Quercetin promotes bone marrow mesenchymal stem cell proliferation and osteogenic differentiation through the H19/miR-625-5p axis to activate the Wnt/β-catenin pathway

Wei Bian1, Shunqiang Xiao1, Lei Yang2, Jun Chen1 and Shifang Deng1*

Abstract

Background: Quercetin and H19 can promote osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs). However, whether quercetin regulates H19 expression to promote osteogenic differentiation of BMSCs is unclear.

Methods: BMSC proliferation, matrix mineralization, and alkaline phosphatase (ALP) activity were assessed using the Cell Counting Kit-8, ALP assay kit, and alizarin red staining kit, respectively. Expression of H19, miR-625-5p, BMP-2, osteocalcin, and RUNX2 were measured by qRT-PCR; β-catenin protein level was measured by western blotting.

Results: Quercetin promoted BMSC proliferation, enhanced ALP activity, and upregulated the expression of BMP-2, osteocalcin, and RUNX2 mRNAs, suggesting that it promoted osteogenic differentiation of BMSCs. Moreover, quercetin increased H19 expression, while the effect of quercetin on BMSCs was reversed by silencing H19 expression. Additionally, miR-625-5p, interacted with H19, was downregulated during quercetin-induced BMSC osteogenic differentiation, which negatively correlated with H19 expression. Silencing miR-625-5p expression promoted BMSC proliferation and osteogenic differentiation, whereas miR-625-5p overexpression weakened the effect of quercetin on BMSCs. Finally, quercetin treatment or downregulation of miR-625-5p expression increased β-catenin protein level in BMSCs. Upregulation or downregulation of miR-625-5p or H19 expression, respectively, inhibited β-catenin protein level in quercetin treated-BMSCs.

Conclusion: H19 promotes, while miR-625-5p inhibits BMSC osteogenic differentiation. Quercetin activates the Wnt/β-catenin pathway and promotes BMSC osteogenic differentiation via the H19/miR-625-5p axis.

Keywords: lncRNA, microRNAs, Osteogenic differentiation, Quercetin

Background

Human bone marrow mesenchymal stem cells (BMSCs) can undergo self-renewal and osteogenic differentiation, and play a vital function for bone formation and remodeling [1]. Some pathophysiological conditions, such as aging, osteoporosis, and some bone defects can inhibit the osteogenic ability of BMSCs [2–4]. Therefore, drugs
that can regulate BMSC osteogenic differentiation need to be developed for the management and treatment of these orthopedic diseases.

Quercetin, a naturally available flavonoid and a well-known phytoestrogen [5], exerts antioxidant and anti-inflammatory properties. In vitro, quercetin promotes osteogenic differentiation of BMSCs via extracellular-signal-regulated protein kinases, adenosine 5’-monophosphate (AMP)-activated protein kinase/sirtuin 1, and estrogen receptor-mediated pathways [6–8]. In vivo, in rat models of postmenopausal osteoporosis, quercetin heightens BMSC osteogenic differentiation to increase the bone mineral density [9]. However, currently available scientific data has not yet elucidated the functional mechanism of quercetin. Therefore, further understanding of the mechanisms underlying quercetin effects will have immense clinical implications.

Long non-coding RNAs (lncRNAs) can regulate protein levels via epigenetic modulations. LncRNAs affect several biological processes, including bone metabolism and osteogenic differentiation of BMSCs [10, 11]. According to lncRNA microarray results reported by Wang et al and Zhang et al, lncRNAs upregulate H19 levels during osteogenic differentiation [12, 13], which in turn, promote osteogenic differentiation of BMSCs [14, 15]. Furthermore, quercetin promotes the apoptosis of prostate cancer cells, colorectal cancer cells, and fibroblast-like synoviocytes by regulating lncRNA expression [16–18]. However, it remains unclear whether quercetin can promote BMSC osteogenic differentiation by regulating H19 expression.

Therefore, in this study, the effect of quercetin and H19 expression on the proliferation and osteogenic differentiation of BMSCs was identified. Further, the mechanism was elucidated by which quercetin affects BMSC osteogenic differentiation.

