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Regular paper

Circ-Sirt1 promotes osteoblast differentiation by activating Sirt1 and Wnt/β-catenin pathway

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Osteoporosis, a bone disease common in the elderly, results in bone loss and damage to bone microstructure. Sirtuin 1 (Sirt1), belongs to Sirtuin family, is involved in regulating bone quality. Circ-Sirt1 is one of the transcripts of Sirt1 host gene. Here, the involvement of circ-Sirt1 was determined in bone disease for the first time, proposing that circ-Sirt1 can activate the Wnt/β-catenin pathway to promote osteogenesis differentiation. This study was aimed to elucidate the potential function of Circ-Sirt1 and Sirt1 regulatory loop in the differentiation of bone marrow mesenchymal stem cells (BMSCs). The differentiation of bone marrow mesenchymal stem cells was detected by ALP, alizarin red staining and qPCR. The dual luciferase reporter assay was applied to reveal the interaction between RNAs. The result showed that Sirt1 promoted osteogenic differentiation of BMSCs. Circ-Sirt1, derived from Sirt1, acted as miR-132 and miR-212 sponge, and up-regulated the expression of Sirt1. Furthermore, Sirt1-mediated circ-Sirt1 promoted osteogenic differentiation. Finally, we unveiled that Circ-Sirt1 facilitates osteogenic differentiation by activating the Wnt/β-catenin pathway. In conclusion, our data suggested that Circ-Sirt1 elevates osteogenic differentiation *via* miR-132/212/Sirt1 and Wnt/β-catenin pathway.

Key word: Circ-Sirt1, Osteoporosis, Wnt/β-catenin pathway, mesenchymal stem cells (BMSCs)

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Abbreviations: BMSCs, bone marrow mesenchymal stem cells; CircRNA, Circular RNA; DPSCs, Dental pulp stem cells; NC, negative control; Sirt1, Sirtuin 1

INTRODUCTION

Osteoporosis is a systemic bone disease which has serious complications in elderly people especially elderly females. It is caused by functional disproportion between osteoblasts and osteoclasts (Liu *et al*., 2020; Zhang *et al*., 2020). Osteoblasts, responsible for bone formation, are not terminally differentiated. However, these osteoblasts can eventually differentiate into osteocytes through physiological activities such as proliferation, secretion of protein, and calcification (Chen *et al*., 2019; Chu *et al*., 2020). Inhibition of the growth and physiological activities of osteoblasts can cause numerous bone diseases, including osteoporosis, osteoarthritis, rheumatoid arthritis and osteogenesis imperfecta (Dadras *et al*., 2020; Edwards *et al*., 2013). Therefore, clarifying the regulatory mechanism of osteoblast differentiation and proliferation may possibly provide novel and effective treatment strategies for osteoporosis.

Sirt1 is a nicotinamide adenine dinucleotide (NAD+) dependent class 3 histone deacetylases which has been demonstrated as regulating lifespan in several model organisms (Koga *et al*., 2015). Sirt1 plays an important role in the physiological activities of bone tissue. It has been revealed that Sirt1 regulates SOX2 to maintain the self-renewal and pluripotency of BMSCs (Yoon *et al*., 2014). Whereas, another study reported that Sirt1 knockout mice shows a low bone mass phenotype, but the specific signaling pathway has not been elucidated (Feng *et al*., 2016). Wnt is a type of secreted protein that is rich in cysteine. It is well known that Wnt/β-catenin signaling pathway affects the differentiation balance of BMSCs in bone tissue. Meanwhile, it is also established that Sirt1 is involved in TNF-α-stimulated osteogenic differentiation of human Dental pulp stem cells (DPSCs) through Wnt/β-catenin signal (Zainabadi *et al*., 2017). As core target of cellular aging, energy metabolism and bone metabolism, Sirt1 is known to play significant role in the pathogenesis of osteoporosis. In this study, we sought to investigate the function and mechanism of Sirt1 in regulating BMSCs differentiation.

Recently, the role of non-coding RNA in osteoporosis has been gradually discovered. Circular RNA (CircRNA) is a new type of non-coding RNA that has regulatory abnormalities in a variety of cancers, frequently involving different carcinogenic processes (Lou *et al*., 2020). CircRNA is a covalent closed loop structure formed by back-splicing of pre-mRNA without 5' and 3' ends (Salzman *et al*., 2013).

With comparison of linear exosomes, the high stability of them is manifested *in vivo* with main existing in the cytoplasm and classification of exosomes (Li *et al*., 2018) Previous studies have affirmed the typical modulation of homologous mRNAs via circRNAs as nuclear transcriptional regulators, platforms for RNA-binding proteins, or cytoplasmic microRNA sponges (Li *et al*., 2015). Using the CircBase database, it was found that Sirt1 host gene may produce 11 circRNAs. However, the function and mechanism of circRNA derived from the Sirt1 host gene is still unclear in the bone related diseases, and the role of Circ-Sirt1 in the regulation of Wnt/β-catenin signaling pathway has not been fully explored.

In this study, it was found that Circ-Sirt1 derived from the Sirt1 gene promotes the expression of Sirt1 by competing with miR-132/212, and activates the Wnt/β-catenin signaling pathway to accelerate the differentiation of bone marrow mesenchymal stem cells. Therefore, Circ-Sirt1 may serve as a new biomarker and therapeutic target in bone related diseases.

