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Original Article

Grem1 inhibits osteogenic differentiation of MBMSCs in OVX rats through BMP/Smad1/5 signaling pathway

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ABSTRACT

Objective: This study aims to explore how Grem1 regulates the differentiation and signaling activity of mandibular bone marrow mesenchymal stem cells (MBMSCs), affecting their osteogenic differentiation capacity and participating in the pathophysiological mechanism of postmenopausal mandibular osteoporosis.

Materials and methods: A postmenopausal osteoporosis (POP) rat model was constructed via bilateral ovariectomy. Techniques such as Western Blot (WB) and Real-Time Quantitative PCR (RT-qPCR) were employed to determine changes in Grem1 expression in MBMSCs of postmenopausal rats and its effect on osteogenic differentiation. Plasmids for Grem1 overexpression and siRNA for Grem1 knockdown were transfected into MBMSCs, and WB was used to assess the regulatory role of Grem1 on MBMSCs osteogenic differentiation.

Results: Grem1 expression was significantly elevated in the MBMSCs and mandibular tissues of POP rats, accompanied by inhibited osteogenic differentiation. Grem1 levels were inversely proportional to osteogenic capacity and BMP/Smad1/5 signaling activity. BMP-2 alleviated Grem1's inhibitory effects on the BMP/Smad1/5 pathway, influencing MBMSCs' osteogenic differentiation. Upregulating Grem1 in MBMSCs suppressed BMP/Smad1/5 pathway activity and osteogenic differentiation, while Grem1 knockdown restored these processes in the OVX group.

Conclusion: Grem1 reduces osteogenic capacity in mandibular POP rats by inhibiting the BMP/Smad1/5 signaling pathway. Targeting Grem1 or enhancing BMP/Smad1/5 signaling activity may improve mandibular bone health in osteoporosis patients.

1. Introduction

Osteoporosis (OP) is a skeletal disorder characterized by reduced bone mass and altered bone microarchitecture, resulting in an increased risk of fractures. Postmenopausal osteoporosis (POP), one of the most common forms of OP, arises from sustained low-grade activation of the immune system and systemic pro-inflammatory states due to estrogen deficiency, leading to bone loss [1]. It is estimated that approximately 200 million women globally suffer from osteoporosis, affecting about 10 % of women in their 60s, 20 % in their 70s, 40 % in their 80s, and 66 % in their 90s [2]. The number of individuals affected by POP is expected to rise significantly in the future, along with an increased risk of fragility fractures, thereby imposing a greater economic burden on families and society.

To address these challenges, modern technological advances must be leveraged to study POP and gain a more accurate understanding of its mechanisms and pathogenesis. This will enable the formulation of effective prevention and targeted treatment strategies to combat bone loss. Traditional theories attribute OP to bone remodeling disorders caused by decreased estrogen or aging. However, recent studies have revealed intricate connections between the skeletal and immune systems [3]. The activities of osteoblasts and osteoclasts are regulated by soluble mediators secreted by immune cells, such as cytokines, chemokines, and growth factors. In turn, osteoblasts and osteoclasts modulate the

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stem cell microenvironment, from which immune cells originate [4]. Immune cells involved include M1 macrophages, dendritic cells (DCs), neutrophils, Th1 cells, Th17 cells, and B cells. These cells release mediators like TNF-α, IL-1β, IL-6, and ROS, promoting osteoclastogenesis [5–7]. Conversely, M1 macrophages, along with Treg cells, facilitate bone formation by releasing TGF-β, IGF-1, CCL2, and CCL5 [8,9]. Increasing evidence indicates that both innate and adaptive immune cells produce inflammatory mediators that drive the development and progression of OP.

OP impacts the skeletal system comprehensively, affecting not only the alveolar bone but extending its reach across the entire body [10]. Research indicates that systemic low bone mineral density (BMD) is linked to alveolar bone loss, where inflammation and its influence on bone remodeling play pivotal roles in both osteoporosis and periodontal disease pathogenesis, serving as central mechanisms bridging these disorders [11]. However, due to the intricate anatomical structures of the jaw, complex movements of facial muscles, and the occlusal dynamics involving the maxillary and mandibular teeth, manifestations of osteoporosis within the jawbones manifest uniquely through decreased trabecular BMD and alterations in bone architecture's complexity, specifically seen through the separation, disruption, and diminution in number and thickness of bone plates [10]. A systematic review highlights how mandibular cortical indices, cortical width, and panoramic mandibular index can serve as effective screening tools for individuals with reduced BMD [12]. Apart from acknowledged systemic osteoporosis risk factors, specific oral conditions such as bacterial infections, periodontal disease, missing teeth, and diminished masticatory function are confirmed to bear close ties with mandibular bone health [13,14]. The manifestation of mandibular osteoporosis significantly jeopardizes oral health, impeding the outcomes of periodontal disease management, orthodontic treatments, dental implant success, and post-extraction recovery [10,15,16]. Current therapeutic agents, encompassing bisphosphonates, raloxifene, denosumab, teriparatide (TPTD), romosozumab, and strontium ranelate, though capable of augmenting mandibular BMD, carry severe adverse effects similar to bisphosphonate-related osteonecrosis of the jaw (BRONJ) [17,18]. Thus, there arises an urgent need for innovative management strategies targeting mandibular osteoporosis.

