, Kim YM *et al*. Phospholipase D1 Inhibition Linked to Upregulation of ICAT Blocks Colorectal Cancer Growth Hyperactivated by Wnt/β-Catenin and PI3K/Akt Signaling. Clinical cancer research : an official journal of the American Association for Cancer Research 2017; 23(23):7340-7350.
25. Graham TA, Clements WK, Kimelman D,Xu W. The crystal structure of the beta-catenin/ICAT complex reveals the inhibitory mechanism of ICAT. Molecular cell 2002; 10(3):563-571.
26. Kim YJ, Kim JT, Bae YC, Suh KT,Jung JS. ICAT participates in proliferation and osteogenic differentiation of human adipose tissue-derived mesenchymal stem cell. Life sciences 2008; 83(25-26):851-858.
27. Huang X, Cen X, Zhang B, Liao Y, Zhu G, Liu J *et al*. Prospect of circular RNA in osteogenesis: A novel orchestrator of signaling pathways. Journal of cellular physiology 2019; 234(12):21450-21459.
28. Xu F, Li W, Yang X, Na L, Chen L,Liu G. The Roles of Epigenetics Regulation in Bone Metabolism and Osteoporosis. Frontiers in cell and developmental biology 2020; 8:619301.

29. Wang A, Zhu J, Li J, Du W, Zhang Y, Cai T *et al*. Downregulation of KIAA1199 by miR-486-5p suppresses tumorigenesis in lung cancer. Cancer medicine 2020; 9(15):5570-5586.
30. Qi L, Gao C, Feng F, Zhang T, Yao Y, Wang X *et al*. MicroRNAs associated with lung squamous cell carcinoma: New prognostic biomarkers and therapeutic targets. Journal of cellular biochemistry 2019; 120(11):18956-18966.
31. Di Stefano AB, Massihnia D, Grisafi F, Castiglia M, Toia F, Montesano L *et al*. Adipose tissue, angiogenesis and angio-MIR under physiological and pathological conditions. European journal of cell biology 2019; 98(2-4):53-64.
32. Kim J, Lee C, Shin Y, Wang S, Han J, Kim M *et al*. sEVs from tonsil-derived mesenchymal stromal cells alleviate activation of hepatic stellate cells and liver fibrosis through miR-486-5p. Molecular therapy : the journal of the American Society of Gene Therapy 2020.
33. Li Q, Li H, Liang J, Mei J, Cao Z, Zhang L *et al*. Sertoli cell-derived exosomal MicroRNA-486-5p regulates differentiation of spermatogonial stem cell through PTEN in mice. Journal of cellular and molecular medicine 2021.
34. Liu HC, Han DS, Hsu CC,Wang JS. Circulating MicroRNA-486 and MicroRNA-146a serve as potential biomarkers of sarcopenia in the older adults. BMC geriatrics 2021; 21(1):86.
35. Panizo S, Naves-Díaz M, Carrillo-López N, Martínez-Arias L, Fernández-Martín JL, Ruiz-Torres MP *et al*. MicroRNAs 29b, 133b, and 211 Regulate Vascular Smooth Muscle Calcification Mediated by High Phosphorus. Journal of the American Society of Nephrology : JASN 2016; 27(3):824-834.