MATERIAL AND METHODS

Cell culture and treatment

BMSCs (Riken Cell Bank, cell line RCB2154, Tsukuba, Japan) were cultured in α-MEM medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate, under 5% CO_2 and 37°C. Ascorbic acid (50 mg/mL), dexamethasone (0.1 mg/mL) and sodium glycerophosphate (10 mM) (Sigma-Aldrich, St. Louis, MI) were applied in the culture medium to stimulate the differentiation of osteoblasts.

RNA extraction and qPCR detection

Total RNA was extracted from BMSCs using TRNzol reagent (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. Reverse transcription kit (TIANGEN Biotech, Beijing, China) was applied to generate cDNA from total RNA. RNA concentration was determined by NanoDrop spectrophotometer (TIANGEN Biotech, Beijing, China). Real-time qPCR was conducted by using FastFire qPCR PreMix (TIANGEN Biotech, Beijing, China). Primer sequences used in this study were listed in Table 1.

ALP activity and ALP staining

To detect the osteogenic differentiation, BMSCs were cultured in osteogenic differentiation induction medium for 21 days. ALP activity was determined using an Alkaline Phosphatase Assay Kit (Beyotime, Beijing, China) by following the manufacturer's instructions.

Alizarin red staining

First, the osteogenic differentiation was induced in BMSCs for 21 days, which were then fixed the cells with 4% paraformaldehyde, and stained with 1% Alizarin Red S (Beyotime, Beijing, China) for 30 minutes. After washing with PBS for 3 times, the cells were visualized, and images were captured under an inverted microscope (Olympus Corporation, Japan). The alizarin red staining was quantified using Image J.

Table 1. The sequences of Primer were provided.

Primer sequence		
Sirt1	Forward	5′-TAGCCTTGTCAGATAAGGAAGGA-3′
	Reverse	5′-ACAGCTTCACAGTCAACTTTGT-3′
Runx2	Forward	5′-TGGTTACTGTCATGGCGGGTA-3′
	Reverse	5′-TCTCAGATCGTTGAACCTTGCTA-3′
OCN	Forward	5′-CACTCCTCGCCCTATTGGC-3′
	Reverse	5′-CCCTCCTGCTTGGACACAAAG-3′
OPN	Forward	5′-CTCCATTGACTCGAACGACTC-3′
	Reverse	5′-CAGGTCTGCGAAACTTCTTAGAT-3′
MiR-132	Forward	5′-ACACTCCAGCTGGGTAACAGTCTA-CAGCCA-3′
	Reverse	5′-TGGTGTCGTGGAGTCG-3′
MiR-212	Forward	5′-ACACTCCAGCTGGGTAACAGTCTC-CAGTC-3′
	Reverse	5′-TGGTGTCGTGGAGTCG-3′
U6	Forward	5′-CTCGCTTCGGCAGCACA-3′
	Reverse	5′-AACGCTTCACGAATTTGCGT-3′
Circ-Sirt1	Forward	5′-TATGACACTGTGGCAGATTG-3′
	Reverse	5′-TTTCATCACCGAACAGAAGG-3′

Luciferase reporter assay

The 3'UTR of circ-Sirt1 or Sirt1 containing miR-132 or miR-212 binding sites was amplified by PCR, cloned into the pgl3 promoter vector of Xba I (circ-Sirt1 WT or Sirt1 WT), The mutant sequence was cloned into pgl3 promoter vector to construct circ-Sirt1 MUT or Sirt1 MUT. Twenty-four hours before transfection, cells were trypsinized (2×10^5 cells/mL) and then plated on 24-well plates. The above plasmids together with Renilla (pRLTK) plasmid, miR-132 mimics, miR-212 mimics, negative control (NC) mimics plasmids were transfected into cells using Lipofectamine 2000 (Invitrogen). After 6 h, the medium was replaced with serum-containing medium, and 48 h after transfection, cells were collected to detect dual-luciferase activity using dual-luciferase assay system (Promega, USA). The relative luciferase activity was calculated from the ratio of firefly to Renilla luciferase signals.

RNA pull down assay

The MagCapture™ RNA Pull Down Kit (whatman, England) was applied for RNA pull-down assay. In brief, BMSCs were washed with precooled PBS, and then incubated with beads and biotinylated miR-132/212 probes for 1 hour. Western blot analysis was used to detect proteins.

Western Blot

Total protein was extracted from BMSCs using RIPA buffer containing 1mM Phenylmethylsulfonyl fluoride (PMSF). BCA kit (Beyotime, Beijing, China) was applied to determine protein concentration. The protein was separated using SDS-PAGE (10% or 12%), and then transferred to PVDF membrane (Millipore, Danvers, MA, USA), and then incubated with primary antibodies Sirt1 (1:1000; ab110304; Abcam), β-catenin (1:1000; ab32572; Abcam), Lamib B1 (1:1000; ab16048; Abcam), β-Tubulin (1:1000; ab6046; Abcam) at 4°C overnight. Later, membranes were washed, and incubated with respective secondary antibodies HRP (anti-rabbit IgG, 1:5000, Cell Signaling Technology, Inc., MA, USA). Finally, the membranes were exposed using ECL system (Beyotime, Beijing, China), and protein bands were analyzed using Quantity One software.