Recent clinical trials have attested to the safety and initial efficacy of stem cell therapy for osteoporosis, particularly highlighting mesenchymal stem cell (MSC) transplantation in accelerating fracture healing rates and alleviating osteoporotic symptoms [19–21]. Nevertheless, definitive protocols concerning the procedure, optimum stem cell sources, appropriate dosage, and methods of delivery require additional scrutiny. Mandible bone marrow mesenchymal stem cells (MBMSCs), derived from the mandibular bone marrow, represent pluripotent stem cells responsive to specific cytokines like BMP and Wnt, which can differentiate into chondrocytes, osteoblasts, or adipocytes, playing essential roles in bone tissue homeostasis [22]. Cao et al. [23] discovered that the reduced osteogenic differentiation capability and enhanced adipogenic propensity of MBMSCs contribute significantly to the onset of mandibular OP. Comparatively, MBMSCs exhibit higher expression levels of osteogenic genes such as Runx2 and osterix, alongside stronger osteogenic and proliferative capacities than iliac crest-derived bone marrow mesenchymal stem cells (BMSCs) [24,25]. This suggests unique characteristics distinct from long bones or other flat bones' stem cells. Hence, exploring key factors governing the biological traits of MBMSCs in mandibular OP will provide insights into the disorder's pathogenesis, facilitating the development of therapeutic strategies. Investigating the regulatory elements orchestrating MBMSC properties in mandibular osteoporosis opens up avenues for understanding the condition's etiology and may offer foundational scientific support for devising targeted treatment approaches. This line of inquiry promises to enrich our comprehension of mandibular OP and potentially revolutionize its clinical management, offering hope for patients afflicted by this debilitating condition.

Grem1, a highly conserved secreted protein and a member of the BMP family antagonists, serves as a specific indicator of changes in tissue or organ microenvironments and plays a critical role in maintaining normal tissue homeostasis [26]. Grem1 interacts with fibroblastic reticular cells to reduce dendritic cell density and quantity, altering the immune microenvironment, indicating that Grem1 is an immune-related cytokine [27,28]. Studies have reported significant upregulation of Grem1 in human and animal models of osteoarthritis [29,30]. Although little research has explored Grem1 expression in BMSCs, one study demonstrated that Grem1 expression was upregulated in osteoblasts treated with miRNA-150 derived from BMSCs exosomes [31]. Gazzerroet al. [32] found that loss of Grem1 in the bone microenvironment enhances BMP signaling and activity, promoting bone formation, whereas overexpression of Grem1 in mouse bone tissue results in severe osteoporotic changes. The authors hypothesized that Grem1 inhibits osteoblast differentiation from BMSCs. In osteoblasts, Grem1 overexpression reduces BMP-2 activity, which can be reversed by siRNA knockdown [33]. In human BMSCs, Grem1 knockdown increases the expression of key osteogenic transcription factors MSX2 and RUNX2 [34]. Further studies have revealed that Grem1 participates in osteoarthritis and bone formation through TGF-β/Smad or BMP-Smad1/5 signaling pathways [35]. These findings indicate that Grem1 plays a critical role in disease development by inhibiting osteoblast differentiation and signaling pathway activity. However, the role of Grem1 in MBMSCs and its impact on mandibular POP remain unexplored. Therefore, we hypothesize that Grem1 plays a crucial role in mandibular POP progression and that the BMP/Smad signaling pathway is involved in this process.

In this study, an ovariectomy rat model was established to simulate the pathological state of postmenopausal osteoporosis. By analyzing Grem1 expression differences in mandibular tissues and MBMSCs of two groups of rats, Grem1 was identified as a key factor regulating MBMSCs' osteogenic characteristics in mandibular POP. Further examination of downstream pathways revealed significant downregulation of BMP/Smad signaling in Grem1-mediated mandibular POP. The objective of this study is to investigate the functional role of Grem1 in MBMSCs and its relationship with mandibular POP.

2. Materials and methods

2.1. Cell culture

Rats in the OVX and Sham groups were euthanized by cervical dislocation at 24 weeks post-surgery. The bodies were immersed in 75 % ethanol for 5 min before being transferred to a sterile workbench. The mandibles were dissected, and soft tissues were carefully removed. The mandibles were placed in α-MEM complete culture medium containing 20 % fetal bovine serum. Bone marrow was flushed from the marrow cavity with a syringe and dispersed by pipetting. The collected bone marrow was placed in a 25 cm^2 plastic culture flask and incubated in a 5 % CO_2 atmosphere at 37 °C. When cell confluence reached approximately 80 %, cells were passaged after digestion with trypsin.

2.2. Cell transfection

Using LipoFiter 3.0 lipid transfection reagent, cells were seeded at a density of 2×10^5 cells/well in a 6-well plate one day prior to

transfection. Once the cells reached approximately 50 % confluence, the culture medium was replaced with 2 mL of fresh DMEM. Appropriate amounts of plasmid DNA and LipoFiter 3.0 lipid reagent were mixed and incubated to form a LipoFiter-DNA complex, which was added to the wells. After 6 h of incubation, the medium was replaced with fresh serum-containing complete culture medium. For siRNA transfection, an RNAFit RNA transfection kit was used. siRNA duplexes and RNAFit reagent were mixed, incubated, and added to the wells. Transfected cells were used for subsequent experiments 24 h later.