Material and methods
BMSCs culture, quercetin treatment, and siRNA transfection
Human BMSCs (catalog number. ZQ0308), complete medium (No. 7501), and osteogenic differentiation medium (No. 7531) were purchased from Zhong-Qiao-Xin-Zhou (Shanghai, China). Quercetin (Aladdin, No: Q111273, purity ≥98.5%, Shanghai, China) was dissolved in DMSO. BMSCs were cultured in complete medium supplemented with 50 μg/mL ascorbic acid and 10 mM β-glycerophosphate (CM medium) and then treated with different concentrations of quercetin (1, 5, and 10 μmol/L). BMSCs in the negative control group were treated with DMSO; BMSCs in the positive control group (positive group) were treated with osteogenic differentiation medium. H19 siRNA (si-H19), negative control siRNA (si-NC), miR-625-5p mimic, miR-625-5p inhibitor, NC mimic, and NC inhibitor were synthesized by Genepharma (Shanghai, China) and transfected into BMSCs.

Proliferation assay
BMSCs (1 × 10⁴ cells/100 μL/well) were cultured for 24 hours. Once BMSCs adhered, BMSCs were treated with DMSO or quercetin (1, 5, and 10 μM) in osteogenic differentiation medium. After 1, 2, and 7 days of treatment, 10 μl CCK-8 solution (Dojindo, Japan) was added and incubated for 4 hours. Finally, optical density at 450 nm (OD₄₅₀) was measured using a Multiskan Mk3 microplate reader (Thermo Fisher).

Alkaline phosphatase assay
An alkaline phosphatase (ALP) assay kit (Beyotime, Shanghai, China) was used to assess the ALP activity. Briefly, BMSCs were lysed and diluted with cell lysate. Standard substance in this kit was added in volumes of 4 μL (20 nmol/L ALP), 8 μL (40 nmol/L), 16 μL (80 nmol/L ALP), 24 μL (120 nmol/L ALP), 32 μL (160 nmol/L ALP), and 40 μL (200 nmol/L ALP), and 50 μL of the lysates was added to the 96-well plate. After mixing, the 96-well plate was incubated at 37 °C. The ALP activity was calculated as the p-nitrophenol produced per min per milligram of protein (unit: nmol/min/mg). The blank acted as negative control.

Matrix mineralization
BMSCs were cultured in CM medium or osteogenic differentiation medium at 37 °C in a 5% CO₂ incubator. Every 3 days, fresh medium (preheated to 37 °C) was used to replace the old CM medium. After 21 days of quercetin treatment, the medium was removed, BMSCs were washed and images were acquired under a microscope (Beijing Cnmicro Instrument Co., Ltd., Beijing, China).

Quantitative real-time polymerase chain reaction (qRT-PCR)
qRT-PCR was performed to analyze the expression of H19, miR-625-5p, bone morphogenetic protein 2 (BMP-2), osteocalcin, and runt-related transcription factor 2 (RUNX2). Briefly, total extractive RNA was reverse-transcribed using EasyScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) and the miRNA First Strand cDNA Synthesis Kit (Sanogen Biotech Co., Ltd. Shanghai, China). qRT-PCR was performed using the SYBR Green qPCR SuperMix (Invitrogen) on the ABI PRISM® 7500 Sequence Detection System (Foster City, CA, USA). Relative expression
levels of lncRNA, miRNA, and miRNA expression were determined using the $2^{-\Delta\Delta CT}$ method [19], according to internal control which used 18s RNA and U6 levels. The information related to the primers used is listed in Table 1.

Bioinformatic database assay and luciferase reporter assay
LncBase Experimental v.2 and Starbase 3.0 were used to find the potential miRNAs binding to H19 [20, 21]. Abnormally expressed miRNAs during BMSC osteogenic differentiation were analyzed using GEO2R in GSE148049 of the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). The potential miRNAs at the intersection of three sets of results were selected.

293T cells (5×10^4 cells/well) were plated and cultured for 24 h. The binding of H19 to miRNAs was analyzed using the luciferase reporter assay. Briefly, the wild type H19 (H19-WT) and mutant H19 (H19-Mut, with mutated binding sites) sequences were cloned onto the luciferase reporter vector psi-CHECK2 and then transfected into 293T cells. Forty-eight hours later, the luciferase activity of renilla or firefly luciferase activity was evaluated by the dual luciferase reporter assay system (Promega). The renilla/firefly luciferase activity was lower in the co-transfected H19 and miR-625-5p mimic groups than that in co-transfected NC mimic and WT-H19 groups, suggesting that H19 can bind to miR-625-5p. The renilla/firefly luciferase rate did not change significantly between the co-transfected H19 and miR-625-5p mimic groups and the co-transfected NC mimic and WT-H19 groups, suggesting that H19 cannot bind to miR-625-5p.