Statistical analyses

Data were analyzed with Graphpad Prism 8 software and expressed as mean ± S.D. One-way ANOVA followed up with a post hoc test was performed for multiple group comparisons. T test was used for comparison between the two groups. $P<0.05$ was considered as statistically significant.

RESULT

Sirt1 promotes BMSCs differentiation

Firstly, it was observed that the mRNA expression of Sirt1 was gradually increased during the differentiation of BMSCs (Fig. 1A), indicating that Sirt1 might play vital role in the differentiation of BMSCs. To evaluate the function of Sirt1 in BMSCs, pcDNA3.1-Sirt1 was used to over-ex-

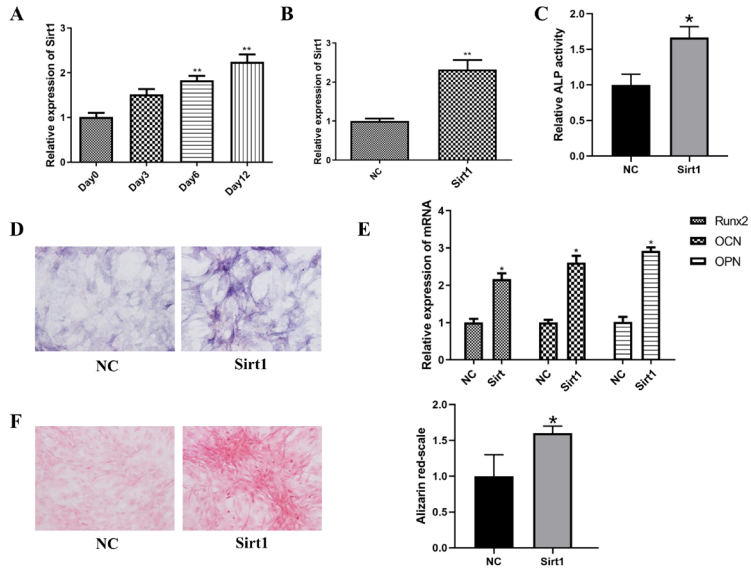

Figure 1. Sirt1 promotes BMSCs differentiation.
(**A**) The relative expression of Sirt1 in the differentiation of BMSCs was detected by qRT-PCR on the 3rd, 6th and 12th day. (**B**) The qRT-PCR was used to detect the overexpression efficiency 24 hours after Sirt1 transfection. (**C**) After overexpression of Sirt1 for 24 hours, the relative activities of alkaline phosphatase in BMSCs were measured with the ALP kit. (**D**) Sirt1 was overexpressed in BMSCs for 24 hours and stained with ALP. (**E**) After overexpression of Sirt1 in BMSCs, qRT-PCR was used to detect the mRNA levels of Runx2, OCN and OPN. (**F**) Sirt1 overexpressed in BMSCs for 24 hours was stained with alizarin red. All results are expressed as mean ± S.D., n=3. **P*<0.05, ***P*<0.01

press Sirt1 (Fig. 1B). Interestingly, over-expression of Sirt1 apparently increased ALP activity and ALP staining intensity in BMSCs as shown in Fig.1C-D. Moreover, osteogenic markers Runx2, OCN and OPN were obviously elevated *via* upregulation of Sirt1 (Fig. 1E). Alizarin red staining showed that BMSCs overexpressing Sirt1 was more likely to develop calcified nodules (Fig. 1F). These data indicated that Sirt1 facilitated osteoblastic differentiation in BMSCs. To study the effect of Sirt1 on Wnt/β-catenin signaling pathway, Western Blot experiment found that β-catenin in cytoplasm and nucleus was also detected after knockdown of Sirt1, affirming that β-catenin in cytoplasm was elevated, while in nucleus was reduced. To further confirm that Sirt1 promoted the nuclear translocation of β-catenin, immunofluorescence experiments was conducted, finding that the immunocytochemical strength of β-catenin in the nucleus of si-Sirt1 group was declined. In short, Sirt1 activates the Wnt/βcatenin pathway to facilitate the nuclear translocation of β-catenin during the osteogenic differentiation of BMSCs (Attached Fig. 1B, C), suggesting that Sirt1 accelerates