2.3. Induction of osteogenic differentiation

When cell confluence reached 80 %, osteogenic differentiation was induced. The osteogenic induction medium contained 1 % penicillin, 0.1 μM dexamethasone, 10 mM β-glycerophosphate, and 0.2 mM vitamin C.

2.4. Alkaline phosphatase (ALP) staining

Seven days after osteogenic induction, MBMSCs were fixed in 4 % paraformaldehyde for 30 min and washed three times with phosphate-buffered saline (PBS). BCIP/NBT staining solution was prepared by mixing BCIP solution (300×, 10 μL), NBT solution (150×, 20 μL), and BCIP/NBT working solution (3.03 mL). The staining solution was added to the cells to completely cover them. Cells were incubated at room temperature in the dark for 5–30 min or up to 24 h until the desired staining intensity was achieved. The reaction was stopped by washing with distilled water 1–2 times. Stained cells were observed and photographed under a microscope.

2.5. Alizarin Red staining

Twenty-one days after osteogenic induction, MBMSCs were fixed in 4 % paraformaldehyde for 30 min and washed three times with PBS. Cells were treated with an appropriate amount of Alizarin Red dye (Solarbio, China) and incubated for 5 min. Excess dye was thoroughly washed off with PBS. Cells were observed and photographed under a microscope.

2.6. Real-Time Quantitative PCR (RT-qPCR)

Total RNA was extracted from samples using the TRIzol method. Reverse transcription was performed using a commercial kit to synthesize cDNA. The extracted RNA was subjected to agarose gel electrophoresis, and sample integrity was confirmed under UV illumination. Bright, sharp, and distinct 28S and 18S bands, with a ratio close to or greater than 2:1, indicated high-quality RNA. RNA with OD 260/280 > 1.8 and OD 260/230 > 1.5 was used for downstream experiments. The RT reaction mixture contained 2 μg of total RNA, and the PCR reaction used 2 μL of cDNA. β-actin was used as an internal control for mRNA amplification during RT-qPCR.

2.7. Western Blot (WB)

Cells were lysed using RIPA buffer supplemented with phosphatase inhibitors and PMSF (100:1 ratio). Protein concentration was determined using a BCA kit (Solarbio, Shanghai, China). Equal amounts of protein (20 μg) were subjected to SDS-PAGE, followed by transfer to PVDF membranes. Membranes were blocked and incubated overnight with primary antibodies (Abcam, Waltham, MA, USA). Secondary antibody incubation and detection were performed the following day (Millipore, China). Protein bands were visualized, and their relative gray values were quantified using Image Lab software.

2.8. Data analysis

Statistical analysis was performed using SPSS 29.5 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Independent sample t-tests were used for comparisons between two groups, while one-way ANOVA was applied for three-group comparisons (non-parametric and two-tailed tests). All experiments were repeated at least three times, and representative results were presented. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Upregulation of Grem1 expression and decreased BMP/Smad1/5 pathway activity in POP rats

Mandibular bone tissues and MBMSCs were extracted from Sham and OVX groups of SD rats (referred to as S-MBMSCs and O-MBMSCs, respectively). Differences in Grem1 expression between the two groups were analyzed using qRT-PCR and WB. Results showed significantly higher Grem1 expression in O-MBMSCs compared to S-MBMSCs (Fig. 1A). Similarly, mandibular bone tissues from the OVX group displayed markedly higher Grem1 expression than those from the Sham group (Fig. 1C), consistent with findings in MBMSCs. Furthermore, elevated Grem1 expression was observed in the spleen and periovarian adipose tissues of OVX rats compared to Sham rats, suggesting tissue-specific expression changes in osteoporosis (Fig. 1B). Subsequently, S-MBMSCs and O-MBMSCs were induced for osteogenesis at 30 min, 7 days, and 21 days, and changes in Runx2 and P-Smad1/5 protein expression were analyzed using WB. Alizarin red staining was used to observe mineralization capacity. Results revealed significantly reduced osteogenic capacity in O-MBMSCs compared to S-MBMSCs, and P-Smad1/5 was inhibited during the osteogenic differentiation process in O-MBMSCs (Fig. 1D and E). These findings suggest that Grem1 expression is upregulated, and BMP/Smad1/5 pathway activity is suppressed in postmenopausal osteoporotic rats.

3.2. Effect of Grem1 expression modulation on osteogenic capacity and BMP/Smad1/5 pathway activity in MBMSCs

To clarify the regulatory effect of Grem1 on BMP/Smad1/5 pathway activity, Grem1 siRNA and plasmid were constructed to knock down or overexpress Grem1 in MBMSCs, respectively (Fig. 2A and B). Osteogenic differentiation was induced for 30 min, 3 days, and 7 days, and Runx2 and P-Smad1/5 expression levels were assessed using qRT-PCR and WB. Results indicated that Grem1 knockdown enhanced Runx2 expression and upregulated P-Smad1/5 protein levels (Fig. 2C and E), while Grem1 overexpression led to the opposite effects (Fig. 2D and F). These results demonstrate that Grem1 regulates BMP/Smad1/5 pathway activity to influence the osteogenic differentiation capacity of MBMSCs.