Figures

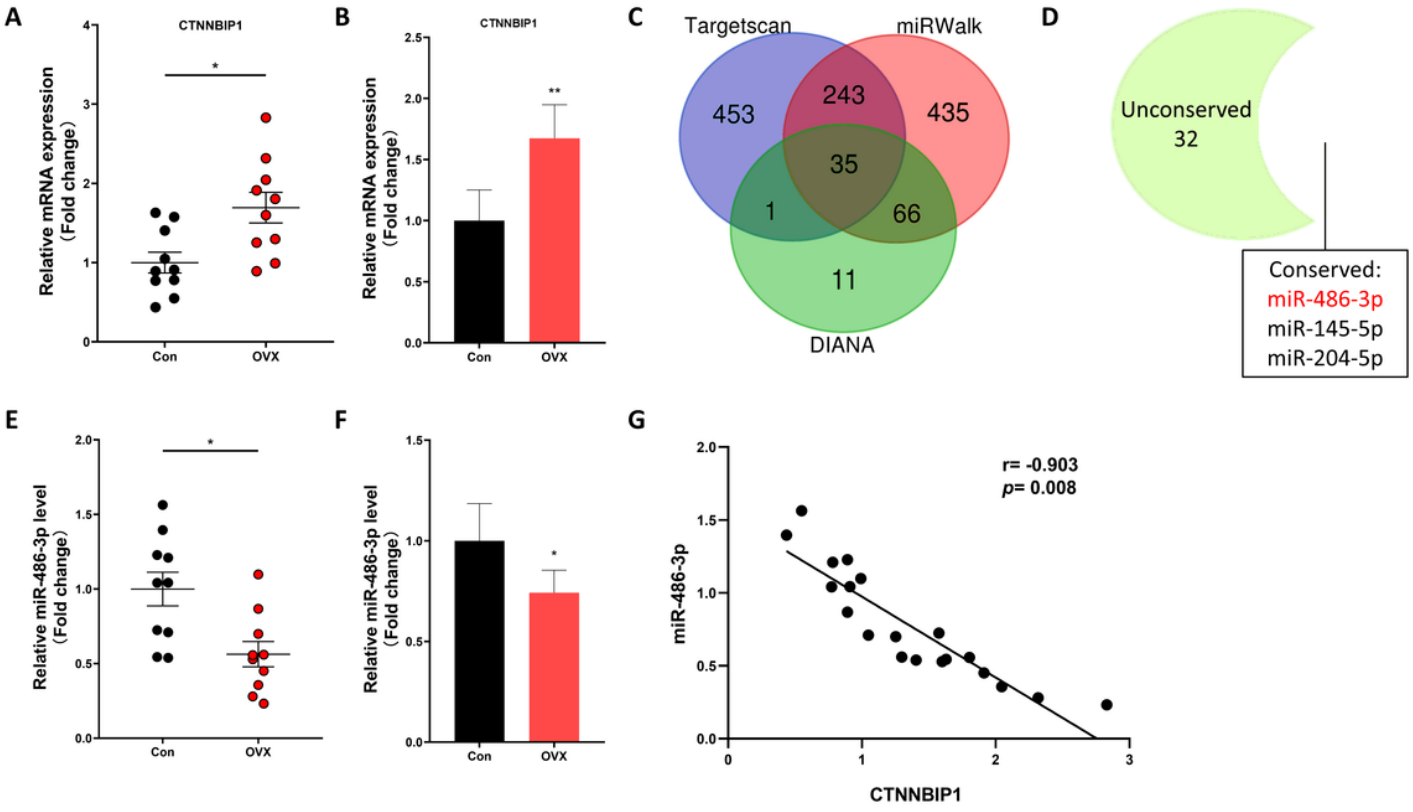

Figure 1

The expression level of CTNNBIP and miR-486-3p in mice osteoporosis model. (A) Representative μCT images of femoral metaphysis of Control and OVX group. (B) Calculations of bone value / total value (BV/TV), trabecular bone mineral density (BMD), trabecular number (Tb.N) and trabecular space (Tb.Sp). n=6. (C) Relative CTNNBIP1 mRNA expression in Con and OVX group. (D) CTNNBIP1 targeted miRNAs predicted by Targetscan, miRWalk and DIANA databases. (E) Relative miR-486-3p expression in Con and OVX group. (F) Pearson's correlation analysis between miR-486-3p and CTNNBIP1 in primary BMSCs isolated from Con and OVX mice. Data are expressed as mean ± SD. * $p < 0.05$; ** $p<0.01$; *** $p<0.001$.