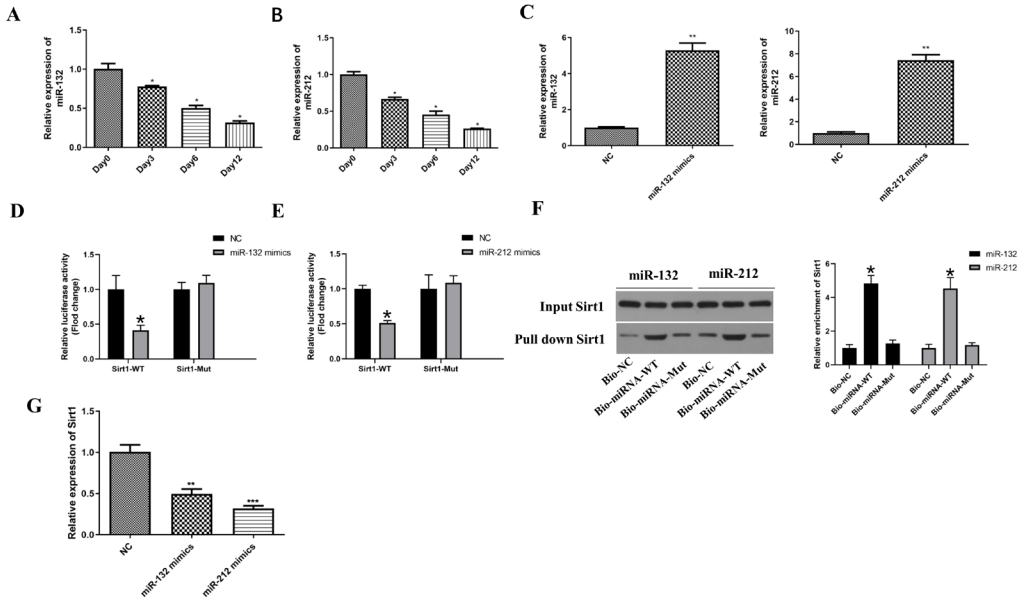

Figure 2. Sirt1 is the direct target of miR-132/212.
(**A, B**) Relative level of miR-132/212 during BMSCs differentiation on the 0, 3rd, 6th and 12th day was detected through qRT-PCR. (**C**) miR-132/212 was overexpressed in BMSCs for 24 hours, and the overexpression efficiency was detected by qRT-PCR. (**D, E**) Effects of Sirt1 luciferase activity were detected by transfection of miR-132/212 mimic in BMSCs for 24 hours. (**F**) RNA pull down detected Sirt1 level by incubating with bio-miR-132/212 (WT or Mut) for 24 hours. (**G**) The effect of overexpression of miR-132/212 for 24 hours on Sirt1 expression in BMSCs was detected by qRT-PCR. All results are expressed as mean ± S.D., n=3. **P*<0.05, ***P*<0.01

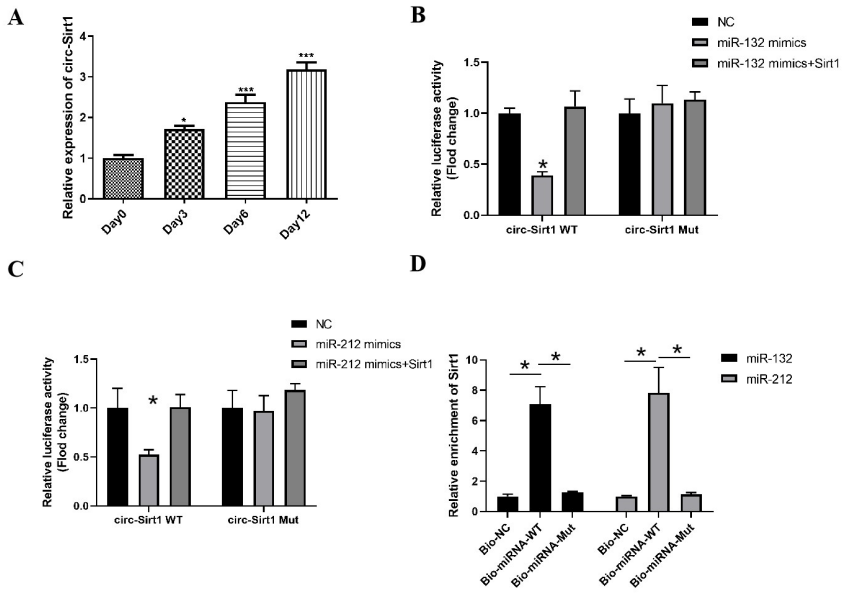

Figure 3. The miR-132/212 are sponged by circ-Sirt1.
(**A**) Relative expression of circ-Sirt1 in BMSC differentiation on the 0, 3rd, 6th and 12th day was detected through qRT-PCR. (**B, C**) The luciferase activities of WT and Mut circ-Sirt1 were detected 24 hours after overexpression of miR-132/212. (**D**) RNA pull down detected circ-Sirt1 level by incubating with bio-miR-132/212 (WT or Mut) for 24 hours. All results are expressed as mean ± S.D., n=3. **P*<0.05, ***P*<0.01

osteogenic differentiation of BMSCs through the Wnt/β catenin pathway.

MiR-132/212 binds to Sirt1 in BMSCs

Next, the binding of upstream miRNAs to Sirt1 was explored. The relative expression of miR-132/212 was decreased during osteogenic differentiation of BMSCs in a time-dependent manner (Fig. 2A–B). Then, miR-132/212 mimics was used to increase the expression of miR-132/212 in BMSCs (Fig. 2C). In order to further investigate the interaction of miR-132/212 and Sirt1, miR-132/212 mimics or mimics-NC was co-transfected with wt-Luc-Sirt1 or mut-Luc-Sirt1 in BMSCs. Our results showed that overexpression of miR-132/212 can clearly inhibit the luciferase activity of Sirt-WT, while overexpression of miR-132/212 had no effect on the relative luciferase activity of Sirt1-MUT in BMSCs (Fig. 2D–E). In addition, RNA pull down assay showed that biotinylated miR-132/212-WT clearly pull down the Sirt1 mRNA, while miR-132/212-MUT did not (Fig. 2F). Finally, it was revealed that miR-123/212 up-regulation apparently reduced the expression of Sirt1 (Fig. 2G). Thus, it was concluded that miR-132/212 reduced the expression of Sirt1 by directly targeting the Sirt1 3'UTR.

