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Original Article

Quercetin stimulates osteogenic differentiation of bone marrow stromal cells through miRNA-206/connexin 43 pathway

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Abstract: The quantity and function of osteoblasts require continuous osteogenic differentiation of bone marrow mesenchymal stem cells. Recent evidence suggests that microRNAs (miRNAs) act as important post-transcriptional regulators in a wide range of biological processes, including osteoblastic differentiation. Quercetin has also been found to prevent bone loss. In this study, we investigated the osteogenesis of quercetin and miR-206 on bone marrow mesenchymal stem cells (BMSCs) and their relationship. We observed quercetin enhanced BMSCs proliferation with a dose-dependent manner in Cell Counting Kit-8 (CCK-8). Alizarin red S staining, alkaline phosphatase (ALP) quantification assay, miR-206 and mRNA levels of osteogenesis marker genes by quantitative real-time PCR (qPCR) were used to analyze osteogenic potential. We observed quercetin significantly elevated bone mineralization and the mRNA expression levels of osteoblast-specific genes including Runt-related transcription factor 2 (Runx2), Osterix (OSX), osteocalcin (OCN), and osteopontin (OPN). Correspondly, Cx43 expression were increased, while miR-206 expression were decreased. In the presence of agomir of miR-206, effects of quercetin on mineralization, alkaline phosphatase activity and osteoblast-specific genes expression were suppressed. Most of all, Cx43 protein level was also blocked while overexpression of miR-206 against quercetin effects. Taken together, these data indicated that quercetin promotes BMSCs proliferation and osteogenic differentiation. The osteogenic effect of quercetin is partly modulated through miR-206/Cx43 pathway.

Keywords: Quercetin, osteogenic differentiation, bone marrow stromal cells, miR-206, connexin 43

Introduction

Bone mesenchymal stem cells (BMSCs), which is a multipotent type of adult stem cell that derived from the bone marrow, has the capacity for self-renewal, proliferative potential and the ability to differentiate into multilineage cells, including osteoblasts, chondrocytes, adipocytes, myoblasts and neurons [1, 2]. Previous research indicates that it is a competing and reciprocal manner between osteoblastogenic and adipogenic differentiation of BMSCs. Integrity of structure and function of bone are mainly maintained by the balance between adipogenic and osteogenic differentiation of BMSCs, which is biased towards the osteogenic differentiation [3, 4]. However, under the pathological class such as osteoporosis, this original balance of differentiation balance is disrupted, resulting in a differentiation malformation and enhanced adipocyte differentiation at the expense of osteoblast differentiation [5, 6]. The disturbance in osteoblast differentiation at last gives rise to decreased osteoblast number and consequent low bone formation is considered to numerous diseases related chronic bone loss [7]. Therefore, it can be inferred that specific enhancement of osteogenesis of BMSCs may provide a potential therapeutic approach for several diseases associated with osteopenic disorders.

It's well-established in recent decades, a number of critical signals and transcription factors are dentified as key determinants in the process of osteoblastogenic or adipogenic differentiation of BMSCs [8]. Changes in the expression of these factors and moderation in the

activation of these signals are associated with bone formation or bone loss. For example, the activation of Wnt signaling and bone morphogenic proteins are essential for osteoblast differentiation during bone formation. Conversely, Notch signaling is suppressed during BMSCs into pre-osteoblastic cells [9]. Additionally, increased expression of runt-related transcription factor 2 (Runx2), a master osteoblastic transcription factor, is associated with osteogenesis [10]. miRNAs are a novel class of small endogenous and noncoding RNAs that govern gene expression by degrading mRNA or by inhibiting translation. Recently, miRNAs have been demonstrated to be closely linked to different biological processes, including cell proliferation, differentiation, activity, apoptosis, and metabolism [11]. MiR-206, previously known as a key muscle-specific miRNA, is downregulated during osteoblast differentiation while its overexpression reduced osteoblast differentiation by targeting connexin 43 (Cx43) both in vitro and vivo [12]. Cx43, encoded by the Gja1 gene, is the most ubiquitous gap junction protein expressed in BMSCs. It has been established that Cx43 plays crucial roles in osteoblastic proliferation and differentiation [13]. Both the magnitude and spatial distribution of gap junction intercellular communication and the osteogenic markers expression were enhanced throughout 3D culturing BMSCs, while Cx43 gene was overexpressed [14].

