β -mercaptoethanol promotes osteogenesis of human mesenchymal stem cells via sirt1-ERK pathway

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Abstract Human umbilical cord-derived mesenchymal stem cells (hUMSCs) hold strong self-renewal capacity and low immunogenicity, which have attracted attention as potential candidates for bone repair and regeneration. However, insufficient osteogenic differentiation markedly hinders the clinical applications of hUMSCs. In the present study, the effect of β -mercaptoethanol (BME), a small molecule antioxidant which has been identified to regulate cell proliferation and differentiation, on osteogenic differentiation of hUMSCs and underlying signaling mechanism were investigated. The results indicated that under osteogenic induction conditions, BME treatment increased the alkaline phosphatase (ALP) activity and promoted calcium mineralization in hUMSCs. The gene and protein expression of osteogenesis-related markers such as ALP, osteopontin (OPN), osteocalcin (OCN) and collagen type I (COLI) were also significantly up-regulated. Besides, BME promoted the protein expression of silent information regulator type 1 (sirt1) and stimulated the activation of extracellular signal-related kinase (ERK), contributing to increased Runx2 expression. Furthermore, blocking the expression of sirt1 attenuated BME-

enhanced ERK phosphorylation and osteogenic differentiation of hUMSCs. These results indicated that BME accelerated osteogenic differentiation of hUMSCs by activating the sirt1-ERK signaling pathway, thereby providing insights into the development of MSCs-based bone regeneration strategies.

Keywords Umbilical cord-derived mesenchymal stem cells · Osteogenic differentiation · β -mercaptoethanol · sirt1 · ERK

Abbreviations

hUMSCs	Human umbilical cord-derived mesenchymal stem cells
BME	β -mercaptoethanol
ALP	Alkaline phosphatase
OPN	Osteopontin
OCN	Osteocalcin
COLI	Collagen type I
sirt1	Silent information regulator type 1
ERK	Extracellular signal-related kinase
ROS	Reactive oxygen species
CCK-8	Cell counting kit-8
OIM	Osteogenic induction medium

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Introduction

Treatment of bone defects caused by trauma, infection or congenital malformations remains a critical challenge in the field of orthopedics (Di Martino et al. 2011). Traditional treatment techniques, including autologous and allogeneic bone transplantation, have several disadvantages in clinical application due to limited bone donor sources, immune rejection or secondary trauma (O'Brien 2011). In recent years, a series of studies have reported that human mesenchymal stem cells (hMSCs) possess the ability to regenerate various damaged tissues, showing great promise to treat orthopedic trauma such as bone defects. hMSCs are multipotent stromal cells with the capacity to differentiate into multiple lineages, such as osteoblasts, adipocytes, chondrocytes and myoblasts, and can be easily isolated from various types of tissues including bone marrow, adipose, umbilical cord and placenta (Brown et al. 2019). Among the sources of hMSCs, human umbilical cord-derived mesenchymal stem cells (hUMSCs) with high expansion capacity, low immunogenicity and strong immunomodulation properties, are becoming a promising cell source for bone repair and regeneration (Marino et al. 2019). However, previous studies showed that hUMSCs displayed weaker osteogenic differentiation capacity compared to other sources of hMSCs (Fazzina et al. 2016; Ma et al. 2019), and hUMSCs seeded on ceramic scaffolds failed to deposit a well-developed bone structure when ectopically implanted in mice (Todeschi et al. 2015). Accordingly, it is essential to identify therapeutic strategies to enhance hUMSCs osteogenesis.

Small molecules have been found to affect stem cell behaviors, such as directing cell differentiation (Chen et al. 2006). Particularly, β -mercaptoethanol (BME), a small thiol-containing molecule, is extensively used as an antioxidant supplement for stem cell culture and differentiation and has been reported to show long-term health benefits in preclinical models, including tumor suppression and immunological functions (Beregi et al. 1991; Heidrick et al. 1984). For example, Guo et al. (2012) demonstrated that BME could inhibit NF- κ B (NF- κ B) activity and induce adipogenic differentiation of murine preadipocytes. By employing BME, it was discovered that bone marrow-derived MSCs could be differentiated into neural-like cells by BME-reduced reactive oxygen species (ROS) level

(Shi et al. 2016). Despite the accumulating evidence related to the biological capabilities of BME, whether BME can affect osteogenic differentiation of hUMSCs remains unknown.