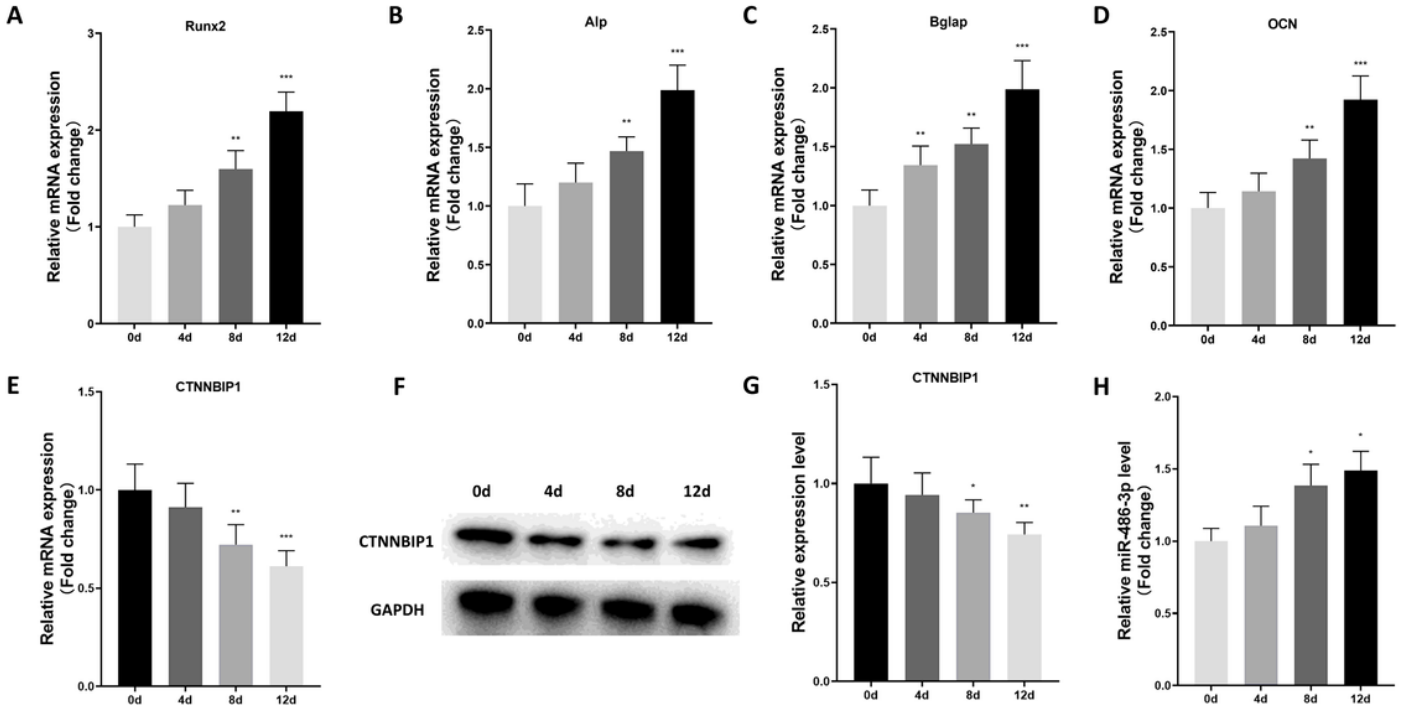

Figure 2

The expression level of CTNNBIP and miR-486-3p during osteogenic differentiation of hBMSCs. (A-D) Relative expression levels of osteogenic related genes including Runx2 (A), Alp(B), Bglap(C) and OCN (D). (E) Relative CTNNBIP1 mRNA expression during osteogenic differentiation of hBMSCs. (F, G) CTNNBIP1 protein level during osteogenic hBMSCs osteogenic differentiation measured by western blot. (H) Relative miR-486-3p expression during osteogenic differentiation of hBMSCs. Data are expressed as mean ± SD. * $p < 0.05$; ** $p<0.01$; *** $p<0.001$.