The excellent and reliable advantage of natural compounds have been widely studied because of its pharmacological properties and beneficial health effects. Quercetin, one of the most common dietary flavonoids, has been shown to have a variety of biological properties, including anti-inflammatory, antioxidant, anticancer and anti-apoptotic activities [15]. Importantly, previous research has established that quercetin play a protective role against bone loss. Kim et al. reported that quercetin increases osteogenic differentiation of adipose stromal cells through ER-independent mechanisms, and efficiently induces the bone formation in a skull defect model of nude mice [16]. A further study demonstrated that rutin, a glycoside derivative of quercetin, inhibits bone mass loss of the femoral trabecular in ovariectomized rats [17]. In addition, a number of studies have shown quercetin plays a significant role in proliferation and differentiation of BMSCs. Hence, quercetin may be a beneficial alternative for the prevention and treatment of several chronic bone loss related diseases, such as osteopenia and osteoporosis. However, the mechanism by which quercetin induces the proliferation and osteogenic differentiation of BMSCs remains obscure. Here we investigated the osteogenic effects of quercetin on cultured BMSCs and elucidated the role of miR-206/Cx43 quercetin exerted in osteogenic differentiation of BMSCs.

Materials and methods

Animals

All animal procedures were conducted according to the guidelines of the institutional animal care committee and were approved by the Animal Use Committee of Shantou University Medical College. Sprague-Dawley rats were raised in the Experimental Animal Center of Shantou University Medical College, and were maintained at an indoor temperature of 22°C, relative humidity 18% to 22%, and a 12-h light/dark cycle.

Isolation and culture of BMSCs

Four-week-old and weighing approximately 100 g male Sprague-Dawley rats were used for isolating the primary BMSCs, according to an established protocol described previously, and more randomly selected 3 rats were used for the in each vitro research. The rats were euthanized using an overdose of chloral hydrate, then the tibias and femurs were immediately removed, and cut off muscles and tissue using a scalpel under sterile conditions. After clipping the metaphyses, the marrow was repeatedly flushed out with basal medium (alpha-MEM (Gibco, #C12571500BT, abbreviated as BAM) supplemented with 10% (v/v) fetal bovine serum (Gibco, #12664025), and 1% (v/v) penicillin-streptomycin solution (Hyclone, #SV30-010)) using a 22-gauge needle. Cell suspension was plated into 10 cm petri dishes and then cultured in an incubator at 37°C, 5% CO_2, and 95% air. The medium supernate was refreshed 3 days later to remove non-adherent cells, and the medium was renewed every 3 days. When the cells reached 80-90% confluence, cells were initially passaged at a split ratio of 1:3 using 0.25% trypsin (AMRESCO, #0458). In the present study, the passages 3 cells were used for subsequent experiments. In-

Table 1. Primer sequence of qPCR

Gene	Forward primer sequence	Reverse primer sequence
Cx43	TGCTTGGGATAGCTGGGCGGA	TGGGGGCAGAGAGAGAAAGCCC
Runx2	GGGACCGTCCACTGTCACTTTAATA	CAAGTGGCCAGGTTCAACGA
OSX	GGAGGCACAAAGAAGCCATA	GGGAAAGGGTGGGTAGTCAT
OCN	AGGTGGTGAATAGACTCCG	GCTGTGCCGTCCATACTTT
OPN	GGAGGGCAGTAAGGTGGTGAA	GAAGCCAATGTGGTCCGCTA
GAPDH	TCCTGCACCACCAACTGCTTAG	AGTGGCAGTGATGGCATGGACT

hibition of Cx43 expression by small-interfering RNA (siRNA) transfection. siRNA transfection was performed to silence the expression of Cx43 (Cx43 siRNA: GCTGGTTACTGGTGACAGA; Control: CCGCAATTACAACAAGAGA). MiR-206 mimics (5'-UGGAAUGUAAGGAAGUGUGUGG-3'), MiR-206 agomir (UGGAAUGUAAGGAAGUGUG-UGG), MiR-206 antagomir (CCACACACUUCCU-UACAUUCCA) and negative controls (NC mimics: 5'-UUCUCCGAACGUGUCACGUTT-3') were synthesized by GenePharma (Shanghai, China). Cells were transfected with 50 μL miR-206 mimics or NC mimics via Lipofectamine 2000.

