Effects of BMP-2 compound with fibrin on osteoporotic vertebral fracture healing in rats

Xiao Ouyang, Yunzhi Ding, Li Yu, Feng Xin, Xiaowei Yang, Peng Sha, Songming Tong, Qi Cheng, Yiqi Xu

Department of Orthopedic Surgery, Xuzhou Third Hospital, Affiliated Xuzhou Hospital of Jiangsu University, Xuzhou, China

Abstract

Objectives: To investigate the effects of bone morphogenetic protein-2 (BMP-2) compound with fibrin on osteoporotic vertebral fracture healing in rats. Methods: For the present study 160 Specific-Pathogen Free 32-week-old female Sprague-Dawley rats were used. 120 rats were randomly divided in three groups (experimental, model and sham operation group- n=40 per group) and were ovariectomized to establish the osteoporosis model. 40 rats served as a control group without treatment. The expression of BMP-2 in the fracture zone at the 4th, 6th, 8th, and 12th weeks was detected by qRT-PCR. The expression of BALP and CTX-I in serum at the 12th week was detected by Elisa. Results: At week 8, the morphology of the sham operation group was the same and the fracture healing occurred more slowly than in the other groups. At week 12, the expression of BMP-2 in the model group was significantly higher than that in the other three groups (p<0.05). At week 12, the maximum load, maximum strain, and elastic modulus of model group were significantly lower than those of the other three groups. Conclusions: BMP-2 compound with fibrin can enhance the timing and quality of bone fracture healing in rats.

Keywords: Bone Morphogenetic Protein-2, Fibrin, Fracture Healing, Osteoporosis, Vertebral Fracture

Introduction

Osteoporosis is a systemic skeletal disorder characterized by low bone mass, compromised bone strength and increased fracture risk. Osteoporosis is becoming an increasing burden on health care globally in view of the demographic changes. Fracture incidence increases due to the ageing of population. A report from the United States shows that osteoporotic patients over 50 years old account for about 3% of the total population in the United States, and another 10% of the population have low bone mass. Osteoporosis, as a multifactorial disorder, has a complex pathogenesis and is the consequence of genetic, hormonal, dietary, lifestyle and physical factors. Bone morphogenetic protein (BMP) is the largest subgroup of Transforming growth factor-β superfamily. BMPs play a key role in bone remodeling and are related to the occurrence and development of osteoporosis. Signaling by BMPs plays an important role in a variety of bone cell-types. The reduction of BMP will cause changes in the number and function of bone cells. BMP-2 belongs to one of BMP family members and is a key factor in bone healing. U.S. FDA approved in 2004 that rhBMP-2 (recombinant human Bone Morphogenetic protein-2) and collagen sponge composite can be used in clinical treatment of open fractures. However, bone morphogenetic protein alone will be diluted by tissue fluid and decomposed by protease after implantation, and cannot maintain a sustained drug concentration. Therefore, we chose to combine fibrin gel to achieve the effect of improving drug durability. However, there are few related researches on whether BMP-2 compound can also improve the condition of osteoporotic vertebral fracture. This study aims to explore the effects of BMP-2 compound fibrin on promoting osteoporotic vertebral fracture healing in rats.
Materials and methods

Animals

160 Specific-Pathogen Free (SPF) grade 32-week-old female Sprague-Dawley (SD) rats (Beijing Weitonglihua Experimental Animal Technology Co., Ltd., SCXK (Beijing) 2016-0006,101) with a mass of 240~260 g were reared in the animal research center of Affiliated Xuzhou Hospital of Jiangsu University after purchase. The rats were exposed to 12 hours of circulating light at a temperature of 21-25°C, a relative humidity of 50-60%, with free to food and water.

Main reagents and materials

RhBMP-2 (China Beijing Zhongke Wuyuan Biotechnology Company, H5001-50), transScript Green Two-step qRT-PCR SuperMix, EasyPure RNA Kit (China Beijing TransGen Biotech company, AQ201-01, ER101-01), rat bone alkaline phosphatase (BALP), rat type I collagen cross-linked carboxy-terminal peptide (CTX-I), Elisa Kit (Shanghai Enzyme-linked Biological Research Institute, China, ml003415, ml003405), chlora hydrate (Sigma company, USA, 47335-U), fibrinogen (self-configured by our hospital), BMP-2 primer sequence designed and synthesized by Shanghai Bio-engineering Biotechnology Co., Ltd., PCR instrument (Applied Biosystems company, USA, 7500).