Western blot analysis
The extractive total proteins were separated by performing SDS-PAGE using 10% resolving gels, then transferred onto polyvinylidene fluoride membranes and incubated with rabbit monoclonal antibody specific to β-catenin (dilution, 1:500, ab68183, Abcam, San Diego, CA, USA) and rabbit monoclonal antibody specific to GAPDH (dilution, 1:10,000, ab128915, Abcam) for 40 min. The membranes were washed three times and incubated with Goat Anti-Rabbit IgG H&L (HRP) (dilution, 1:10,000, ab205718, Abcam). Finally, protein bands were visualized using an enhanced chemiluminescent reagent (PerkinElmer Life Sciences, MA, USA).

Statistical analysis
Data, conform to normal distribution, are presented as the mean ± standard deviation. One-way analysis of variance was performed using SPSS 19.0 statistical software (IBM, Inc.) to analyze the statistical difference between more than three groups, followed by Tukey’s post-hoc test. Statistical significance was set at $P < 0.05$.

Results
Quercetin enhances BMSC osteogenic differentiation
The molecular structural formula of quercetin is shown in Fig. 1A. Cell proliferation increased significantly in quercetin treatment groups (both 5 and 10 μM) compared with that in the blank group from day 1 to day 7, whereas cell proliferation in the positive group increased significantly on day 1 and 2 but decreased on day 7 (Fig. 1B). ALP activity and the transcription of BMP-2, osteocalcin, and RUNX2 was significantly enhanced in the quercetin treatment and positive groups compared with those in the blank group (Fig. 1A-D). Additionally, calcium nodules were observed in all groups after treatment for 21 days (Fig. 2E). Compared with that in the blank group, the number and area of calcium nodules notably increased in the quercetin treatment and positive groups (Fig. 2E). These results suggest that quercetin treatment significantly increased cell proliferation and osteogenic differentiation.

Quercetin promotes H19 expression
The H19 levels was measured by performing qRT-PCR after treating cells with quercetin and osteogenic differentiation medium (positive group). H19 expression increased significantly in the quercetin treatment and positive groups compared with that observed in the blank group (Fig. 3A). Furthermore, H19 expression had a positive relationship with ALP activity, and the

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Table 1 Sequences of primers used in the study

| Gene            | Sequence (5′-3′) | Size |
|-----------------|-----------------|------|
| miR-625-5p-Forward | ACTCCAGCTGGGAGGGGAAGTTCTTA TAG | 71 bp |
| miR-625-5p-Reverse | CTCAGCTGGTGTCGGA | 71 bp |
| U6-Forward      | CTCAGCTGGGACGACA | 96 bp |
| U6-Reverse      | AACGCTCTACGTTCTGCTG | 96 bp |
| BMP2-Forward    | ACGCCTTAAGTCAGCTGA | 160 bp |
| BMP2-Reverse    | GGCATTAGTTGGAGTTCCA | 160 bp |
| Runx2-Forward   | TCTAATCCTGGGAGTTCTCAG | 250 bp |
| Runx2-Reverse   | GAGGCCTACTCCCAAGGA | 250 bp |
| Osteocalcin-Forward | CTCACATCCTGGGCCATT | 139 bp |
| Osteocalcin-Reverse | TGGGTCTCTCTCAGTCTCAG | 139 bp |
| H19-Forward     | GCGGCTCTTTCTTCTTCTTCTTCTTC | 171 bp |
| H19-Reverse     | GTGTTGTTAAAGTGTCAGCAT | 171 bp |
| 18s-Forward     | CCTGATACCGGAGCTTAGGA | 112 bp |
| 18s-Reverse     | GCGGCGCAATACGAAATGCC | 112 bp |
mRNA expression of BMP-2, RUNX2, and osteocalcin (Fig. 3B–E).