3.3. Grem1 knockdown promotes osteogenic differentiation in O-MBMSCs

To investigate whether Grem1 knockdown could improve the osteogenic capacity of O-MBMSCs, Grem1 siRNA and si-NC were transfected into O-MBMSCs, followed by osteogenic induction for 30 min, 7 days, and 21 days. Results from WB and Alizarin red staining showed that Grem1 knockdown significantly enhanced the osteogenic capacity of O-MBMSCs (Fig. 3A and B). Additionally, P-Smad1/5 activity was significantly elevated (Fig. 3A). These findings indicate that reducing Grem1 expression in MBMSCs from

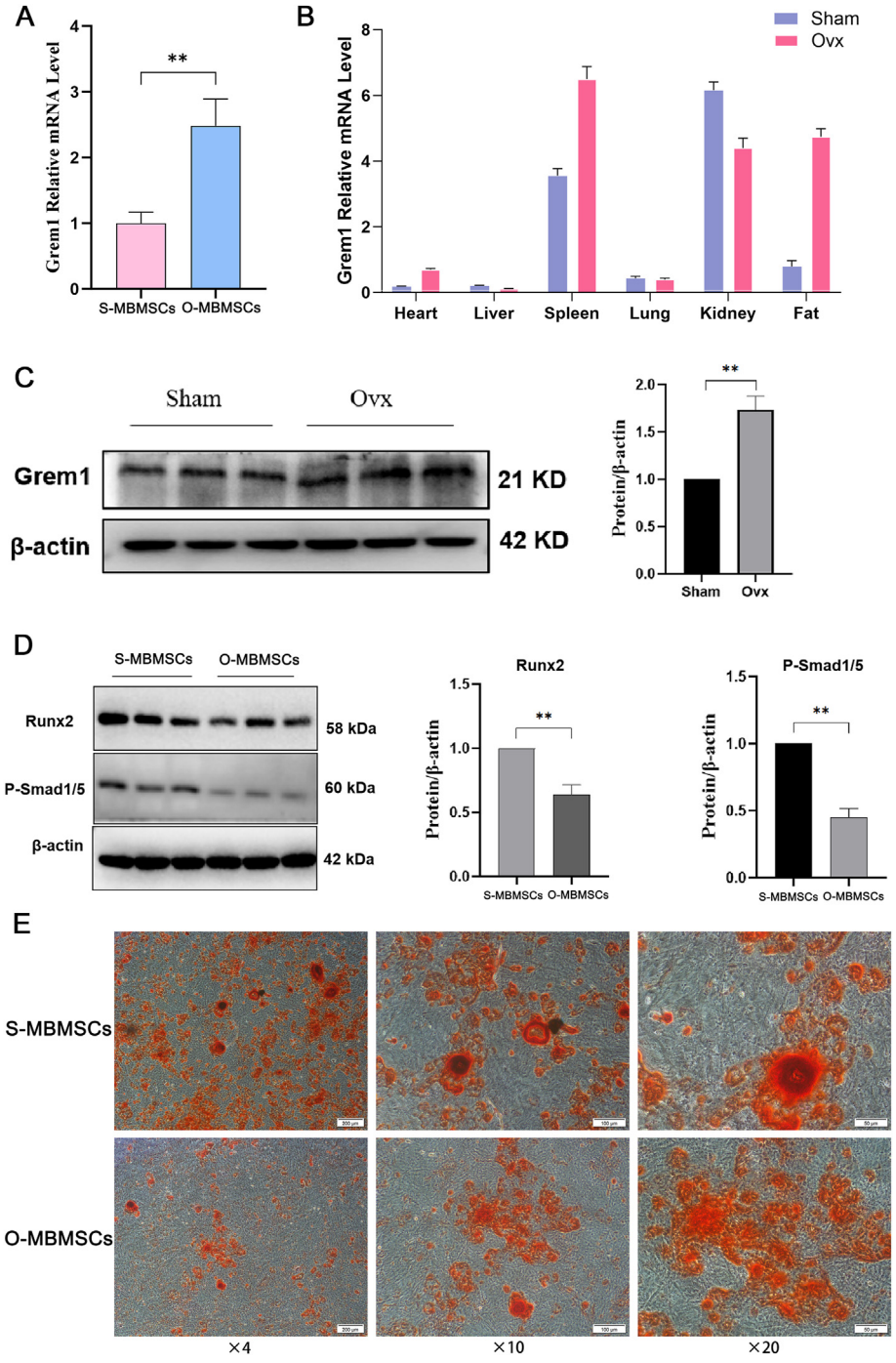

Fig. 1. Upregulation of Grem1 Expression and Downregulation of BMP/Smad1/5 Pathway Activity in POP Rats. (A) qRT-PCR detection of Grem1 mRNA expression in MBMSCs from Sham and OVX groups. (B) qRT-PCR detection of Grem1 mRNA expression in the heart, liver, spleen, lungs, kidneys, and periovarian adipose tissues of Sham and OVX rats. (C) WB detection of Grem1 protein expression in mandibular bone tissues from Sham and OVX rats, along with statistical analysis of grayscale values for protein bands. (D) WB detection of P-Smad1/5 and Runx2 protein expression in MBMSCs from Sham and OVX groups after 30 min and 7 days of osteogenic induction, with statistical analysis of grayscale values for protein bands. (E) Alizarin red staining of MBMSCs from Sham and OVX groups after 21 days of osteogenic induction. *P < 0.05, **P < 0.01.

postmenopausal osteoporotic rats can partially restore BMP/Smad1/5 pathway activity and osteogenic capacity.