Establishment of rat animal model

A total of 160 rats were randomly divided into 4 groups: control group, sham operation group, model group and experimental group. All rats were anesthetized with 10% chloral hydrate (300 mg/kg) intraperitoneally. Ovaries were removed in the experimental group and the model group. Only part of soft tissues around the ovaries were removed in the sham operation group, and then the incision was sutured. The control group was not interfered and the rats were kept in cages. Three months later, the rats in the control group did not undergo surgery. The sham operation group, model group and experimental group were anesthetized with 10% chloral hydrate (0.3 mL/100 g) intraperitoneally. The lateral position was taken and disinfected. The external oblique muscle and internal oblique muscle were surgically incised. The fat layer and sacrospinalis muscle gap were bluntly separated until the sacrospinalis muscle root and paravertebral muscle border. A fenestration was performed on the side of L5 vertebral body, with a size of about 4.5 mm in length and 4 mm in width. Gelatin sponge (1.5*3.0) was used for hemostasis. The experimental group was tapered 20 μg+ fibrin 20 μg gel mixture. The model group and the sham operation group were treated with equal volume of physiological saline, the window bone flap was sealed, and the incision was sutured layer by layer. After the operation was completed, the rats were kept in cages. All the operations were performed under aseptic conditions. After the operation, neomycin (2 mg/mL) was added to the drinking water for 7 days to prevent bacterial infection.

Collection of samples

The rats were sacrificed at the 4th, 6th, 8th and 12th weeks of the rat modeling respectively. 5 rats were sacrificed at a time, 10% chlora hydrate (300 mg/kg) was used to anesthetize them; the rats were decapitated, the L5 vertebral body of the rats was taken for subsequent detection, and the peripheral blood of the rats at the 12th week was collected to detect the expression of BALP and CTX-I.

Observation indicators

qRT-PCR detection

The collected tissues were extracted with EasyPure RNA Kit reagent to extract total RNA. The purity, concentration and integrity of the extracted total RNA were detected by UV spectrophotometer and agarose gel electrophoresis. TransScript Green Two-Step qRT-PCR SuperMix was used to reverse transcription of total RNA, and the specific steps were carried out according to the kit instructions. 5X TransScript® II All-in-One SuperMix for qPCR and gDNA Remover kits to carry out reverse transcription operation procedures in strict accordance with manufacturers' kits. Then PCR amplification experiment was carried out. PCR reaction system was as follows: cDNA 1μL, upstream and downstream primers 0.4μL each, 2XTransScript® Tip Green qPCR SuperMix 10μl, Passive Reference Dye (50X). Finally, Nuclease-free Water was added to make up to 20μL. PCR reaction conditions were as follows: 94°C pre-denaturation for 30s, 94°C denaturation for 5s, 60°C annealing extension for 30s, for a total of 40 cycles. Each sample was provided with 3 repeated wells, and the experiment was carried out 3 times. GAPDH was used as an internal reference and 2^-ΔCT was used to analyze the data. GAPDH upstream primer 5'-AAGAAGGGTGGTACAGGCGAG-3', downstream primer 5'-TCCACCCACGTCTGTGTGA-3', BMP-2 upstream primer 5'-CGTGAGATTAGCAAGTCTT-3', downstream primer 5'-GGCGTTTCCGCTTTT-3'.