Silencing H19 expression reverses the effect of quercetin on BMSCs
To study the effect of H19, BMSCs were transfected with si-H19, followed by treatment with 10 μM quercetin; si-NC was used as control. H19 expression was successfully silenced by si-H19 (Fig. 4A). Following H19 silencing, the proliferation of BMSCs was inhibited on days 1, 2, and 7 (Fig. 4B). Moreover, on day 21 after H19 silencing, ALP activity and mRNA levels of BMP-2, RUNX2, and osteocalcin were significantly reduced (Fig. 4C–D). The number and area of calcium nodules were notably decreased following H19 silencing on day 21 (Fig. 4E).

H19 interacts with miR-625-5p
Firstly, we identified 254 miRNAs in the GSE148049 dataset that were differentially expressed during BMSC osteogenic differentiation. Additionally, 105 and 237 potential miRNAs binding to H19 were identified. miR-625-5p and miR-483-3p were selected through the intersection of the three sets of results (Fig. 5A). Although miR-625-5p and miR-483-3p expression did not change significantly between day 0 and day 7 during BMSC osteogenic differentiation (P=0.064>0.05 and P=0.097>0.05, respectively), miR-625-5p expression was higher while that of miR-483-3p was lower on day 7 than on day 0 (Fig. 5B). This result suggested that miR-483-3p promotes BMSC osteogenic differentiation, whereas miR-625-5p inhibits BMSC osteogenic differentiation. Since H19 plays a role in promoting osteogenic differentiation, we aimed to identify miRNAs that inhibit osteogenic differentiation to elucidate the function of H19. Therefore, miR-625-5p was selected for further evaluation. The binding site between H19 and miR-625-5p is shown in Fig. 5C. Renilla/firefly luciferase activity rate was lower in the miR-625-5p mimic+WT-H19 group than in the NC mimic+WT-H19 group, and it comparable between the NC+Mut-H19 group and miR-625-5p mimic+Mut-H19 group, suggesting that H19 can bind to miR-625-5p (Fig. 5D). Finally, we observed that miR-625-5p expression gradually decreased during quercetin-induced BMSC osteogenic differentiation and negatively correlated with H19 expression (Fig. 5E and F). During quercetin-induced BMSC osteogenic differentiation, downregulation of H19 expression promoted miR-625-5p expression (Fig. 5G).

miR-625-5p overexpression reverses the effect of quercetin on BMSCs
Next, miR-625-5p inhibitor was transfected into BMSCs to decrease the miR-625-5p level, while the transfection of NC inhibitor was used as control. miR-625-5p level was successfully decreased by miR-625-5p inhibitor (Fig. 6A), which promoted BMSC proliferation, enhanced ALP activity, and increased mRNA expression of BMP-2, osteocalcin, and RUNX2 (Fig. 6B and C). The number and area of calcium nodules were notably increased in BMSCs transfected with the miR-625-5p inhibitor (Fig. 6D). Additionally, we investigated the effect of miR-625-5p overexpression on quercetin-induced BMSC proliferation and osteogenic differentiation. miR-625-5p mimic was transfected into BMSCs to overexpress miR-625-5p and subsequently, BMSCs were treated with 10 μM quercetin; NC mimic was used as control. miR-625-5p levels were successfully increased by miR-625-5p mimic (Fig. 6A). The proliferation, ALP activity, and mRNA expression of BMP-2, osteocalcin, and RUNX2 were significantly decreased by miR-625-5p mimic (Fig. 6B and C). In addition, the number and area of calcium nodules were notably decreased by miR-625-5p mimic (Fig. 6D). Collectively, these results suggested that silencing miR-625-5p can promote BMSC proliferation.
and osteogenic differentiation, whereas miR-625-5p overexpression reverses these effect of quercetin.

**Quercetin promotes BMSC osteogenic differentiation by targeting the H19/miR-625-5p axis and activating the Wnt/β-catenin signaling pathway**

Finally, β-catenin protein levels were notably enhanced during quercetin-induced BMSC osteogenic differentiation (Fig. 7A). β-catenin protein level was significantly increased in response to treatment with miR-625-5p inhibitor (Fig. 7B). In addition, β-catenin protein levels decreased significantly after BMSCs were treated with si-H19 or miR-625-5p mimic followed by treatment with 10 μM quercetin (Fig. 7C).