3.4. BMP-2 partially reverses the inhibitory effect of Grem1 on BMP/Smad1/5 pathway and osteogenesis

BMP-2, as an antagonist of Grem1, was used to examine whether it could mitigate the inhibitory effect of Grem1 on the BMP/Smad1/5 pathway and osteogenic differentiation in MBMSCs. MBMSCs were treated with varying concentrations of BMP-2 (0, 10, 20, 40, 80, and 160 ng/mL) for 7 days. qRT-PCR and ALP staining results showed that Runx2 mRNA expression was significantly increased in the 80 ng/mL and 160 ng/mL BMP-2 groups, with no significant difference between these two groups (Fig. 4A and B). Therefore, 80 ng/mL was chosen for subsequent experiments. Next, MBMSCs were divided into four groups: Control, BMP-2-treated, Grem1-

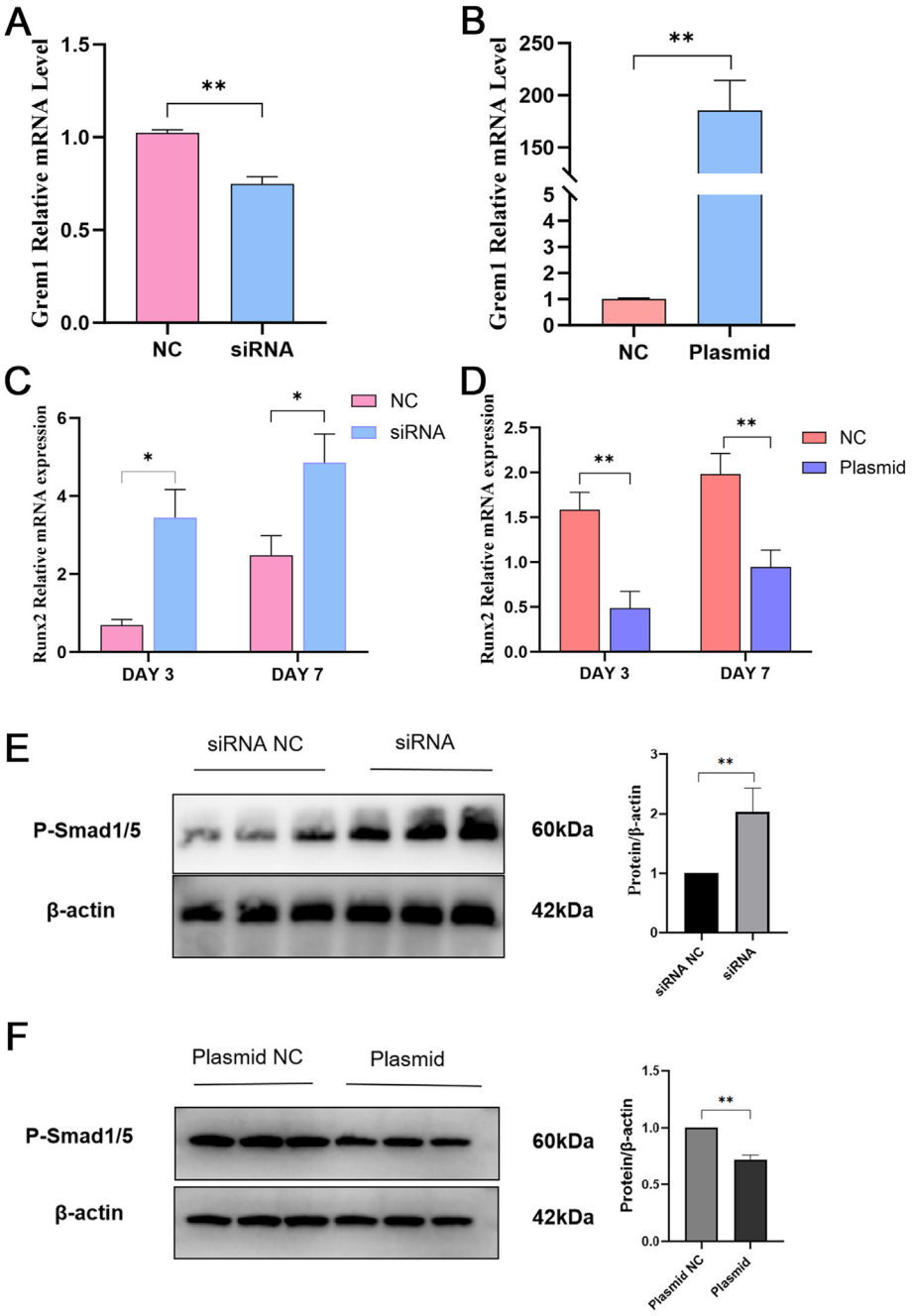

Fig. 2. Effect of Grem1 on Osteogenic Differentiation and BMP/Smad1/5 Pathway Activity in MBMSCs. (A) Validation of Grem1 knockdown in MBMSCs using qRT-PCR after transfection with Grem1 siRNA. (B) Validation of Grem1 overexpression in MBMSCs using qRT-PCR after transfection with Plasmid-Grem1. (C, D) qRT-PCR detection of Runx2 expression after 3 and 7 days of osteogenic induction in MBMSCs transfected with Grem1 siRNA or Plasmid-Grem1. (E, F) WB detection of P-Smad1/5 protein expression after 30 min of osteogenic induction in MBMSCs transfected with Grem1 siRNA or Plasmid-Grem1, along with statistical analysis of grayscale values for protein bands. *P < 0.05, **P < 0.01.