Cell proliferation assay

Cells (2×10^3 per well) were seeded in a 96-well plate and cultured in BAM for 12 h. Then cells were treated with BAM or BAM containing-quercetin at different concentrations (0, 1, 2.5, 5, 7.5, and 10 μM), and cell proliferation was determined after 1, 2, 3, 4 and 5 days using the CCK-8 (Dojindo, #C0038) assay as instructed by the manufacturer. The 0 μM group, which was treated with DMSO, served as a control group. Absorbance was measured at 450 nm using a microplate reader (Thermo Scientific, Beijing, China). Cell proliferation was expressed as the optical density (OD) value.

Osteogenic differentiation

BMSCs plated at a density of 5000 cells/cm^2 and differentiation into osteoblasts was induced by osteogenic induction medium consisting of a-MEM, 10% FBS, 50 μM ascorbic acid (Sigma, #A4544), 10 mM beta-glycerophosphate (Sigma, #G9422), 100 nM dexamethasone (Sigma, #D1756), and 1% (v/v) penicillin-streptomycin solution with or without quercetin (final concentration at 0, 1, 2, 5, and 10 μM). Medium were replaced every 2-3 days with OIM until cells were harvested for experiments.

Alkaline phosphatase (ALP) activity assay

At the designated time, ALP activity was evaluated using an ALP measurement kit (Beyotime, #P0321). Briefly, cells were dissolved with 1% Triton X-100 and centrifuged at 4°C, 1000 rpm. Supernatants were transferred to a non-polluting tube, then quantified the ALP activity according to the manufacturer's protocol. The absorbance was measured at 405 nm by a 96-well plate microplate reader (Thermo Scientific).

Alizarin red S staining and quantification

After 14 days of osteogenic differentiation or incubation period, cells were gently washed twice with phosphate-buffered saline (PBS) and fixed in absolute ethanol for 15 minutes. The cells were then incubated with Alizarin red S solution (0.1%, Panera, #AAPR122-A1) for 30 min at room temperature. To quantify the stained Alizarin red S in calcium nodules, stained cells were destained with cetyl pyridinium chloride (Sigma, #588393) in 10 mM sodium phosphate for 30 min and transfer eluent to 96-well plate, absorbance was then measured at 540 nm with a microplate reader (Thermo Scientific).

Quantitative real-time PCR (qPCR)

We investigate Runx2, OSX, and OPN mRNA expression at 14 days' osteogenic differentiation phase. Total RNA was extracted with an RNA extraction kit (TaKaRa, #9767) according to the manufacturer's instructions, and total miRNA was isolated using a miRNeasy Mini Kit (Qiagen, K157001) in accordance to the manufacturers' instructions. cDNA was prepared with a PrimeScript™RT Master Mix (TaKaRa, #RR036A) from 1000 ng total RNA. For real-timePCR, a total reaction volume of 25 μL containing SYBR® Premix Ex TaqTM (TaKaRa, #RR420A) was reacted using a CFX96 qPCR Detection System Detection System (BIO-RAD Laboratories). The primers sequences are shown in **Table 1**. Relative mRNA levels were quantized by means of the $2^{-\Delta\Delta Ct}$ method and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalized gene.

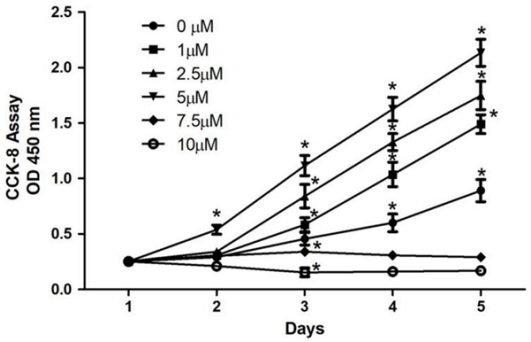

Figure 1. Quercetin enhanced cell proliferation. BMSCs were treated various concentrations of quercetin for 1-5 days, and the proliferation rate were assessed by the CCK-8 test. The 0 μM treated group was as the control group, data are represented as mean ± SD in three times independent experiments (n = 3), (**P*<0.05, ***P*<0.01).