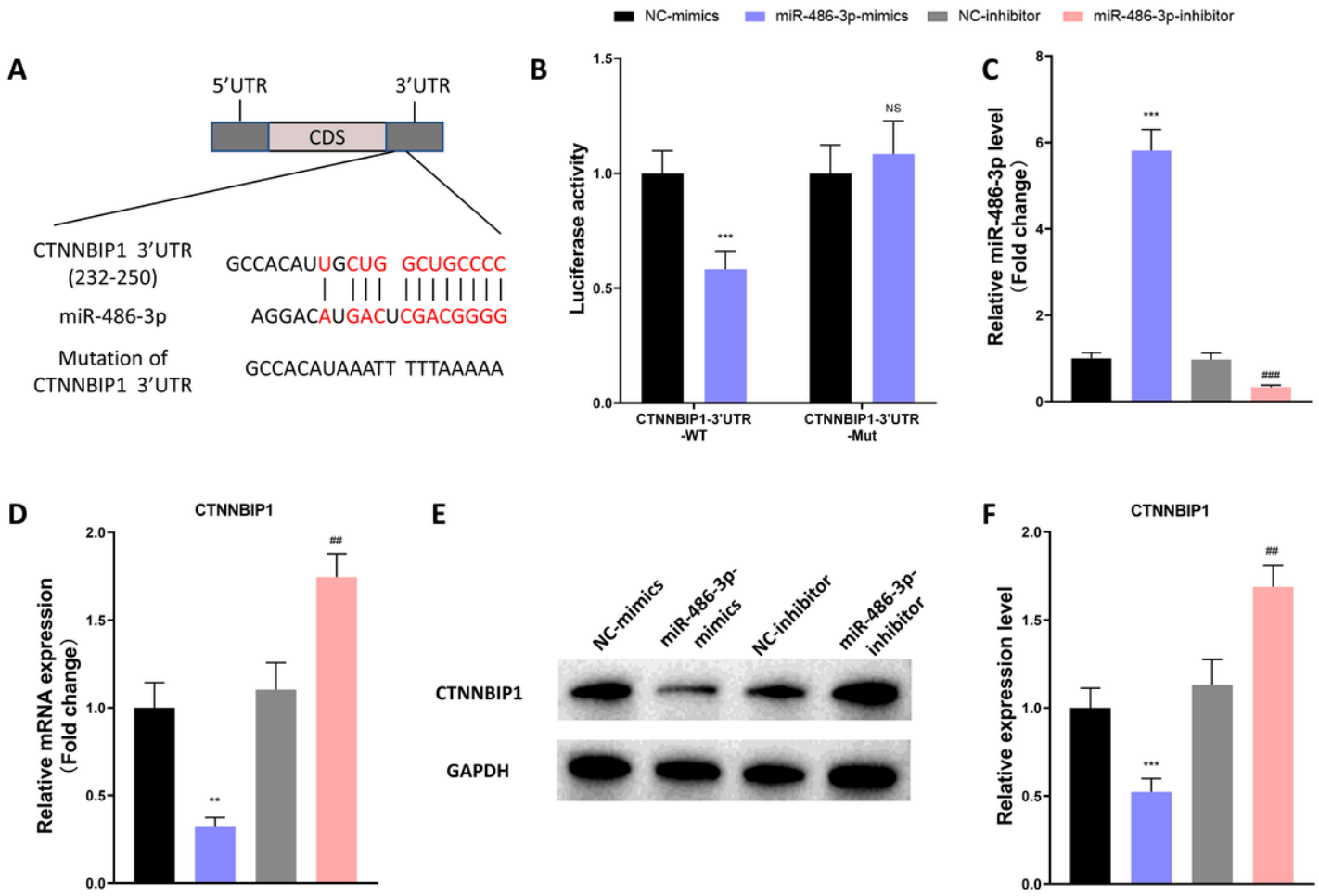

Figure 3

CTNNBIP is a direct target of miR-486-3p. (A) Binding sequences between 3'UTR of CTNNBIP1 and miR-486-3p. (B) Luciferase activity of the CTNNBIP1-WT and CTNNBIP1-Mut after transfection of NC mimics or miR-486-3p mimics. (C) Relative miR-486-3p expression after transfection of NC mimics, miR-486-3p mimics, NC inhibitor or miR-486-3p inhibitor. (D) Relative CTNNBIP1 mRNA expression after transfection of NC mimics, miR-486-3p mimics, NC inhibitor or miR-486-3p inhibitor. (E) CTNNBIP1 protein level after transfection of NC mimics, miR-486-3p mimics, NC inhibitor or miR-486-3p inhibitor measured by western blot. Data are expressed as mean ± SD. * $p < 0.05$; ** $p<0.01$; *** $p<0.001$.