Sirtuins (sirt) are widely distributed nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylases, and there are presently seven mammalian sirtuins family members, sirt1 to sirt7 (Borkowski and Sinclair 2016). Among them, sirt1 is the founding member and is also the most adequately studied one. Sirt1 is an important regulator of cell proliferation and differentiation by deacetylation of histones or transcription factors such as NF- κ B and forkhead box O transcription factors 3a (FoxO3a) (Sun et al. 2018). Sirt1 has also been shown to be involved in osteogenesis of MSCs. For example, Cohen-Kfir et al. (2011) found that sirt1 up-regulated osteogenic differentiation of mesenchymal stem cells by repressing the expression of *sost* (a critical inhibitor of bone formation) via H3K9 deacetylation. Inhibition of sirt1 increases the number of adipocytes and reduces the expression of osteoblast markers. Moreover, studies have shown that sirt1 expression could be affected in abnormal bone tissue and osteoblasts due to excessive ROS levels. High level of ROS has been linked to reduced expression of sirt1 and impaired bone generative potential in MSCs (Chen et al. 2017), implying that BME may have a positive effect on sirt1 expression and osteogenic differentiation in MSCs due to its antioxidant effect.

In the present study, the role and underlying mechanism of BME on hUMSCs osteogenic differentiation were investigated. The results demonstrated that BME enhanced Runx2 expression levels and promoted the osteogenic differentiation of hUMSCs via the sirt1-ERK signaling pathway, providing a valuable application of BME in MSCs osteogenesis.

Materials and methods

Cell culture

hUMSCs were purchased from ScienCell (USA) and cultured in minimum essential medium α medium (α -MEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Hyclone, USA) at 37 °C in a humidified atmosphere of 5% CO₂. Passage 3 to 5 of hUMSCs were used in this study.

Cell proliferation assay

To assess the effect of BME on hUMSCs proliferation, cell counting kit-8 (CCK-8, Yeasen, China) assay was performed to detect cell viability. Cells were inoculated into a 24-well plate at 1×10^4 cells/well and 1 mL growth medium supplemented with indicated concentrations of BME (5, 10, 20, 50 and 100 μM) (Sigma-Aldrich, USA) was added to each well. After 4 days of culture, 200 μL α -MEM comprising 10% (v/v) CCK-8 solution was added to treat the cells for 2 h at 37 °C, and the absorbance was measured at 450 nm using ELx800 ELISA microplate reader (BioTek).

Osteogenic induction

For osteogenic induction, cells were seeded at 5×10^3 cells/ cm^2 in growth medium. After cell confluence reached over 80%, the growth medium was replaced with osteogenic induction medium (OIM) consisting of Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% FBS, 1 μM dexamethasone (Sigma-Aldrich, USA), 10 mM sodium β -glycerophosphate (Sigma-Aldrich, USA), 50 mg/L L-ascorbic acid (Sigma-Aldrich, USA) and BME at various concentrations mentioned above. Medium of each group was refreshed every 2 days.

Detection of ALP activity

After the osteogenic induction for 7 days, the cells in 24-well plates were washed with PBS and then fixed in 4% paraformaldehyde for 10 min. After washing with PBS, ALP staining was performed with BCIP/NBT alkaline phosphatase color development kit (Beyotime, China) according to the manufacturer's instructions. To quantify ALP activity, cells after 7 days of osteogenic induction were washed twice with PBS and lysed with 0.2% Triton X-100 for 1 h. Then lysate was centrifuged at 10,000 rpm for 10 min. The ALP activity was quantified with the alkaline phosphatase assay kit (Nanjing Jiancheng Biological Engineering Research Institute, China) according to the manufacturer's instructions. ALP activity was normalized based on the total protein concentration.

Alizarin red S staining and determination of calcium deposition

Cells after 14 days of osteogenic induction were washed twice with PBS and fixed in 4% paraformaldehyde for 10 min. Then, the cells were incubated with 1% w/v Alizarin red S (pH 4.2, Sigma-Aldrich, USA) at room temperature for 30 min. To quantify the deposition of calcium, cells were treated with 0.1 M HCl for 1 h to dissolve the calcium matrix. After centrifugation at 1000 r/min for 5 min, 20 μL supernatant was taken out to measure the calcium ion concentration using a calcium assay kit (Nanjing Jiancheng Biological Engineering Research Institute, China) according to the manufacturer's instructions. Calcium content was normalized based on the total protein concentration.