Transfection

The BMSCs were transfected with an agomir (RiboBio, Guangzhou, China) of miR-206 at a concentration of 50 nM (RiboBio), Lipofectamine® 2000 Transfection Reagent (Gibco Life Technologies) was used to transfect cells according to the manufacturer's instructions. Nonspecific microRNA (miR-control; RiboBio) was used as a control.

Western blotting analysis

In brief, cells were washed 2 times with PBS and were lysed with RIPA buffer (Beyotime, #P0013B) containing 1% (v/v) protease inhibitor cocktail (Biotool, #B14001). The lysates were centrifuged for 15 minutes at 4°C, 15000 rpm, and protein concentrations were determined using the Bradford method (Bio-Rad Laboratories). These samples were then separated by was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to polyvinylidene difluoride membranes (Millipore, #IPVH00010), and immunoblotted with the corresponding antibodies. Primary antibodie is anti-Cx43 (1:1000, Cell Signaling Technology, #3512) and rabbit anti-GAPDH (1:1000, Panera, #SF-PA005), secondary antibodie was horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, #111-035-003). At last, protein bands were visualized using an enhanced chemiluminescence reagent (Thermo Scientific), imaged with Bio-Rad ChemiDoc-XRS+, and analyzed using Image Lab software (Bio-Rad). Protein values were normalized to GAPDH.

Dual luciferase reporter assay

Sequences of wildtype or mutant 3'-UTR of Cx43 were cloned into pmirGLO luciferase reporter vector (Promega, Madison, Wisconsin, USA). Cells were seeded into 24-well plates and the cell density was 70-80% one day before transfection, and co-transfected miR-206 mimics or NC mimics with pmirGLO-wt-Cx43, pmirGLO-mut-Cx43 via Lipofectamine 2000. 48 hours after transfection, the luciferase activities were performed with the Lucifer Reporter Assay System (Promega) and normalized to Renilla luciferase activity.

Statistical analysis

All experiments were performed no less than three times independently. All values are expressed as the fold change over the control, and data are shown as the mean ± SD. The software SPSS version 20.0 was used for statistical analysis. Statistical analysis was calculated using Student's two-tailed t-test, one-way or two-way analysis of variance (ANOVA) to determine the statistical significance of differences between groups as appropriate. Differences were considered significant when P< 0.05.

Results

Quercetin enhanced cell proliferation

In the CCK-8 assay, as shown in **Figure 1**, quercetin at 1, 2.5, and 5 μM caused a dose and time-dependent increase in the proliferation of BMSCs. Quercetin at 5 μM caused a statistically significant increase in the growth of BMSCs at days 3 to 5, as compared to controls (P<0.05). Nevertheless, quercetin at a higher dose (7.5, 10 μM) markedly depressed the proliferation of BMSCs. Thus, treatment with 7.5, 10 μM quercetin was removed for the remaining experiments.

Quercetin inhibits miR-206 to enhance BMSCs osteogenic differentiation

MiR-206 is considered to play an important negative regulatory role in osteogenic differentiation. We investigated the association be-