MiR-132/212 is sponged by circ-Sirt1 in BMSCs

It was previously reported that miR-132 and miR-212 are sponged by circ-Sirt1 in vascular smooth muscle cells (VSMCs) (Kong *et al*., 2019). The detection for circRNAs candidates from Sirt1 host genes in the human genome was conducted. Meanwhile, the identification of three circRNAs (hsa_circ_0093883, hsa_circ_0093887, hsa_circ_0093890) from Sirt1 host genes was exposed in human BMSCs (attached Fig. 1A). The up-regulation of Sirt1 *via* circ-Sirt1 was assured in BMSCs by sponging miR-132/212. As shown in Fig. 3A, it was found the elevation of circ-Sirt1 during osteogenic differentiation of BMSCs in a time-dependent manner. It was further determined that the ectopic expression of miR-132/212 reduced the relative luciferase activity of circ-Sirt1-WT, while Sirt1 overexpression further enhanced it. Whereas, similar experimental conditions had not affected the relative luciferase activity of MUT-circ-Sirt1 (Fig. 3B–C). RNA pull down assay manifested that circ-Sirt1 was clearly pulled down *via* biotinylated miR-132/212-WT, while not affected *via* miR-132/212-MUT (Fig. 3D). Taken together, these results indicated that miR-132/212 was sponged by circ-Sirt1 in BMSCs.

Sirt1 plays an essential role in circ-Sirt1-promoted osteoblastic differentiation

To further examine the effect of Sirt1 in circ-Sirt1 dependent BMSCs differentiation, sh-Sirt1 was applied to transfect BMSCs. Our results showed that silencing of Sirt1 reduced the stimulatory effects of up-regulatory circ-Sirt1 on ALP activity and staining intensity as shown in Fig. 4A–B. Moreover, silencing of Sirt1 also restored the expression of Runx2, OCN and OPN upregulation as induced by circ-Sirt1 upregulation (Fig. 4C–E). These data suggested that Sirt1 played an essential role in the BMSC differentiation as regulated by circ-Sirt1.

Circ-Sirt1 promotes BMSCs differentiation by activating Wnt/β-catenin pathway

Finally, it was investigated whether the Wnt/β-catenin pathway mediated circ-Sirt1 dependent BMSCs differentiation, finding that β-catenin was apparently declined via silencing of circ-Sirt1 in the nucleus, while an opposite trend manifested in the cytoplasm (Fig. 5A). The detection results of TOP/FOP Flash showed that silencing of circ-Sirt1 obviously reduced the TOP luciferase activity in BMSCs (Fig. 5B), indicating that silencing circ-Sirt1 inhibited Wnt/β-catenin pathway. Finally, it was found that WIF-1 (Wnt/β-catenin pathway inhibitor) treatment obviously reduced the stimulatory effects of circ-Sirt1 up-regulation on osteogenic differentiation of BMSCs in Fig. 5C–H. In summary, these results indicated that circ-Sirt1 up-regulates Sirt1 through miR-132/212, and activates Wnt/β-catenin pathway to promote BMSCs differentiation.

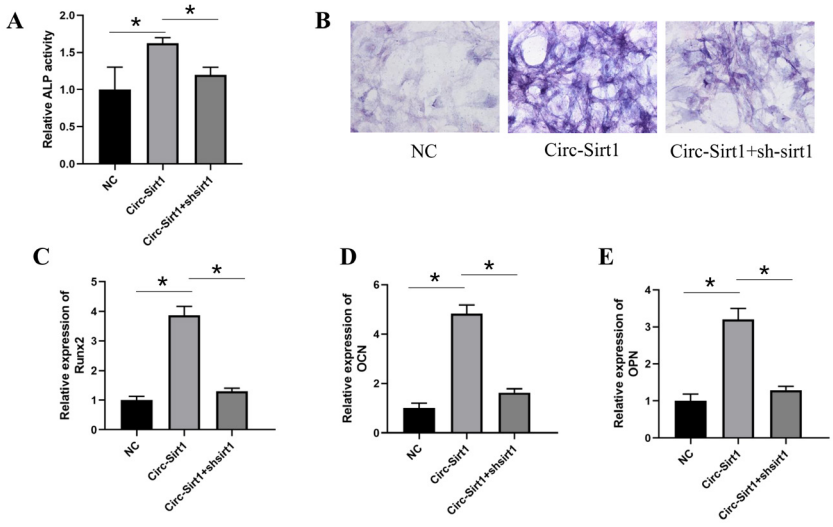

Figure 4. Sirt1 is essential in Circ-Sirt1-induced BMSC differentiation.
(**A, B**) The ALP activity and ALP staining of BMSCs were detected 24 hours after transfection of circ-Sirt1 and sh-Sirt1. (**C–E**) The mRNA levels of osteogenic differentiation marker were detected 24 hours after transfection of circ-Sirt1 and sh-Sirt1. All results are expressed as mean ± S.D., n=3. **P*<0.05, ***P*<0.01.