**Discussion**

Quercetin can heighten the BMSCs osteogenic differentiation and increase bone mineral density [6–9]. However, optimal concentration of quercetin treatment remains debatable. Treatment with 2, 2.5, and 5 μM quercetin promoted osteogenic differentiation of mouse BMSCs [7, 8]. Zhou et al reported that treatment with 2 μM quercetin promoted osteogenic differentiation of rat BMSCs, and this effect was even better than that observed following...
treatment with 5 or 10 μM quercetin [6]. Notoya et al reported that treatment with 5 and 10 μM concentration quercetin inhibited the osteogenic differentiation of Rob cells, whereas treatment with 0.1 and 1 μM concentration quercetin had no obvious effect on the proliferation and differentiation of Rob cells [22]. Treatment with 0.1, 1, and 10 μM concentration quercetin enhanced osteogenic differentiation of rat BMSCs, particularly at 10 μM [23]. We observed that quercetin treatment induced osteogenic differentiation in BMSCs and found that 10 μM quercetin was the optimal concentration to achieve these effects. The inconsistent effects of quercetin treatment on rat, mouse, and human BMSCs may be attributed to the differential tolerance of quercetin in different species. Casado-Díaz et al reported that BMSC differentiation medium supplemented with 10 μM quercetin decreased BMSC proliferation and differentiation, while BMSC differentiation medium supplemented with 0.1 μM quercetin promoted BMSC proliferation and differentiation [24]. Casado-Díaz's study indirectly shows that quercetin promotes osteogenic differentiation. However, when present at a higher concentration (10 μM quercetin), in presence of another strong inducer of osteogenic differentiation (BMSC differentiation medium), it inhibits osteogenic differentiation. In our study, treatment with 1, 5, and 10 μM quercetin or BMSC differentiation medium treatment promoted BMSC proliferation and differentiation. Although based on our results we inferred that that 10 μM is the optimal quercetin concentration to induce osteogenic differentiation of BMSCs, the toxicity of high concentrations of quercetin (more than 2 μM) should be considered in animals and humans based on the above studies. Based on these observations, we propose that 1~2 μM quercetin may be the effective concentration to induce osteogenic differentiation without causing cytotoxicity. Further studies are warranted to validate these observations and inferences.

Previous studies also reported that H19 heightened osteogenic differentiation of BMSCs [14, 25, 26]. Consistently, H19 expression was upregulated during BMSC osteogenic differentiation in this study. Additionally, silencing H19 expression in BMSCs reversed the osteogenic differentiation-inducing effects of quercetin. This result suggested that H19 participates in the regulation of quercetin-induced BMSCs osteogenic differentiation. Functionally, H19 heightened BMSCs osteogenic differentiation by inhibiting the expression of miR-140-5p and miR-149 [14, 26]. In our study, H19 was found to interact with miR-625-5p during quercetin-induced BMSC osteogenic differentiation. The role of miR-625-5p on BMSC osteogenic differentiation was previously unclear. Our results revealed that silencing miR-625-5p promotes osteogenic differentiation of BMSCs, suggesting that miR-625-5p inhibits BMSCs osteogenic differentiation. Furthermore, miR-625-5p overexpression can reverse quercetin-induced osteogenic differentiation of BMSCs.
Collectively, quercetin promotes BMSCs osteogenic differentiation by targeting the H19/miR-625-5p. In our study, H19 adsorbed miR-625-5p to regulate quercetin-induced osteogenic differentiation. Consistently with our results H19 can adsorb miRNAs include miR-140-5p, miR-149, and miR-532-3p to regulate osteogenic differentiation induced by other factors or drugs [14, 26, 27]. These results indicate that H19 regulated osteogenic differentiation can be further modulated by different factors by adsorbing different miRNAs.

H19 too can activate Wnt/β-catenin pathway to promote BMSCs osteogenic differentiation [28–30]. Quercetin can activate Wnt/β-catenin pathway to alleviate cerebral ischemia reperfusion injury and post-menopausal osteoporosis (PMOP) [9, 31]. Furthermore, quercetin treatment enhances the osteoblast differentiation of MC3T3-E1 cells via enhancing β-catenin protein level and activating the Wnt/β-catenin pathway [32]. These studies suggest that the Wnt/β-catenin pathway is the main downstream pathway in H19 and
quercetin-induced osteogenic differentiation. We found that quercetin treatment or downregulation of miR-625-5p expression promoted β-catenin protein level in BMSCs, whereas upregulation of miR-625-5p expression or downregulation of H19 expression inhibited β-catenin protein level in quercetin-treated BMSCs. These results provide evidence that Wnt/β-catenin signaling is a downstream pathway regulated by quercetin and H19, consistent with previous studies [9, 28, 29, 31].