overexpressed (Plasmid), and BMP-2 + Grem1-overexpressed (BMP-2 + Plasmid). Osteogenic induction was performed for 30 min, 7 days, and 21 days. WB and Alizarin red staining results showed that BMP-2 treatment significantly upregulated Runx2 and P-Smad1/5 expression, while Grem1 overexpression significantly inhibited their expression. Subsequently, we investigated the expression of Grem1 after 7 days of osteogenic differentiation induced by 80 ng/ml BMP-2 in MBMSCs via qRT-PCR. Our findings revealed a notable downregulation of Grem1 under the stimulation of BMP-2 (Fig. 4C), further suggesting that BMP-2 might facilitate the osteogenic differentiation of MBMSCs by suppressing the expression of Grem1. Notably, BMP-2 partially reversed the inhibitory effects of Grem1 on P-Smad1/5 and Runx2 expression in the BMP-2 + Plasmid group compared to the Plasmid group (Fig. 4D and E). These findings suggest that exogenous BMP-2 can partially rescue the inhibitory effects of Grem1 on the BMP/Smad1/5 pathway and osteogenic capacity during MBMSCs osteogenesis.

4. Discussion

This study revealed the upregulation of Grem1 expression and the downregulation of BMP/Smad1/5 signaling activity in

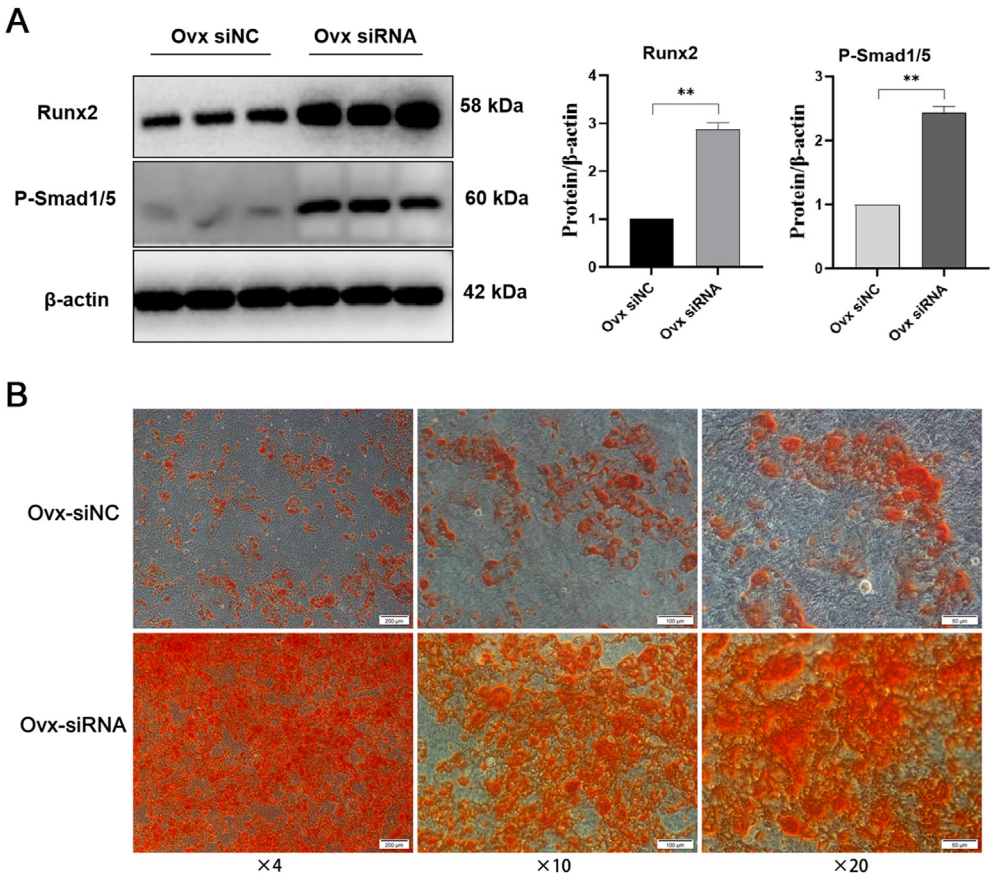

Fig. 3. Grem1 Knockdown Promotes Osteogenic Differentiation in O-MBMSCs. (A) WB detection of P-Smad1/5 and Runx2 protein expression in O-MBMSCs after Grem1 knockdown, along with statistical analysis of grayscale values for protein bands. (B) Alizarin red staining to observe changes in mineralization capacity in O-MBMSCs after Grem1 knockdown. *P < 0.05, **P < 0.01.

postmenopausal osteoporotic rats. Specifically, we observed significantly higher Grem1 expression in O-MBMSCs compared to S-MBMSCs, and similarly elevated Grem1 levels in the mandibular bone tissues of OVX rats compared to Sham rats. These findings align with prior studies reporting increased Grem1 expression in other tissues, suggesting a crucial role for Grem1 in the pathogenesis of POP.