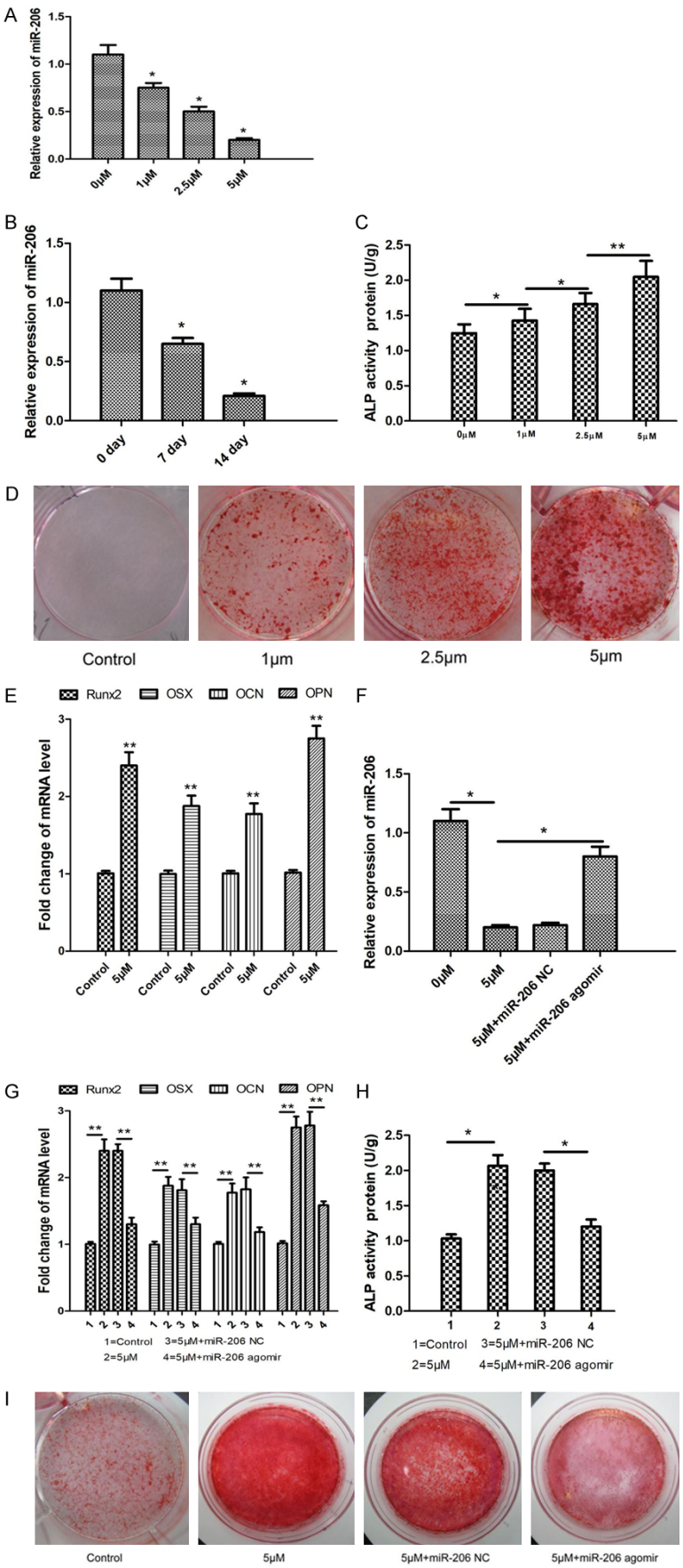

Figure 2. Effect of quercetin on osteogenic differentiation in BMSCs. A. qPCR analysis of miR-206 levels in BMSCs treated with quercetin at different concentrations for 14 days. B. qPCR analysis of miR-206 expression in BMSCs at the indicated time points after treatment with quercetin at 5 μM. C. ALP activity was tested on BMSCs at 14 days following various concentrations of quercetin treated differentiation. D. Alizarin red S staining was performed 14 days after induction. E. qPCR analysis of Runx2, OSX, OCN and OPN mRNA expression in BMSCs after treatment with quercetin at 5 μM. F, G. qPCR analysis of miR-206, Runx2, OSX, OCN and OPN mRNA levels in BMSCs after treatment with quercetin together with 10 μM miR-206 agomir or miR-206 NC in osteogenic medium for 14 days. H. ALP activity was tested in BMSCs after treatment with quercetin together with 10 μM miR-206 agomir or miR-206 NC for 14 days. I. Alizarin red staining of BMSCs after treatment with quercetin together with 10 μM miR-206 agomir or miR-206 NC for 14 days. The 0 μM treated group was as the control group, data are represented as mean ± SD in three times independent experiments (n = 3), (*P<0.05, **P<0.01).

tween quercetin and miR-206 expression by treating BMSC with different doses of quercetin for 7 and 14 days. Subsequently, the qPCR analysis showed that quercetin treatment inhibits the expression of mir-206 in a dose-dependent and time-dependent manner (**Figure 2A** and **2B**).