RNA extraction and real-time PCR

Cells were harvested after 7 and 14 days of osteogenic induction and total RNAs were extracted using Trizol reagent (Invitrogen, USA). Total RNA (1 μg) was reverse-transcribed into cDNA by using M-MLV Reverse Transcriptase (Promega, USA) according to the manufacturer's instructions. The real-time PCR experiment was performed on a real-time PCR system (Bio-Rad CFX96) under the following reaction conditions: 95 °C for 4 min, followed by 40 cycles of amplification at 95 °C for 10 s and 60 °C for 40 s. The relative quantification of gene expression was analyzed according to the $\Delta\Delta\text{CT}$ method, which was normalized to *GAPDH* mRNA expression level. The sequences of primers are shown in Table 1.

Intracellular ROS detection

The levels of intracellular ROS were measured using ROS probe DCFH-DA (Beyotime, China). After cultured in OIM with or without BME for 3 days, the cells were collected and then incubated in 10 μM of DCFH-DA solution at 37 °C for 20 min. After washing twice with α -MEM, the fluorescence intensity was measured using a flow cytometer (FACSCalibur, BD) at an excitation wavelength of 488 nm.

Table 1 Sequences of primers for real-time PCR

Gene	Forward and reverse primers (5' → 3')	Product size (bp)
<i>GAPDH</i>	GAGAAGGCTGGGGCTCATTT AGTGATGGCATGGACTGTGG	231
<i>OPN</i>	GACAGCCAGGACTCCATT GATGTCAGGTCTGCGAAA	246
<i>OCN</i>	GCGGTGCAGAGTCCAGCAAA CCCTAGACCGGGCCGTAGAA	229
<i>COLI</i>	GGACAGCGTGGTGTGTGGTCGG CCTTGGCGCCAGGAGAACCG	235
<i>ALP</i>	GAACCCGGACTTCTGGAACC	212

Western blotting analysis

Cells were cultured in OIM with or without BME until harvested and lysed in RIPA lysis buffer (Beyotime, China) containing 10 mM phenylmethylsulfonyl fluoride (PMSF, Beyotime, China). After centrifugation for 30 min at 12,000 rpm, the supernatant was collected and protein concentrations were determined by using the BCA protein assay kit (Beyotime, China) following the manufacturer's instructions. After being heated for 5 min at 95 °C, equal amounts of protein were loaded in a 10% SDS polyacrylamide gel. The separated proteins were then electrically transferred onto a polyvinylidene difluoride membrane (PVDF, Millipore) and blocked with 5% (w/v) non-fat dry milk in TBST buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween-20) for 1 h at room temperature. Then, the membranes were incubated with primary antibodies against phosphorylated ERK (p-ERK), total ERK, Runx2, sirt1, β -actin (all from Cell Signaling Technology, USA, 1:1000 dilution) and OCN, OPN, COLI (all from Abcam, Britain, 1:1000 dilution) overnight at 4 °C. The membranes were then washed with TBST and incubated for 1 h with secondary antibodies conjugated with horseradish peroxidase (HRP) (Abcam). The antibody-reactive bands were visualized by enhanced chemiluminescence (Millipore) and band intensity was quantified by using Image J software. β -actin was used as an internal control, and its expression was used to standardize input protein to analyze relative protein expression.

Statistical analysis

The results of the experimental data were presented in the form of mean $\pm$ standard deviation (SD). Unless

otherwise specified, three parallel samples were set up for each experiment. Statistical significance was evaluated using a two-tailed student's *t* test and a value of $P < 0.05$ was considered to represent a statistically significant difference.

Results

Effect of BME on cell proliferation of hUMSCs

To investigate the effect of BME on cell proliferation, hUMSCs were exposed to growth medium with various concentrations of BME (0–100 μ M) for 4 days and the CCK-8 assay was performed to detect cell proliferation. As shown in Fig. 1, BME at concentrations of 5 μ M and 10 μ M showed no

Fig. 1 Viability of hUMSCs treated by BME at various concentrations (0–100 μ M). The cells were incubated with BME for 4 days, then CCK-8 assay was performed to test the cell viability. Cells cultured in medium without BME were included as control. * $P < 0.05$, compared with control

significant effect on cell proliferation in comparison with the BME-free group. Nevertheless, BME significantly facilitated cell growth when it was used at concentrations between 20 and 50 μM .