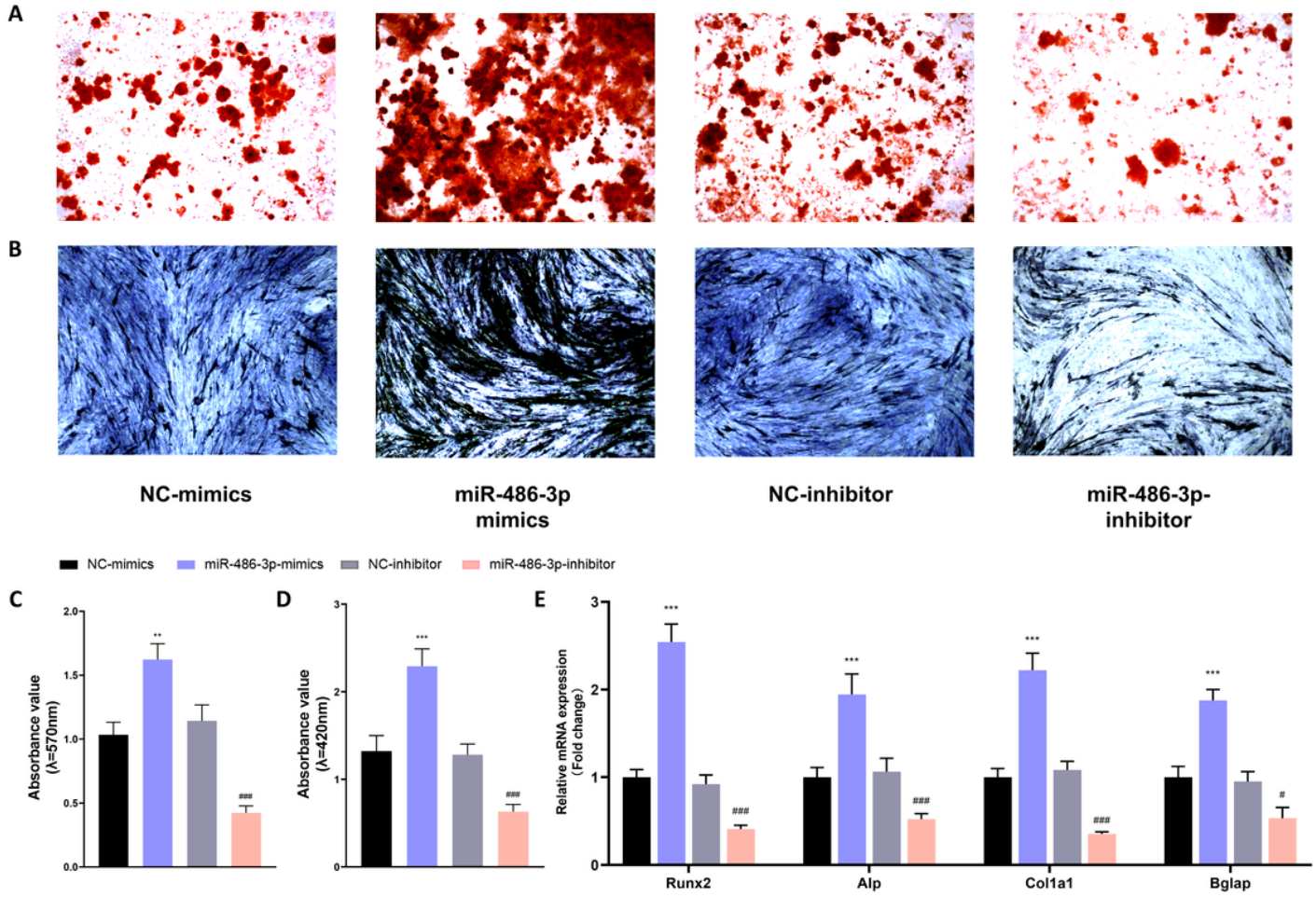

Figure 4

MiR-486-3p overexpression promotes while miR-486-3p knockdown inhibits osteogenesis of hBMSCs. Osteogenic assay was performed on hBMSCs were transfected with NC mimics, miR-486-3p mimics, NC inhibitor or miR-486-3p inhibitor. (A, C) ARS staining and its quantification. (B, D) ALP staining and quantified ALP activity quantification. (E) Relative expression of osteoblastic marker genes. Data are expressed as mean ± SD. * $p < 0.05$; ** $p<0.01$; *** $p<0.001$.