We further tested the effect of quercetin on osteogenic differentiation. ALP activity was investigated with or without quercetin for 14 days under OIM conditions. ALP activity was increased by being treated with quercetin in a dose-dependent manner, with significant enhancement at 1 μM, and maximal enhance-

ment at 5 μM (**Figure 2C**). Meanwhile, the similar result was also come out in Alizarin red S staining (**Figure 2D**). Next, we observed that quercetin treatment significantly up-regulated Runx2, OSX, OCN and OPN mRNA expression in BMSCs compared to controls (**Figure 2E**). We utilized miR-206 agomir to further demonstrate whether quercetin promotes osteogenic differentiation of BMSCs by inhibiting miR-206. The results revealed that BMSCs in the quercetin group displayed lower mRNA levels of miR-206 while miR-206 agomir increased miR-206 expression (**Figure 2F**). Moreover, the mRNA levels of Runx2, OSX, OCN and OPN were also analyzed. Results showed that miR-206 agomir could inhibit the increased expression of Runx2, OSX, OCN and OPN which was induced by quercetin (**Figure 2G**). ALP activity assay also observed similar results (**Figure 2H**). Further-more, Alizarin Red staining showed that BMSCs treated with quercetin exerted increased number of mineralized nodules and mineralized matrix accumulation while miR-206 agomir significantly attenuated the induction effects of quercetin treatment on osteogenesis differentiation (**Figure 2I**). Taken together, our results suggest that miR-206 has a negative regulatory effect on osteogenic differentiation of BMSCs induced by quercetin.

Quercetin enhances osteogenesis of BMSCs by inhibiting miR-206 to up-regulated the expression of connexin 43

MiRNA target gene prediction software TargetScan showed that there was a potential binding site in the 3'-UTR of Cx43 for the miR-206 sequences. To verify the interaction between mir-206 and Cx43, we constructed a luciferase reporter vector with Cx43 3'-UTR (**Figure 3A**). Co-transfection with miR-206 + Cx43-3'-UTR resulted in significantly decreased relative luciferase activity compared with control + Cx43-3'-UTR or miR-206 + Cx43-3'-UTR mut co-transfections, confirming that miR-206 particularly binds to the predicted 3'-UTR region of Cx43 (**Figure 3B**).

In addition, transfection with mir-206 agomir showed that the up-regulation of mir-206 significantly reduced the expression of Cx43 at mRNA and protein levels compared with NC, while miR-206 antagomir induced the opposite effect (**Figure 3C** and **3D**). This all confirms that miR-206 regulates Cx43 expression. To investigate the effect of quercetin on Cx43 by inhibiting miR-206, we performed a luciferase assay in BMSC supplemented with quercetin. The results showed that miR-206 agomir reduced luciferase activity (**Figure 3E**). Besides, reduced Cx43 protein expression was also observed in cells treated with miR-206 agomir (**Figure 3F**).

Next, in order to investigate whether miR-206/Cx43 did indeed help osteogenic differentiation, we transfected BMSCs with Cx43 siRNA alone or combined with miR-206 agomir in the presence of quercetin. The results of qRT-PCR analysis showed that Cx43 silence could decrease the expression of Runx2, OSX, OCN and OPN, which was elevated by miR-206 treatment (**Figure 3G**). ALP activity assay also observed similar results (**Figure 3H**). Consistently, Alizarin Red staining also showed that miR-206 could enhance the osteogenic differentiation of BMSCs in the presence of quercetin while Cx43 silence could abolish this effect (**Figure 3I**).

Taken together, our results confirm that Cx43 is a direct target gene of miR-206 and that miR-206 in BMSCs was inhibited during quercetin treatment, which increased the expression of Cx43 and helped osteoblasts to differentiate.