BME increased ALP activity and mineralization of hUMSCs

To further clarify whether BME influences the osteogenesis of hUMSCs, cells were treated with OIM containing different concentrations of BME. ALP is an early-stage marker of osteogenesis that promotes mineralization during the osteogenic process and plays a vital role in bone formation (Orimo 2010), so we measured ALP activity after osteogenic induction for 7 days. The results showed that ALP activity was significantly increased by the BME treatment at concentrations ranging from 20 to 50 μM with BCIP/NBT staining and quantitative detection, with the maximal effect observed at a concentration of 50 μM (Fig. 2a, b). In addition, we examined the

effect of BME on calcium mineralization, which verifies late-stage osteogenic differentiation (Yun et al. 2015), of hUMSCs on day 14. As shown in Fig. 2c, mineral deposition was significantly increased by the BME treatment in the range of 20–100 μM . The strongest effect on calcium deposition was observed following treatment with 50 μM BME. Taken together, these data indicated that 50 μM of BME was an appropriate concentration to promote ALP activity and mineralization of hUMSCs. Therefore, 50 μM of BME was selected as the optimum dose for subsequent experiments.

BME up-regulated osteogenesis-related markers of hUMSCs

Furthermore, we investigated the effect of BME on expression of osteogenesis-related markers. The results of real-time PCR showed that compared with the BME-free group, the mRNA expression levels of *ALP* and *OCN* were promoted obviously in those cells

Fig. 2 Effect of BME on ALP activity and mineralization of hUMSCs. **a** Under osteogenic induction conditions, cells were treated with the indicated concentrations of BME and then stained with BCIP/NBT dye and Alizarin red S. Scale bar,

100 μm . Cells cultured in OIM without BME were included as control. **b** Quantification of ALP activity on day 7. **c** Quantification of calcium content on day 14. * $P < 0.05$, compared with control. (Color figure online)

cultured in OIM containing 50 μ M BME for 7 days. With the prolongation of culture time to 14 days, the mRNA expression levels of *OPN*, *OCN* and *COLI* were all significantly up-regulated by BME treatment (Fig. 3a–d). We also measured the protein expression of these markers and Western blotting results showed that BME treatment obviously promoted the protein expression of OPN and OCN on day 7, with a stimulatory effect on OCN and COLI on day 14, respectively (Fig. 3e–f). Together, these findings

demonstrated that BME could effectively enhance osteogenic differentiation of hUMSCs.

Sirt1-ERK1/2 pathway was involved in the BME-enhanced osteogenesis of hUMSCs

To investigate the underlying molecular mechanisms involved in BME-enhanced osteogenesis of hUMSCs, we first evaluated the effect of BME on intracellular ROS production of hUMSCs. The results showed that BME treatment significantly reduced intracellular

Fig. 3 Effect of BME on osteogenesis-related markers of hUMSCs. In OIM, hUMSCs were treated with BME at concentrations of 50 μ M for 7 days and 14 days. Cells cultured in OIM without BME were included as control. The mRNA levels of osteogenesis-related marker genes, including *ALP*,

OPN, *OCN* and *COLI* were determined by real-time PCR (a–d). The protein levels of OPN, OCN and COLI were detected by western blotting with the indicated antibodies (e) and the band intensities were further quantified using Image J software (f). * $P < 0.05$, compared with control

ROS levels (Fig. 4a, b). Since ROS plays an important role in regulating sirt1 expression, we subsequently determined the effect of BME on sirt1 expression. The western blotting assay showed that the protein level of sirt1 in cells was significantly up-regulated after BME treatment (Fig. 4c, d). ERK1/2 has been widely

reported to promote the Runx2 expression, a key transcription factor for osteogenic differentiation, and plays an important role in MSCs osteogenesis (Fan et al. 2018). Herein, we explored the effect of BME on ERK1/2 phosphorylation and Runx2 expression. The results showed that the expression of phosphorylated