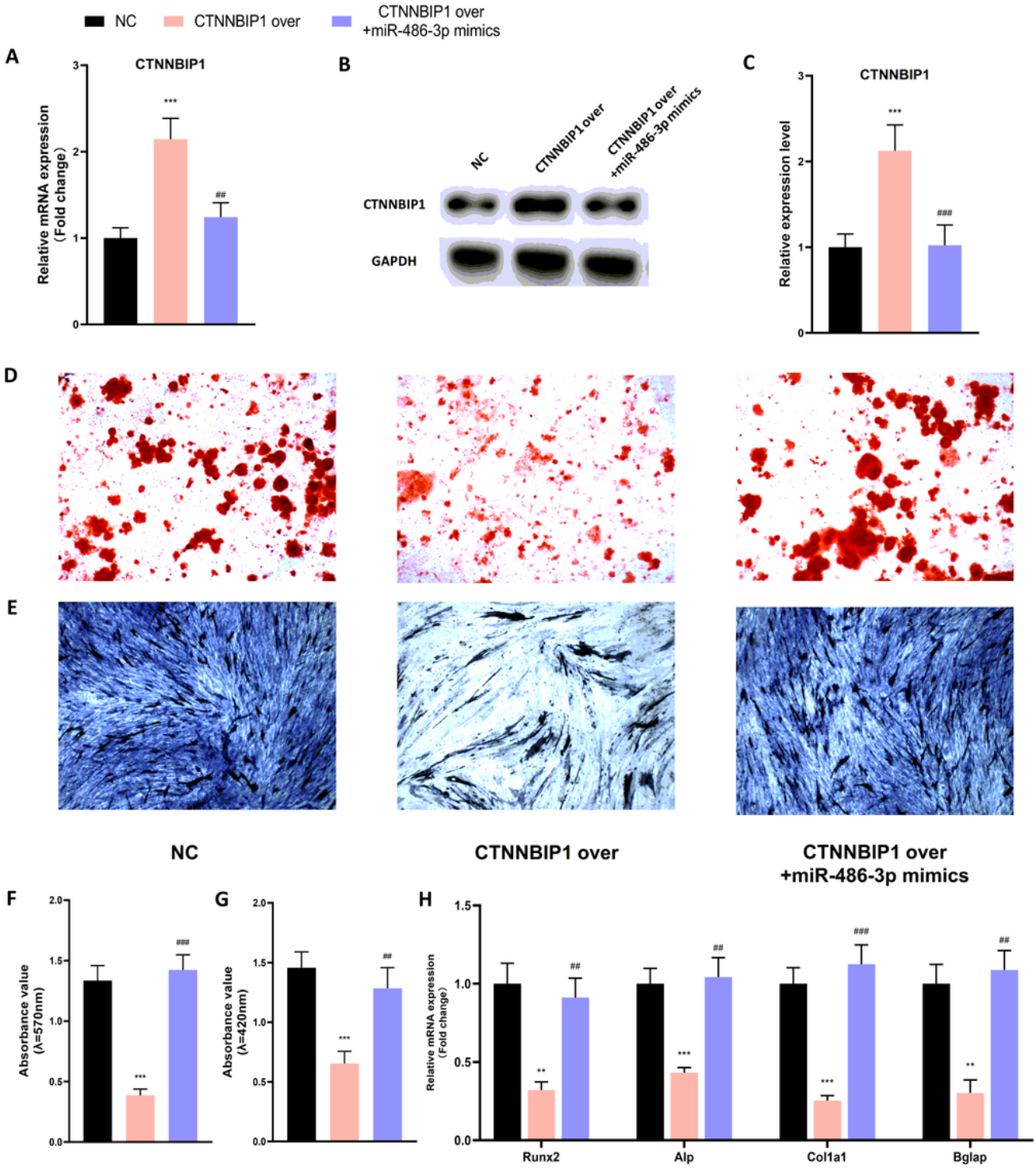

Figure 5

CTNNBIP1 overexpression impedes BMSCs osteogenic differentiation, which could be reversed by miR-486-3p. HBMSCs were transfected with NC lentivirus, CTNNBIP1 overexpression lentivirus and CTNNBIP1 overexpression lentivirus plus miR-486-3p mimics. Then osteogenic assay was performed. (A) Relative CTNNBIP1 mRNA expression after transfection. (B, C) CTNNBIP1 protein level after transfection (D, F) ARS staining and its quantification from osteogenic transfected hBMSCs. (E, G) ALP staining and

quantified ALP activity quantification from osteogenic transfected hBMSCs. (H) Relative expression of osteoblastic marker gene from osteogenic transfected hBMSCs. Data are expressed as mean ± SD. * p < 0.05; ** p<0.01; *** p<0.001.