Discussion

The slower growth rate of BMSCs, decreased ability to differentiate into osteoblasts, and increased bone resorption due to elevated osteoclast function play an important role in the pathogenesis of osteoporosis [6]. Currently, drugs used for the treatment of osteoporosis, such as vitamin D analogs, calcitonin [18], estrogen, bisphosphonates [19], have little effect on the saving proliferation and osteogenesis of BMSCs, and only unilaterally inhibit bone absorption by inhibiting osteoclast activity [20]. Therefore, enhancement osteogenic differentiation and proliferation of BMSCs may be an alternative therapeutic target for osteoporosis. It has been reported that plant flavonoid is a protective compounds involing in against bone loss [21]. In this study, we investigated the role of quercetin in the osteogenic differentiation and proliferation of bone marrow mesenchymal stem cells, and identified it as a new drug for

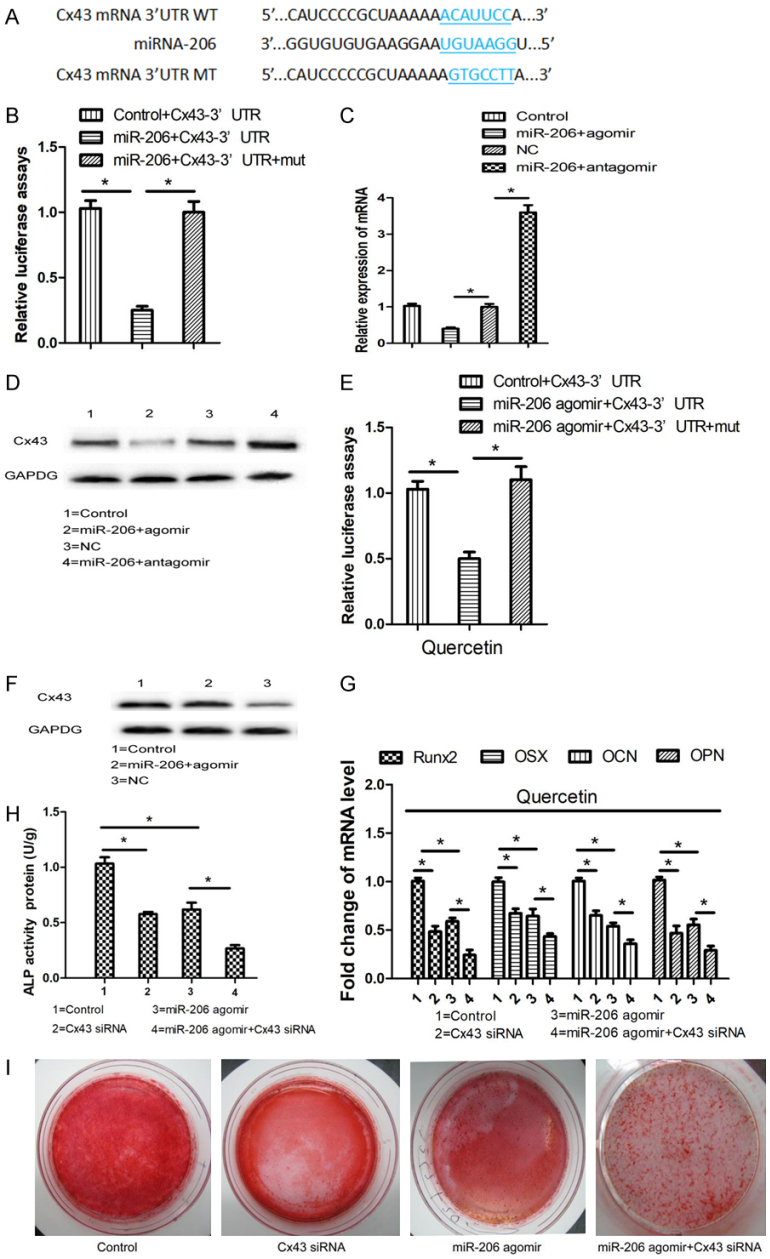

Figure 3. Quercetin Enhances Osteogenesis of BMSCs by Inhibiting miR-206 to Up-regulated the expression of Connexin 43. A. 3'UTR of Cx43 mRNA is complementary with miR-206. The sequences highlighted in blue represent the nucleotides altered in order to generate the mutant reporter plasmid. B. Luciferase activity in BMSCs after treatment with 5 μM quercetin. C, D. qPCR and western bolt analysis of Cx43 in BMSCs after treatment with 10 μM miR-206 agomir or miR-206 antagomir for 14 days. E. Luciferase activity in BMSCs after treatment with 5 μM quercetin together with 10 μM miR-206 agomir. F. Western bolt analysis of Cx43 in BMSCs after treatment with 5 μM quercetin together with 10 μM miR-206 agomir. G. qPCR analysis of Runx2, OSX, OCN and OPN in BMSCs after treatment with Cx43 siRNA and miR-206 agomir in osteogenic medium supplemented with 5 μM quercetin. H. ALP activity was tested in BMSCs treatment with Cx43 siRNA and miR-206 agomir in osteogenic medium supplemented with 5 μM quercetin. I. Alizarin red staining of BMSCs after treatment with Cx43 siRNA and miR-206 agomir in osteogenic medium supplemented with 5 μM quercetin. The data are represented as mean ± SD in three times independent experiments (n = 3), (*P<0.05, **P<0.01).

the treatment of osteoporosis.

Our results demonstrated that quercetin enhance osteogenic and mineralization potential of BMSCs in a dose-dependent manner. Our investigation verified that the effects of quercetin below 5 μM were non-cytotoxicity on BMSCs and 5 μM quercetin exert the biggest influence on BMSCs. Therefore, the concentration of 5 μM was selected in subsequent experiments.