Fig. 4 BME up-regulated the expression of sirt1, p-ERK1/2 and Runx2 in hUMSCs. In OIM, hUMSCs were treated with BME at concentration of 50 μ M for 3 days. Cells cultured in OIM without BME were included as control. **a** Intracellular ROS levels of hUMSCs were determined by flow cytometry and

quantitative analysis (**b**). **c** The protein levels of sirt1, p-ERK1/2, total ERK1/2 and Runx2 were detected by western blotting with the indicated antibodies and the band intensities were further quantified using Image J software (**d–f**). * $P < 0.05$, compared with control

ERK1/2 and Runx2 were also significantly up-regulated in hUMSCs with BME treatment (Fig. 4e, f). These results suggested that sirt1-ERK1/2 pathway may contribute to increased Runx2 expression following stimulation with BME, therefore enhancing osteogenic differentiation of hUMSCs.

Inhibition of sirt1 attenuated the stimulatory effect of BME on hUMSCs osteogenesis

To further confirm the involvement of the sirt1-ERK1/2 signaling pathway in BME-enhanced osteogenic differentiation of hUMSCs, nicotinamide (NAM), an antagonist used to inhibit sirt1 activity in cells, was added to the OIM through the entire cultivation period. As for the NAM concentration, we adopted the concentration of NAM as 10 mM, referring to other

reported studies (Wang et al. 2018). As shown in Fig. 5a–c, treatment with NAM at the dose of 10 mM decreased the protein level of sirt1 and phosphorylation of ERK1/2. Consistently, the expression of Runx2 in hUMSCs was also down-regulated by treatment with NAM (Fig. 5d), indicating that down-regulation of sirt1 can decrease the expression of p-ERK1/2 and Runx2.

Furthermore, we examined whether inhibition of sirt1 by NAM would block the effect of BME on osteogenic differentiation of hUMSCs. The ALP activity assay and Alizarin Red S staining showed that treatment with NAM significantly reduced BME-stimulated ALP activity and mineralization (Fig. 5e). Moreover, the results of real-time PCR showed that the increased mRNA expression levels of *ALP*, *OPN*, *OCN* and *COL1* by BME treatment were significantly

Fig. 5 NAM inhibited the effect of BME on the osteogenesis of hUMSCs. Cells were induced toward osteogenic differentiation in the presence of 50 μM BME and 10 mM NAM for 3 days. **a** The protein levels of sirt1, p-ERK1/2, total ERK1/2 and Runx2 were detected by western blotting with the indicated antibodies and the band intensities were further quantified using

Image J software (**b–d**). **e** Representative images of ALP activity stained by BCIP/NBT dye on day 7 and mineralization stained by Alizarin Red S on day 14. Scale bar, 100 μm. **f** Gene expression of *ALP* (day 7), *OPN*, *OCN* and *COL1* (day 14) were determined by real-time PCR. * $P < 0.05$, compared with control. (Color figure online)

suppressed by NAM (Fig. 5f). Taken together, these results suggested that the stimulatory effect of BME on hUMSCs osteogenic differentiation was mediated through the sirt1-ERK1/2 pathway.

Discussion

hUMSCs are emerging as a promising source for bone regeneration in the treatment of bone defects as it does not require ethical issues to obtain and can alleviate patients' morbidities (Ansari et al. 2018). However, several studies have shown that hUMSCs displayed weaker osteogenic differentiation capacity compared with other sources of MSCs, halting the further development of hUMSCs into therapeutics. So, it is important to develop an efficient induction process and understand the mechanism of osteogenesis in hUMSCs towards bone regeneration. In the present study, our results showed that BME (50 μ M) significantly promoted ALP activity, calcium deposition and the expression of osteogenesis-related markers. Moreover, we found that the sirt1-ERK1/2 pathway was involved in the BME-enhanced osteogenic differentiation of hUMSCs.

Since many researchers have observed that BME promoted cell growth and differentiation such as murine B cells (Sugama et al. 1987), human marrow osteoprogenitor cells (Inui et al. 1997) and fetal neurons (Ni et al. 2001), high concentrations of BME can also induce cell shrinkage and apoptosis (Lu et al. 2004). Therefore, the optimal concentration of BME on the growth and osteogenic differentiation of hUMSCs in our study remains to be determined. The results indicated that BME enhanced hUMSCs proliferation at 20–50 μ M, which this proliferative effect was also present in BMSCs (Khang et al. 2012). Moreover, the expression of osteogenesis-related markers that are expressed at different stages of the differentiation process, including ALP, OPN, OCN and COLI were significantly up-regulated by 50 μ M BME treatment, clarifying that BME could also promote the osteogenic differentiation of hUMSCs in both early and late stages.