DISSCUSSION

In this study, it was uncovered that Circ-Sirt1 promoted osteoblast differentiation by activating Sirt1 and Wnt/β-catenin pathway (Fig. 6). It has been determined that Sirt1 is widely involved in the physiological activities of bone tissue, and the biological functions of bone cells (Qu *et al.*, 2020). Inhibition of Sirt1 accelerates the acetylation and ubiquitination of SOX2, and represses the osteogenic differentiation of BMSCs. In current study, the

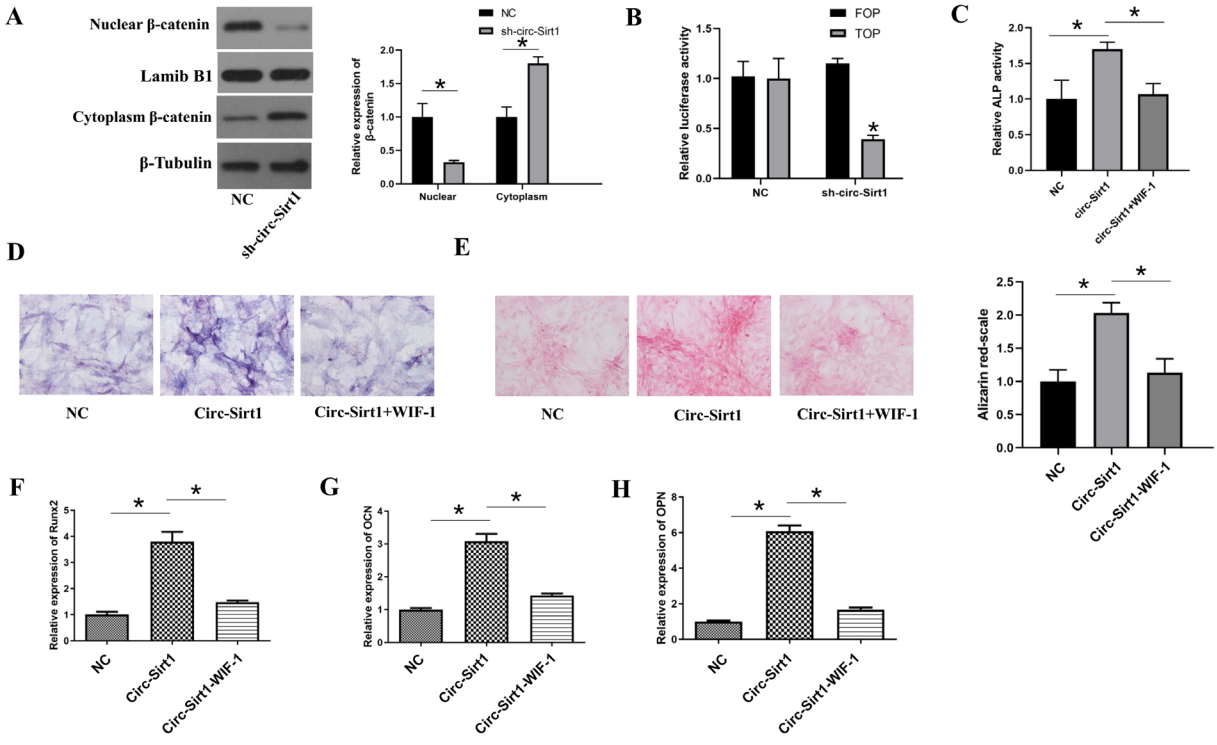

Figure 5. Circ-Sirt1 regulates BMSC differentiation via regulate Wnt/β-catenin pathway.
(**A**) Relative expression of nuclear β-catenin and cytoplasm β-catenin were detected by Western blot 24 hours after transfection of sh-circ-Sirt1 in BMSCs. (**B**) The relative activity of TOP and FOP luciferase were detected 24 hours after transfection of sh-circ-Sirt1 in BMSCs. (**C–D**) ALP activity and ALP staining were detected in BMSCs treated with circ-Sirt1 and WIF-1 for 24 hours. E. Alizarin red was detected in BMSCs treated with circ-Sirt1 and WIF-1 for 24 hours. (**F–H**) The mRNA levels of osteogenic differentiation marker were detected in BMSCs treated with circ-Sirt1 and WIF-1 for 24 hours. All results are expressed as mean ± S.D., n=3. **P*<0.05, ***P*<0.01.

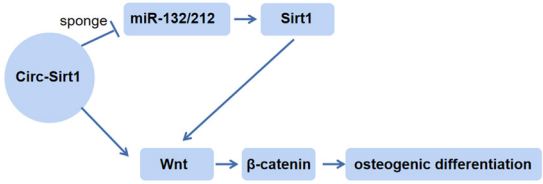

Figure 6. Schematic diagram of circ-Sirt1 activating Wnt/β catenin signaling pathway through direct and indirect mechanisms

role and molecular mechanism of Sirt1 was explored in BMSCs differentiation, affirming that Sirt1 was elevated with BMSCs differentiation. Moreover, miR-132 and miR-212 restrain the Sirt1 dependent BMSCs differentiation.

Increasing evidence showed that host genes can be regulated by circRNA which are formed by non-classical splicing. For example, circGFRA1 regulates GFRA1 in breast cancer by sponging miR-34a (Bai *et al.*, 2018). Similarly, CircFBLIM1 promotes HCC progression by sponging miR-346 (He *et al.*, 2017). It has been studied that Circ-Sirt1 enhances Sirt1 expression through sequence-specific interaction with miR-132/212 binding in Vascular smooth muscle cells. Therefore, the study was aimed to investigate if Circ-Sirt1, spliced from Sirt1, could sponge miR-132/212 to up-regulate Sirt1 in BMSCs. Sirt1 and miR-132/212 have been closely associated with human aging and Alzheimer's disease (Hernandez *et al.*, 2016).However, the function of miR-132/212 in osteoporosis remains ambiguous. In this study, it was confirmed that circ-Sirt1 can sponge miR-132/212 as the ceRNA of Sirt1. Circ-Sirt1 promoted BMSCs differentiation, and the silencing of Sirt1 inhibited the stimulatory effects of circ-Sirt1 on the differentiation of BMSCs.