ELISA detection

5 mL of collected peripheral blood was allowed to stand for 30 min and then was centrifuged at 3000 rpm for 10 min. The collected supernatant was used to detect the expression of BALP and CTX-I in rats by ELSA kit. The ELISA detection method was as follows: Blank wells, standard wells and sample wells to be detected were respectively arranged. Accurately 50 μl of standard substance was added to the enzyme-labeled coating plate, 40 μl of sample diluent was added to the sample well to be tested, and then 10 μl of sample to be tested was added. The sample was added to the bottom of the well of the enzyme-labeled plate. Try not to touch the wall of the well, and it was gently shaken and mixed. After sealing the plate with sealing plate membrane, it was incubated at 37°C for 30 minutes. The sealing film was removed, the liquid was discarded, then it was spin-dried. Each well was filled with washing liquid, standing for 30 seconds, and then it was discarded, repeating this for 5 times, and then it was patted dry. 50 μl of enzyme
Results

Bone morphology of rats

The histological morphology of the fracture zone of the four rats groups at the 4th and 8th week was observed. The histological morphology of the fracture zone of the sham operation group and the experimental group was basically the same at the 4th week, with chondrocytes, cartilage islands and bone fibers appearing, and reticular bone-like trabecular structure formation (fibrocallus) appeared between the fibers and chondrocytes, while a large number of chondrocytes were formed in the model group after 4 weeks of fracture, but only a small part of fibrocallus and structural disorder existed. At the 8th week, the bony callus of the sham operation group and the experimental group was completely mature, the fibrous calus disappeared, and the fracture had basically healed. Compared with the bone tissue of the control group, the morphology of the sham operation group was basically the same. At the 8th week, the model group had partial bone defects, a large number of fibrous callus, and no mature callus. Compared with the sham operation group and the experimental group, the fracture healing was slower. The bone tissue of rats in the control group was normal.

Expression of BMP-2 in bone tissue of rats at different time points

The expression of BMP-2 in rat tissues at various time points were detected, and it was found that there were statistical differences between groups at various time points (p<0.001). At the 4th, 6th and 8th weeks, the expression of BMP-2 in sham operation group, model group and experimental group was significantly higher than that in control group, and there were differences. The expression of BMP-2 in sham operation group and experimental group was significantly lower than that in model group (p<0.05). There were no statistical differences among the other groups (p>0.05). At the 12th week, the expression of BMP-2 in the model group was significantly higher than that in the other three groups (p<0.05), and there were no significant differences among the other groups (p>0.05). The comparison in each group showed that there were no significant differences in BMP-2 expression in the control group (P>0.05). BMP-2 peak value in the sham operation group and the experimental group reached and then gradually decreased at the 6th week, while BMP-2 peak value in the model group reached and then gradually decreased at the 8th week.
The results of detecting the expression of BALP and CTX-I in serum of rats in each group at the 12th week showed that the expression of BALP and CTX-I in serum of rats in the sham operation group and the experimental group had no significant differences with those in the control group (p>0.05), while the expression of BALP and CTX-I in serum of rats in the model group were significantly lower than those in other groups, and the expression of CTX-I was significantly higher than that in other three groups (p<0.05), as shown in Figure 1.

Detection of maximum load, maximum strain and elastic modulus

The maximum load, the maximum strain and the elastic modulus of the rats at the 6th, 8th and 12th weeks after the operation were detected and found that there were statistical differences between the groups at each time point (p<0.001). The maximum load, the maximum strain and the elastic modulus of the control group at the 6th and 8th weeks were significantly higher than those of the sham operation group, the model group and the experimental group (p<0.05). The maximum load, maximum strain and elastic modulus of the sham operation group and the experimental group were significantly higher than those of the model group (p<0.05), while there were no statistical differences among the other groups (p>0.05). At the 12th week, the maximum load, maximum strain and elastic modulus of the model group were significantly lower than those of the other three groups (p<0.05), and there were no significant differences among the other groups (p>0.05). The intra-group comparison found that the maximum load, maximum strain and elastic modulus of the sham operation group, model group and experimental group increased gradually with the increase of time, and there were statistical differences (p<0.001) in

### Table 1. Expression of BMP-2 in the four rats groups at different time points.

| Group                  | Week 4     | Week 6     | Week 8     | Week 12    | F value | P value |
|------------------------|------------|------------|------------|------------|---------|---------|
| Control group          | 1.038±0.061| 1.053±0.085| 1.013±0.120| 1.067±0.112| 1.775   | 0.229   |
| Sham operation group   | 1.430±0.059*| 1.636±0.085*| 1.503±0.054*| 1.145±0.125| 84.946  | <0.001  |
| Model group            | 1.247±0.075*#| 1.455±0.056**#| 1.695±0.085**#| 1.403±0.066**#| 38.941  | <0.001  |
| Experimental group     | 1.515±0.086*Δ| 1.729±0.082*Δ| 1.522±0.051*Δ| 1.182±0.077Δ| 94.865  | <0.001  |
| **F value**            | 44.495     | 73.917     | 63.211     | 11.764     |         |         |
| **P value**            | <0.001     | <0.001     | <0.001     | 0.001      |         |         |

*indicates that there is a difference compared with the control group (p<0.05), # indicates that there is a difference compared with the sham operation group (p<0.05), Δ indicates that there is a difference compared with the model group (p<0.05).