Osteogenic differentiation of BMSCs is a continuous process, and undergo differentiation into osteo-progenitor cells, and then preosteoblasts and osteoblasts followed by matrix mineralization [22]. ALP is a bone-specific enzyme and is considered an early osteogenic marker that plays a role in osteogenic differentiation [23]. Runx2, a member of the runt family, is necessary for osteoblast differentiation and bone formation and is expressed in the early stages of bone formation to control the expression of Bglap and OPN genes [24]. Osterix is a novel zinc finger-containing transcription factor that is also essential for early osteoblast differentiation [25]. OPN, OCN expression and mineralization were the late-stage marker of bone formation. In order to evaluate the quercetin effect on the early-stage and late-stage stage of osteogenic differentiation, ALP activity, and Runx2, Osterix, OPN, OCN expressions were detected. In the present study, quercetin was shown to stimulate osteogenic differentiation of BMSCs by regulating the expression of osteogenic transcription factors.

The mechanism by which quercetin enhances osteogenic differentiation has not yet been whole established. We then sought to determine how quercetin regulates the osteogenic differentiation of BMSCs. Many pathways have been identified involving the effect of quercetin in the process of osteogenic differentiation by BMSCs, like MAPK pathway, BMP pathway, Wnt/β-catenin and estrogen receptor pathway [26, 27]. Specific miRNAs have been implicated as the modulators of cell proliferation and apoptosis, stem cell maintenance and differentiation. MiRNAs are a new class of endogenous small non-coding RNAs that regulate gene expression through translation inhibition or degradation of their targets [28]. Cx43 is one of the targets of miR-206, and the knockdown of miR-206 expression partially promotes the differentiation potential of osteoblasts by increasing the accumulation of Cx43 [12]. Rossello RA et al. reported that overexpression of Cx43 in BMSCs enhances both the magnitude and spatial distribution of gap junction intercellular communication and the expression of osteogenic markers throughout the 3D cultures. Here, we hypothesize that mir-206/Cx43 is involved in the stimulation of osteoblast differentiation by quercetin. Interestingly, our results suggest that increased expression of miR-206 suggests that quercetin induced osteogenesis is inhibitory, depending on the accumulation of Cx43 expression.

Taken together, our results indicated that quercetin may be a suitable selection for the treatment of osteoporosis patients. Since BMSCs used in this study were isolated from adult healthy rats, further study was needed to determine the effect of quercetin on osteoblast differentiation in osteoporotic rats. Furthermore, for quercetin anti-osteoporosis, more bone formation related empirical models need to be used to verify quercetin mediated acceleration bone formation, including osteoclasts and in vivo. Even so, in the present study, quercetin was shown to enhanced osteoblastic differentiation in a dose-dependent manner, and miR-206/Cx43 participate in effect of quercetin in BMSCs osteogenesis differentiation. Our research suggested that quercetin may be a satisfactory bone building agents for the treatment of osteoporosis.

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Disclosure of conflict of interest

None.

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