Clinically, osteogenic ability of BMSCs is weakened under some pathophysiological conditions, such as aging, menopause, trauma, and inflammation, all of which can lead to bone defects and osteoporosis [2–4, 33]. We found that quercetin treatment induced BMSC proliferation and osteogenic differentiation, suggesting that quercetin can be used for the clinical treatment of osteoporosis and bone defects. H19 expression is inhibited in patients with osteoporosis and bone defects, suggesting...
Fig. 6  miR-625-5p overexpression reversed quercetin effects on BMSCs.  

A.  miR-625-5p expression was measured by qRT-PCR at 24 h after transfection.

B.  Proliferation of transfected BMSCs was measured using the Cell Counting Kit-8 on days 1, 2, and 7 following quercetin treatment.

C.  ALP activity was measured using the Alkaline Phosphatase Assay Kit, and BMP-2, RUNX2, and osteocalcin mRNA expressions were measured by qRT-PCR after transfection and quercetin treatment for 21 days.

D.  Calcium nodule formation was detected using Alizarin Red staining (400×) after transfection and quercetin treatment for 21 days. *P < 0.05 and ***P < 0.001
that H19 is a therapeutic target for these diseases [27, 34–36]. Since quercetin treatment increased H19 expression during quercetin-induced BMSCs osteogenic differentiation, quercetin may be used for the clinical treatment of osteoporosis and bone defects.

Although our study presents some credible data, it had some limitations. First, the target genes of miR-625-5p remain unclear. Additionally, quercetin may also activate the ERK and p38 MAPK pathways to enhance osteogenic differentiation of BMSCs, which can also be activated by H19 in cardiomyoblasts [23, 37], suggesting that the MAPK pathway may be another downstream pathway regulated by H19 in quercetin-induced osteogenic differentiation. Thus, to elucidate the relationship between quercetin, H19, and the ERK and p38 MAPK signaling pathways, further studies are warranted. Moreover, understanding the effect of quercetin on BMSC osteogenic differentiation in animal models and humans require further studies, and the nontoxic dose of quercetin remains to be confirmed.

Conclusions
Our study demonstrated that H19 promoted, while miR-625-5p inhibited osteogenic differentiation of BMSCs. Quercetin promoted BMSC proliferation and osteogenic differentiation via the H19/miR-625-5p axis to activate the Wnt/β-catenin pathway. Additionally, 1–2 μM quercetin may be the effective concentration to induce osteogenic differentiation without causing cytotoxicity. However, the concentration and mechanism by which quercetin facilitates the osteogenic differentiation of BMSCs in vivo requires further exploration.

Abbreviations
ALP: Alkaline phosphatase; AMP: Adenosine 5'-monophosphate; BMP-2: Bone morphogenetic protein 2; BMSCs: Bone marrow mesenchymal stem cells; lncRNA: Long noncoding RNA; RUNX2: Runt-related transcription factor 2.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12906-021-03418-8.

Acknowledgements
None.

Authors' contributions
WB made substantial contributions to the conception and design of the study, performed experiments, analyzed the data, and drafted the manuscript. SX and LY made substantial contributions to the conception and design of the work and performed experiments. JC made substantial contributions to the acquisition and analysis of data. SD designed the study, interpreted the data, and revised the manuscript. All authors read and approved the final manuscript.

Funding
This work was funded by the Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (grant number 2021B1212040007).

Availability of data and materials
The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

Declarations
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Traditional Chinese Medicine, Shenzhen People’s Hospital (The Second Clinical Medical College, Jinan University, The First Affiliated Hospital, Southern University of Science and Technology), No. 1017, Dongmen North Road, Luohu District, Shenzhen 518020, China. 2Department of Gynecology in Luohu Hospital of Traditional Chinese Medicine/Shenzhen Hospital of Shanghai University of traditional Chinese Medicine, Shenzhen 518000, China.