**Expression of BALP and CTX-I in rat serum**

The ELISA test results showed that the expression of BALP in model group was significantly lower than that in other three groups (p<0.001). ELISA results showed that the expression of CTX-I in model group was significantly higher than that in other three groups (p<0.001).
the intra-group comparison, while there were no significant differences (p>0.05) in each time point of the control group (see Tables 2, 3 and 4).

Expression of volume percentage, surface percentage, average width and mineralization deposition rate of rat trabecular bone

The volume percentage, surface percentage, average width and mineralization deposition rate of trabeculae in each group of rats at the 12th week were detected. It was found that the volume percentage and average width of trabeculae in the control group, sham operation group and experimental group were significantly higher than those in the model group (p<0.05). The surface percentage and mineralization deposition rate in the control group, sham operation group and experimental group were significantly lower than those in the model group (p<0.05). The indexes in the control group, sham operation group and experimental group had no difference (p>0.05) (Table 5).

Discussion

Osteoporosis is a primary disorder of the skeleton and is the most common cause of fractures. Due to the aging of the
Table 5. Comparison of volume percentage, surface percentage, average width and mineralization deposition rate of rat trabecular bone.

| Group              | Trabecular volume percentage (%) | Surface percentage (%) | Average width (μm) | Mineralization sedimentation rate (%) |
|--------------------|----------------------------------|------------------------|-------------------|--------------------------------------|
| Control group      | 19.68±2.44                       | 3.30±1.16              | 200.40±7.86       | 0.72±0.08                            |
| Sham operation group| 19.17±2.56                       | 3.28±1.41              | 197.39±7.69       | 0.71±0.06                            |
| Model group        | 14.95±2.31*#                     | 8.54±1.43*#           | 154.74±9.70*#     | 1.15±0.14*#                          |
| Experimental group | 19.18±2.14A                      | 3.20±1.07A            | 203.70±9.86A      | 0.74±0.10A                           |

*indicates that there is a difference compared with the control group (p<0.05), #indicates that there is a difference compared with the sham operation group (p<0.05), ^indicates that there is a difference compared with the model group (p<0.05).

population, the prevalence of osteoporosis and low bone mass is expected to increase. Gillespie et al. study showed that the incidence rate gradually increased with the increase of patients’ age through a 2-year survey of 1,638,454 women over 50 years old who had not experienced osteoporosis. At present, the clinical plan for osteoporotic vertebral fracture is limited by many factors, such as cement leakage. Although the pain of postoperative patients is relieved, kyphosis may still occur. Therefore, it is hoped to find a new clinical plan for treatment.

Bone morphogenetic protein (BMP) is the earliest and most in-depth growth factor and has become the preferred protein factor for bone tissue engineering. BMP plays a regulatory role in different stages of development of various tissues, especially in inducing bone formation. In vitro studies show that both embryonic cells and mature cells will increase their differentiation activity after being induced by BMP. BMPs are suggested to have therapeutic potential to enhance fracture healing in osteoporotic patients. BMP-2 is one of the most important extracellular signal molecules in BMP family to promote bone formation and induce osteoblast differentiation. Some countries have approved the treatment of rhBMP-2 in open fractures, but it is not clear whether rhBMP-2 has the same efficacy in osteoporotic vertebral fractures. Therefore, this study aims to provide references for clinicians’ treatment by exploring the improvement of BMP-2 compound fibrin on the condition of osteoporotic vertebral fracture in rats.