Previous studies reported that Sirt1 could trigger the Wnt/β-catenin pathway (Hwang *et al.*, 2015). β-catenin, the core molecule of Wnt/β-catenin pathway, is widely implicated with osteoblast differentiation and proliferation (Feng *et al.*, 2020). demonstrated that microRNA-378 inhibits osteogenesis and bone formation damage of BMSC by inactivating the Wnt/β-catenin signaling pathway (Wang *et al.*, 2021). Whereas, miR-130a-3p regulates Wnt/β-catenin pathway to induce osteogenic differentiation among adipose stem cells (Yang *et al.*, 2020). Additionally, a study has manifested the participation of Sirt1 in osteoblast differentiation through Wnt/β-catenin signaling in endodontic stem cells. . The first detection of β-catenin in cytoplasm and nucleus in this study was conducted after Sirt1 knockdown, affirming up-regulation of β-catenin in cytoplasm and decline in nucleus. Furthermore, the promotion of the nuclear translocation of β-catenin via Sirt1 was determined. Moreover, elevation of β-catenin in the cytoplasm and reduction in the nucleus via down-regulation of circ-Sirt1 were founded. Briefly, our experimental results demonstrated that circ-Sirt1 elevates BMSCs differentiation by activating Wnt/β-catenin signaling pathway.

In summary, our study demonstrated that circ-Sirt1 up-regulates Sirt1 as a sponge for miR-132/212, and activates the Wnt/β-catenin pathway to promote osteoblastic differentiation of BMSCs, suggesting that circ-Sirt1 may be associated with bone formation. Circ-Sirt1 activates the Wnt/βcatenin signaling pathway through direct and indirect mechanisms, respectively. However, the direct and indirect effects of circ-Sirt1 are performed on Wnt/β catenin signaling pathway, and of them which is the main way remains to be further studied. The possible application of circ-Sirt1 in clinic needs further study. Clinical samples and in vivo experiments will be further explored with the permission of experimental conditions later.Overall, these findings provide novel directions and insights into potential osteoporosis therapeutics.

Declarations

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Conflict of interest. All authors declare no conflict of interest

REFERENCE

Bai N, Peng E, Qiu X, Lyu N, Zhang Z, Tao Y, Li X, Wang Z (2018) circFBLIM1 act as a ceRNA to promote hepatocellular cancer progression by sponging miR-346. *J Exp Clin Cancer Res* **37**: 172. https://doi.org/10.1186/s13046-018-0838-8.

Chen PC, Liu JF, Fong YC, Huang YL, Chao CC, Tang CH (2019) CCN3 Facilitates Runx2 and osterix expression by inhibiting miR-608 through PI3K/Akt signaling in osteoblasts. *Int J Mol Sci* **20**. https://doi.org/10.3390/ijms20133300.

Chu Y, Gao Y, Yang Y, Liu Y, Guo N, Wang L, Huang W, Wu L, Sun D, Gu W (2020) β-catenin mediates fluoride-induced aberrant osteoblasts activity and osteogenesis. *Environ Pollut* (Barking, Essex: 1987). **265** (Pt A): 114734. https://doi.org/10.1016/j.envpol.2020.114734.

Dadras M, May C, Wagner JM, Wallner C, Becerikli M, Dittfeld S, Serschnitzki B, Schilde L, Guntermann A, Sengstock C, Köller M, Seybold D, Geßmann J, Schildhauer TA, Lehnhardt M, Marcus K, Behr B (2020) Comparative proteomic analysis of osteogenic differentiated human adipose tissue and bone marrow-derived stromal cells. *J Cell Mol Med* **24**: 11814–11827. https://doi.org/10.1111/jcmm.15797.

Edwards JR, Perrien DS, Fleming N, Nyman JS, Ono K, Connelly L, Moore MM, Lwin ST, Yull FE, Mundy GR, Elefteriou F (2013) Silent information regulator (Sir)T1 inhibits NF-κB signaling to maintain normal skeletal remodeling. *J Bone Mineral Res* **28**: 960–969. https://doi.org/10.1002/jbmr.1824.

Feng G, Zheng K, Song D, Xu K, Huang D, Zhang Y, Cao P, Shen S, Zhang J, Feng X, Zhang D (2016) SIRT1 was involved in TNF-α-promoted osteogenic differentiation of human DPSCs through Wnt/β-catenin signal. *In Vitro Cell Dev Biol Anim* **52**: 1001–1011. https://doi.org/10.1007/s11626-016-0070-9.

Feng L, Zhang JF, Shi L, Yang ZM, Wu TY, Wang HX, Lin WP, Lu YF, Lo JHT, Zhu DH, Li G (2020) MicroRNA-378 suppressed osteogenesis of MSCs and impaired bone formation *via* inactivating Wnt/β-catenin signaling. *Mol Therapy Nucleic Acids* **21**: 1017–1028. https://doi.org/10.1016/j.omtn.2020.07.018.

He R, Liu P, Xie X, Zhou Y, Liao Q, Xiong W, Li X, Li G, Zeng Z, Tang H (2017) circGFRA1 and GFRA1 act as ceRNAs in triple negative breast cancer by regulating miR-34a. *J Exp Clin Cancer Res* **36**: 145. https://doi.org/10.1186/s13046-017-0614-1.