Received: 30 April 2021  Accepted: 17 September 2021
Published online: 30 September 2021

References
1. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells. 2001;19:180–92.
2. Zhang Y, Ravikumar M, Ling L, Nunncombe V, Cool SM. Age-related changes in the inflammatory status of human mesenchymal stem cells: implications for cell therapy. Stem Cell Res. 2021;16:694–707.
3. Tian F, Yang HT, Huang T, Chen FF, Xiong FJ. Involvement of CB2 signalling pathway in the development of osteoporosis by regulating the proliferation and differentiation of hBMSCS. J Cell Mol Med. 2021;25:2426–35.
4. Houdek MT, Wyles CC, Packard BD, Terzic A, Behfar A, Sierra RJ. Decreased osteogenic activity of mesenchymal stem cells in patients with corticosteroid-induced osteonecrosis of the femoral head. J Arthroplast. 2016;31:893–8.
5. Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care. 2008;11:733–40.
6. Zhou Y, Wu Y, Jiang X, Zhang X, Xia L, Lin K, et al. The effect of quercetin on the osteogenic differentiation and angiogenic factor expression of bone marrow-derived mesenchymal stem cells. PLoS One. 2015;10:e0126905.
7. Pang XG, Cong Y, Bao NR, Li YG, Zhao JN. Quercetin stimulates bone marrow mesenchymal stem cell differentiation through an estrogen receptor-mediated pathway. Biomed Res Int. 2018;2018:4178021.
8. Wang N, Wang L, Yang J, Wang Z, Cheng L. Quercetin promotes osteogenic differentiation and antioxidant responses of mouse bone marrow stem cells through activation of the AMPK/SIRT1 signaling pathway. Phytother Res. 2021. https://doi.org/10.1002/ptr.7010.
9. Yuan Z, Min J, Zhao Y, Cheng Q, Wang K, Lin S, et al. Quercetin rescued TNF-alpha-induced impairments in bone marrow-derived mesenchymal stem cell osteogenesis and improved osteoporosis in rats. Am J Transl Res. 2018;10:4313–21.
10. Yang Y, Yujiao W, Fang W, Linhua Y, Ziqi G, Zhichen W, et al. The roles of miRNA, IncRNA and circRNA in the development of osteoporosis. Biol Reprod. 2020;93:40.
11. Li Z, Huang C, Yang B, Hu W, Chan MT, Wu WKK. Emerging roles of long non-coding RNAs in osteonecrosis of the femoral head. Am J Transl Res. 2020;12:5984–91.
12. Zhang W, Dong R, Diao S, Du J, Fan Z, Wang F. Differential long non-coding RNA/miRNA expression profiling and functional network analysis during osteogenic differentiation of human bone marrow mesenchymal stem cells. Stem Cell Res Ther. 2017;8:60.
13. Wang L, Wang Y, Li J, Li Z, Yu B. Differential expression of long noncoding ribonucleic acids during osteogenic differentiation of human bone marrow mesenchymal stem cells. Int Orthop. 2015;39:1013–9.
14. Li G, Yun X, Ye K, Zhao H, An J, Zhang X, et al. Long non-coding RNA-H19 stimulates osteogenic differentiation of bone marrow mesenchymal stem cells via the microRNA-140-SDF-1 axis. J Cell Mol Med. 2020;24:4946–55.
15. Weng W, Di S, Xing S, Sun Z, Shen Z, Dou X, et al. Long non-coding RNA DANC2 modulates osteogenic differentiation by regulating the miR-1301-3p/PROX1 axis. Mol Cell Biochem. 2021;476:2503–12.
16. Pan F, Zhu L, Lv H, Pei C. Quercetin promotes the apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis by upregulating IncRNA MALAT1. Int J Mol Med. 2016;38:1507–14.
17. Lu X, Chen D, Yang F, Xing N. Quercetin inhibits epithelial-to-mesenchymal transition (EMT) process and promotes apoptosis in prostate cancer via downregulating IncRNA MALAT1. Cancer Manag Res. 2020;12:1741–50.
18. Zhang Z, Li B, Xu P, Yang B. Integrated whole transcriptome profiling and bioinformatics analysis for revealing regulatory pathways associated with quercetin-induced apoptosis in HCT-116 cells. Front Pharmacol. 2019;10:798.