An increasing body of evidence indicates that Grem1 induces inflammation and is associated with various pathological mechanisms and diseases, including fibrosis in the heart, lungs, and liver, as well as processes like osteogenesis, angiogenesis, and cancer progression [36–38]. Importantly, chronic inflammation and reduced osteogenic differentiation of BMSCs are key features promoting POP [39,40]. Notably, in this study, significant upregulation of Grem1 expression was also observed in the spleen and periovarian adipose tissues of OVX rats. Osteoporosis is recognized not only as a skeletal disorder but also as a systemic metabolic disease involving complex interactions among the skeletal, immune, and endocrine systems. Immune cells, such as T cells and B cells, secrete cytokines that influence bone formation and resorption, a field termed “osteoimmunology” [41]. Furthermore, the spleen, as a vital immune organ, may affect systemic immune states and bone metabolism. Adipose tissue, known for secreting adipokines such as leptin and adiponectin, also plays a role in bone metabolism [42].

Grem1, a BMP antagonist, exhibits tissue-specific expression. During development, its expression varies across different organs and tissues [43]. However, limited research exists on the changes in Grem1 expression in immune organs and adipose tissue under osteoporotic conditions. Although direct evidence is lacking, given Grem1's role in BMP signaling and the significance of immune and adipose tissues in bone metabolism, it is plausible that changes in Grem1 expression contribute to the systemic metabolic regulation of osteoporosis. Our study is the first to report significant upregulation of Grem1 in the spleen and periovarian adipose tissues of OVX rats, suggesting that Grem1 may not only act locally in bone tissues but also indirectly influence bone metabolism by modulating immune function and adipose metabolism.

In functional experiments involving Grem1, few studies have explored its expression in BMSCs. One study showed that Grem1 expression was upregulated in osteoblasts treated with exosomal miRNA-150 from BMSCs [31]. However, no prior research has examined the relationship between Grem1 expression in MBMSCs and mandibular POP. Our findings demonstrate that O-MBMSCs exhibit significantly reduced osteogenic capacity compared to S-MBMSCs, with suppressed P-Smad1/5 expression during osteogenic differentiation. Bone morphogenetic proteins (BMPs) are key initiators of cellular and signaling cascades [44]. The upregulation of Grem1, as a BMP antagonist, likely inhibits BMP/Smad1/5 signaling, impairing osteogenic differentiation. This aligns with previous studies suggesting that Grem1 negatively regulates bone formation. Derynck et al. [45] noted that BMP-2 and TGF-β1 activate classical Smad1/5/8 and Smad2/3 pathways, respectively, in BMSCs. These pathways, though coupled with Smad4, perform distinct and sometimes opposing functions in bone homeostasis. Ligand antagonists can further regulate Smad signaling in a synergistic manner [46]. For example, Wang et al. [47] found that