Previous researches have shown that BME, as a thiol compound, stimulated the intracellular glutathione (GSH) synthesis and reduced ROS levels, leading to low oxidative stress in many species (Patel et al. 2015). Studies have reported that ROS

production affected the sirt1 activity, consequently inducing age-related pathology and cellular senescence (Salminen et al. 2013). Furthermore, excessive ROS levels inhibited sirt1 protein expression and led to increased expression of peroxisome proliferator-activated receptor gamma 2 (PPAR γ 2) and a decreased Runx2 expression, thereby regulating the adipogenic/osteogenic lineage differentiation of MSCs (Lin et al. 2018). In our study, BME treatment reduced intracellular ROS levels and increased expression of sirt1 in hUMSCs. Increasing evidence has demonstrated that sirt1 played a crucial role in modulating the lineage commitment of MSCs to osteogenesis and promoting bone formation. In a study conducted by Zainabadi et al. (2017), mice lacking sirt1 displayed an osteoporotic phenotype associated with decreased osteoblast numbers and reduced bone formation rate. Osteoblasts lacking sirt1 showed decreased osteogenic differentiation associated with reduced expression of Runx2 targets. Runx2 is a pivotal transcription factor and has been shown to regulate the expression of osteogenesis-related markers including *COLI*, *OCN*, *OPN*, playing a crucial role in osteogenic differentiation and bone formation (Liu and Lee 2013). Sirt1 can up-regulate *Runx2* expression through binding of a sirt1/FOXO3a complex to a novel FOXO response element (FRE) on the recently promoter of *Runx2* (Tseng et al. 2011). Consistently, our study found that inhibition of sirt1 by NAM attenuated the BME-enhanced Runx2 expression and osteogenesis, highlighting the importance of sirt1 in promoting Runx2 expression in hUMSCs.

ERK1/2, a member of the mitogen-activated protein kinase (MAPK) family, has been intensively investigated to regulate many cellular functions, such as cell proliferation, differentiation and apoptosis (Lai et al. 2001; Lu et al. 2010). Furthermore, inhibition or activation of ERK1/2 has been reported to control the commitment of hMSCs into the adipogenic or osteogenic lineages, respectively (Jaiswal et al. 2000). Studies have found that Runx2 is one of the important downstream target of the ERK1/2 pathway. The activated ERK1/2 can directly enter the nucleus and increase the phosphorylation and transcription potential of Runx2, thereby regulating downstream gene expression and subsequent mineralization (Greenblatt et al. 2013). With regard to the relationship between sirt1 and ERK signaling, it has been demonstrated that sirt1 could increase the

phosphorylation of ERK1/2 to promote human embryonic stem cells self-renewal (Safaeinejad et al. 2017) and protect cardiomyocytes from oxidative injury (Becatti et al. 2012). Similarly, the western blotting assay in our study showed that blocking the expression of sirt1 with NAM treatment decreased ERK1/2 phosphorylation and Runx2 expression. The results suggest that sirt1 may also indirectly up-regulate Runx2 expression through phosphorylation of ERK1/2. While the mechanism of sirt1-induced ERK1/2 phosphorylation remains to be elucidated, studies have demonstrated that during the osteogenic differentiation of MSCs, ERK signaling pathway was modulated by AMPK (Kim et al. 2012). The activity of AMPK could be regulated by its upstream regulator, liver kinase B1 (LKB1), which has been reported to be deacetylated by sirt1 (Lan et al. 2008). Thus, it is tempting to speculate that sirt1 may promote ERK phosphorylation by regulating AMPK activity and further investigations are required to ascertain this conjecture.

Conclusion

In conclusion, our study demonstrated that small-molecule antioxidant BME facilitated osteogenic differentiation of hUMSCs. Furthermore, the BME-enhanced osteogenic differentiation was mediated by increased Runx2 expression via the sirt1-ERK1/2 signaling pathway. These findings highlight the critical influence of BME on osteogenic induction, suggesting potential implications in bone defect repair.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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