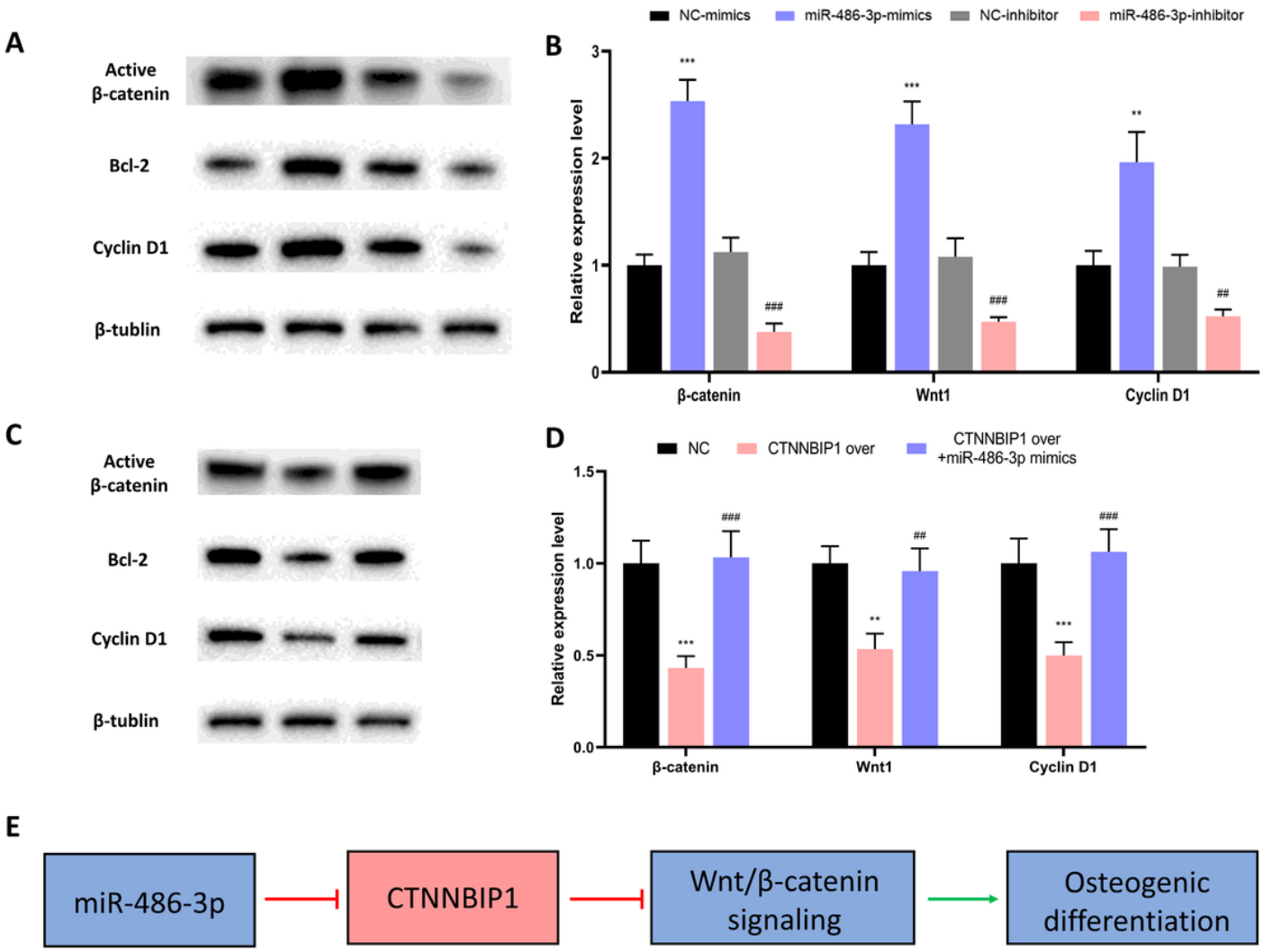

Figure 6

CTNNBIP1 blocks but miR-486-3p enhances the Wnt/β-catenin signaling. The expression of key proteins in Wnt/β-catenin including active β-catenin, Bcl-2 and Cyclin D were measured after transfection of (A, B) NC mimics, miR-486-3p mimics, NC inhibitor or miR-486-3p inhibitor or (C, D) NC lentivirus, CTNNBIP1 overexpression lentivirus and CTNNBIP1 overexpression lentivirus plus miR-486-3p mimics. (E) Proposed mechanism of miR-486-3p/CTNNBIP1 axis: miR-486-3p sponges CTNNBIP1 thus activating Wnt/β-catenin signaling pathway to promote osteogenesis of BMSCs. Data are expressed as mean ± SD. * p < 0.05; ** p<0.01; *** p<0.001.

Supplementary Files

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