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25:402–8.
20. Paraskevoopoulos MD, Vlachos IS, Karagioudi D, Georgakilas G, Kanellos I, Vergoulis T, et al. DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts. Nucleic Acids Res. 2016;44:D231–8.
21. Li JH, Liu S, Zhou H, Du LH, Yang JH. starBase v2.0. decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. 2014;42:D92–7.
22. Notoya M, Tsukamoto Y, Nishimura H, Woo JT, Nagai K, Lee IS, et al. Quercetin, a flavonoid, inhibits the proliferation, differentiation, and mineralization of osteoblasts in vitro. Eur J Pharmacol. 2004;485:89–96.
23. Li Y, Wang J, Chen G, Feng S, Wang P, Zhu X, et al. Quercetin promotes the osteogenic differentiation of rat mesenchymal stem cells via mitogen-activated protein kinase signaling. Exp Ther Med. 2015;9:2072–80.
24. Casado-Diaz A, Arter J, Dorado G, Quesada-Gómez JM. Effects of quercetin, a natural phenolic compound, in the differentiation of human mesenchymal stem cells (MSC) into adipocytes and osteoblasts. J Nutr Biochem. 2016;32:151–62.
25. Zhou Z, Hossain MS, Liu D. Involvement of the long noncoding RNA H19 in osteogenic differentiation and bone regeneration. Stem Cell Ther. 2021;12:74.
26. Bi HL, Wang D, Liu X, Wang G, Wu X. Long non-coding RNA H19 promotes osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by regulating microRNA-140-5p/SATB2 axis. J Biolog. 2020;45:56.
27. Li T, Jiang H, Li Y, Xiao H, Ding H. Estrogen promotes IncRNA H19 expression to regulate osteogenic differentiation of BMSCs and reduce osteoporosis via miR-352-3p/SIRT1 axis. Mol Cell Endocrinol. 2021;527:111171.
28. Xie X, Liu M, Meng Q. Angelica polysaccharide promotes proliferation and osteoblast differentiation of mesenchymal stem cells by regulation of long non-coding RNA H19: An animal study. Bone Joint Res. 2019;8:323–32.
29. Ma X, Bian Y, Yuan H, Chen N, Pan Y, Zhou W, et al. Human amniotic mesenchymal stem cells promote osteogenic differentiation of human bone marrow mesenchymal stem cells via H19/miR-675/APC axis. Aging. 2020;12:10527–43.
30. Liang WC, Fu WM, Wang YB, Sun YY, Xu LL, Wong CW, et al. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep. 2016;6:20121.
31. Jin Z, Ke J, Guo P, Wang Y, Wu H. Quercetin improves blood-brain barrier dysfunction in rats with cerebral ischemia reperfusion via Wnt signaling pathway. Am J Transl Res. 2019;11:4683–95.
32. Guo C, Yang RJ, Jiang K, Zhou XL, Liu YZ. Protective effects of pretreatment with quercetin against lipopolysaccharide-induced apoptosis and the inhibition of osteoblast differentiation via the MAPK and Wnt/B-catenin pathways in MC3T3-E1 cells. Cell Physiol Biochem. 2017;43:1547–61.
33. Wang C, Meng H, Wang X, Zhao C, Peng J, Wang Y. Differentiation of bone marrow mesenchymal stem cells in osteoblasts and adipocytes and its role in treatment of osteoporosis. Med Sci Monit. 2016;22:226–33.
34. Xiaolong G, Shuaibin L, Kailu L. MicroRNA-19b-3p promotes cell proliferation and osteogenic differentiation of BMSCs by interacting with IncRNA H19. BMC Med Genet. 2020;21:11.
35. Zhou QF, Zhang F, Zhang J, Ma D. H19 promotes the proliferation of osteocytes by inhibiting p53 during fracture healing. Eur Rev Med Pharmacol Sci. 2018;22:226–32.
36. Zhang M, Guo JM, Zhang SH, Zou J. [Research progress on the effect of long non-coding RNA H19 on osteogenic differentiation and bone diseases]. Sheng Li Xue Bao 2018, 70:531–538.
37. Yuan L, Yu L, Zhang J, Zhou Z, Li C, Zhou B, et al. Long non-coding RNA H19 protects H9c2 cells against hypoxia-induced injury by activating the PI3K/AKT and ERK/p38 pathways. Mol Med Rep. 2020;21:1709–16.

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