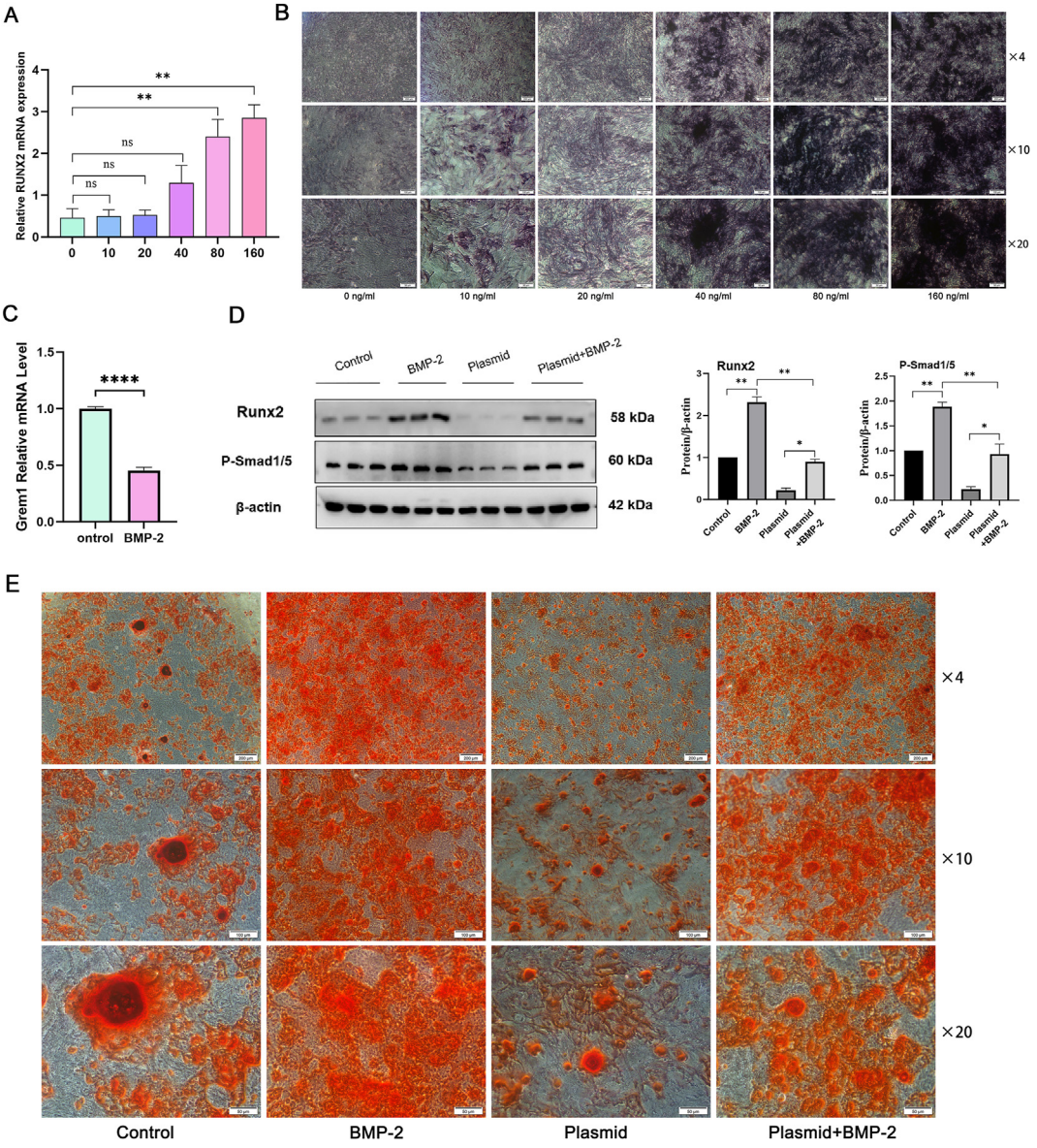

Fig. 4. BMP-2 Partially Reverses the Inhibitory Effects of Grem1 on Osteogenesis and BMP/Smad1/5 Pathway Activity in MBMSCs. (A, B) qRT-PCR and ALP staining to determine the optimal BMP-2 concentration for promoting osteogenic differentiation in MBMSCs. (C) qRT-PCR analysis of Grem1 mRNA expression changes after 7 days of osteogenic induction under 80 ng/ml BMP-2. (D) Western blot detection of P-smad1/5 and Runx2 protein expression levels, along with quantification of band densities, in MBMSCs subjected to different treatments at 30 min and 7 days post-induction. (E) Alizarin red staining to observe changes in mineralization capacity after 21 days of osteogenic induction under different treatment conditions. *P < 0.05, **P < 0.01.

excessive activation of TGF-β-mediated Smad3 signaling in primary osteoporotic BMSCs counteracted the induction of Noggin, a BMP antagonist.

In our study, Grem1 knockdown enhanced Runx2 expression and P-Smad1/5 activation in MBMSCs, improving osteogenic differentiation. Conversely, Grem1 overexpression suppressed these processes. These results underscore the pivotal role of Grem1 in regulating BMP/Smad1/5 signaling and osteogenic differentiation. Additionally, reducing Grem1 expression in O-MBMSCs restored their osteogenic capacity and BMP/Smad1/5 activity. This suggests that targeting Grem1 expression may have therapeutic potential in reversing impaired osteogenesis and BMP signaling in osteoporosis. Furthermore, we observed that exogenous addition of BMP-2 was able to downregulate Grem1 expression and counteract, to some extent, the suppressive effect of Grem1 on the BMP/Smad1/5 pathway. Acting as a functional antagonist to Grem1, BMP-2 upregulated the expression of Runx2 and P-Smad1/5, thereby enhancing osteogenic differentiation. Although Grem1 upregulation inhibited these effects, intervention with BMP-2 partially rescued this suppression. These findings suggest that BMP-2 may serve as a potential therapeutic agent to counteract Grem1-mediated inhibition of bone formation. However, further exploration is needed to determine whether other TGF-β family signaling pathways are dysregulated and interact with BMP/Smad1/5 signaling in mandibular POP.

5. Limitations

This study has several limitations. First, it primarily focuses on in vitro cellular experiments, lacking in vivo validation of Grem1's role. Second, BMP/Smad1/5 signaling regulation may involve additional factors, and Grem1's specificity requires further

investigation. Lastly, clinical application of BMP-2 raises concerns regarding dosage and safety, necessitating careful evaluation. Future studies should aim to validate the therapeutic efficacy of Grem1 inhibition in animal models and explore other regulatory mechanisms. Developing specific Grem1 inhibitors or gene therapies may provide new strategies for treating osteoporosis.

6. Conclusion

In summary, this study demonstrates that Grem1 inhibits osteogenic capacity in mandibular POP rats by suppressing the BMP/Smad1/5 signaling pathway. This mechanism provides a novel therapeutic target for osteoporosis. Targeting Grem1 or enhancing BMP/Smad1/5 pathway activity may improve mandibular bone health in osteoporosis patients.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors contributions

SSY and MLH: methodology, validation, formal analysis, investigation, data curation, writing-original draft, writing-reviewing and editing, and project administration. XHH: analyzed the data, prepared figures and/or tables. ZW: authored or reviewed drafts of the article, and approved the final draft. SSY and XHY: conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft. XHY and XHH: project administration. All authors read and approved the final manuscript.

Institutional review board statement

This study has been conducted in full compliance with the ethical principles established by the Declaration of Helsinki and has been formally approved by the Ethics Review Committee of School and Hospital of Stomatology, Zunyi Medical University (No.: Lun Shen (2020) 2–473). The approval validates that the study's design, execution, and data collection processes adhere to ethical standards. We pledge to consistently uphold these ethical guidelines throughout the research and assume accountability for the accuracy and impartiality of the findings.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Not applicable.

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