Hernandez-Rapp J, Rainone S, Goupil C, Dorval V, Smith PY, Saint-Pierre M, Vallée M, Planel E, Droit A, Calon F, Cicchetti F, Hébert SS (2016) microRNA-132/212 deficiency enhances Aβ production and senile plaque deposition in Alzheimer's disease triple transgenic mice. *Sci Rep* **6**: 30953. https://doi.org/10.1038/srep30953.

Hwang JH, Cha PH, Han G, Bach TT, Min do S, Choi KY (2015) Euodia sutchuenensis Dode extract stimulates osteoblast differentiation *via* Wnt/β-catenin pathway activation. *Exp Mol Med* **47**: e152. https://doi.org/10.1038/emm.2014.115.

Koga T, Suico MA, Shimasaki S, Watanabe E, Kai Y, Koyama K, Omachi K, Morino-Koga S, Sato T, Shuto T, Mori K, Hino S, Nakao M, Kai H (2015) Endoplasmic reticulum (ER) stress induces sirtuin 1 (SIRT1) expression *via* the PI3K-Akt-GSK3β signaling pathway and promotes hepatocellular injury. *J Biol Chem* **290**: 30366–3074. https://doi.org/10.1074/jbc.M115.664169.

Kong P, Yu Y, Wang L, Dou YQ, Zhang XH, Cui Y, Wang HY, Yong YT, Liu YB, Hu HJ, Cui W, Sun SG, Li BH, Zhang F, Han M (2019) circ-Sirt1 controls NF-κB activation *via* sequence-specific interaction and enhancement of SIRT1 expression by binding to miR-132/212 in vascular smooth muscle cells. *Nucleic Acids Res* **47**: 3580–3593. https://doi.org/10.1093/nar/gkz141.

Li X, Yang L, Chen LL (2018) The biogenesis, functions, and challenges of circular RNAs. *Mol Cell* **71**: 428–442. https://doi.org/10.1016/j.molcel.2018.06.034.

Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, Chen D, Gu J, He X, Huang S (2015) Circular RNA is enriched and stable in exosomes:

a promising biomarker for cancer diagnosis. *Cell Res* **25**: 981–984. https://doi.org/10.1038/cr.2015.82.

Liu Z, Li C, Huang P, Hu F, Jiang M, Xu X, Li B, Deng L, Ye T, Guo L (2020) CircHmbox1 targeting miRNA-1247-5p is involved in the regulation of bone metabolism by TNF-α in postmenopausal osteoporosis. *Frontiers Cell Develop Biol* **8**: 594785. https://doi.org/10.3389/fcell.2020.594785.

Lou J, Hao Y, Lin K, Lyu Y, Chen M, Wang H, Zou D, Jiang X, Wang R, Jin D, Lam EW, Shao S, Liu Q, Yan J, Wang X, Chen P, Zhang B, Jin B (2020) Circular RNA CDR1as disrupts the p53/MDM2 complex to inhibit gliomagenesis. *Mol Cancer* **19**: 138. https://doi.org/10.1186/s12943-020-01253-y.

Qu B, He J, Zeng Z, Yang H, Liu Z, Cao Z, Yu H, Zhao W, Pan X (2020) MiR-155 inhibition alleviates suppression of osteoblastic differentiation by high glucose and free fatty acids in human bone marrow stromal cells by upregulating SIRT1. *Pflugers Archiv* **472**: 473–480. https://doi.org/10.1007/s00424-020-02372-7.

Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO (2013) Cell-type specific features of circular RNA expression. *PLoS Genet* **9**: e1003777. https://doi.org/10.1371/journal.pgen.1003777.

Wang K, Zhao Z, Wang X, Zhang Y (2021) BRD4 induces osteogenic differentiation of BMSCs *via* the Wnt/β-catenin signaling pathway. *Tissue Cell* **72**: 101555. https://doi.org/10.1016/j.tice.2021.101555.

Yang S, Guo S, Tong S, Sun X (2020) Exosomal miR-130a-3p regulates osteogenic differentiation of Human Adipose-Derived stem cells through mediating SIRT7/Wnt/β-catenin axis. *Cell Prolifer* **53**: e12890. https://doi.org/10.1111/cpr.12890.

Yoon DS, Choi Y, Jang Y, Lee M, Choi WJ, Kim SH, Lee JW (2014) SIRT1 directly regulates SOX2 to maintain self-renewal and multipotency in bone marrow-derived mesenchymal stem cells. *Stem Cells* (Dayton, Ohio) **32**: 3219–3231. https://doi.org/10.1002/stem.1811.

Zainabadi K, Liu CJ, Caldwell ALM, Guarente L (2017) SIRT1 is a positive regulator of *in vivo* bone mass and a therapeutic target for osteoporosis. *PloS One* **12**: e0185236. https://doi.org/10.1371/journal.pone.0185236.

Zhang L, Xie H, Li S (2020) LncRNA LOXL1-AS1 controls osteogenic and adipocytic differentiation of bone marrow mesenchymal stem cells in postmenopausal osteoporosis through regulating the miR-196a-5p/Hmga2 axis. J Bone Mineral Metabol **38**: 794–805. https://doi.org/10.1007/s00774-020-01123-z.