In the study of Cheng et al, BMP-2 delivered by sucrose acetate isobutyrate significantly improved bone repair in rats with open fractures. In this study, we established a rat model of osteoporotic vertebral fracture. After treatment with BMP-2 and fibrin, we observed the histological changes of the rats at the 4th and 8th weeks after the model was established. It was found that in the 4th week of the model group, there were a large number of chondrocytes, only a small amount of fibrous callus, and the structure was disordered. However, the experimental group and the sham operation group have the same tissue morphology in the fracture zone, with a small number of chondrocytes, cartilage islands and bone fibers, and fibrous callus appeared on the fibers and chondrocytes. At the 8th week, some bone defects and a large amount of fibrous callus appeared in the fracture tissue area of rats in the model group, but no mature callus appeared. Compared with the model group, the sham operation group and the experimental group, the bony callus was completely mature, the fibrous callus disappeared, and the fracture basically healed. Compared with the bone tissue of the control group, the morphology was basically the same, which indicated that BMP-2 had the same effect in treating osteoporotic vertebral fracture. Furthermore, we further detected the expression of BMP-2 in fracture tissues of rats in each group at different time points, and found that the intra-group comparison found that the expression of BMP-2 in rats in each group increased continuously with the increase of time except for the control group, and the expression of BMP-2 in sham operation group and experimental group reached a peak in the 6th week and then gradually decreased, while BMP-2 in model group reached a peak in the 8th week. The inter-group comparison found that the rats in sham operation group and experimental group reached the 4th, 6th. Compared with the control group, it was obviously increased at the 8th week, but there were no differences at the 12th week. Compared with the control group, the model group had differences at all time points, which indicated that the metabolism of rats after ovariectomy was out of balance, and the BMP-2 growth factor released after fracture was obviously decreased, while the healing of osteoporotic rats’ vertebral fracture was effectively promoted by exogenous increase of BMP-2 expression.

Furthermore, we also tested the maximum load, maximum strain and elastic modulus of rats in each group at weeks 6, 8 and 12. We found that the maximum load, maximum strain and elastic modulus of rats in other groups at weeks 6, 8 and 12 except the control group increased gradually with time. The maximum load, maximum strain and elastic modulus of rats in the experimental group and the sham operation group showed no obvious difference at each time point, and showed no difference compared with the control group at week 12. Although the indexes of rats in the model group gradually improved, the degree of change was

http://www.ismni.org
obviously lower than that in the experimental group and the sham operation group. The maximum load, the maximum strain and the elastic modulus are important indexes for evaluating the biological function of bone. Detection of these indexes also shows that the recovery of the biological function of bone in rats can be improved after treatment with BMP-2 composite fibrin. Studies by Huang et al. show that the maximum load, the maximum strain and the elastic modulus of the ovariectomized fracture mice treated with rhBMP-2 combined with psoralen are significantly improved compared with the model group. This is similar to the results of our study, but relative to his study, we have increased the detection at various time points, and observed the changes of maximum load, maximum strain and elastic modulus of rats through dynamic detection at multiple time points, further proving the role of BMP-2 composite fibrin in the treatment of osteoporotic vertebral fracture healing.

We also detected the expression of BALP and CTX-I in serum of rats in each group at the 12th week, as well as the volume percentage, surface percentage, average width and mineralization deposition rate of trabecular bone. BALP is secreted by osteoblasts, and its expression changes are markers reflecting the maturation and activity of osteoblasts. CTX-I is a bone turnover marker and is an important predictor of bone loss rate and fracture risk. The results showed that the expression of BALP and CTX-I in model group were significantly lower than that in other groups, and the expression of CTX-I was higher than that in each group. This indicated that the expression of BALP and CTX-I in rats were effectively improved after BMP-2 combined with fibrin therapy. This may be that BMP can indirectly stimulate osteoclast formation through the osteoblast nuclear factor receptor activator to stimulate κ-B (RANK)/RANKL ligand (RANKL) pathway, thus causing changes in the expression of BALP and CTX-I in rats. However, we found that the volume percentage, surface percentage, average width and mineralized deposition rate of trabecular bone in the sham operation group and the experimental group were significantly higher than those in the model group at the 12th week, while the surface percentage and mineralized deposition rate in the sham operation group and the experimental group were significantly lower than those in the model group, and there were no significant differences in each index between the experimental group and the sham operation group and the control group, which indicated that BMP-2 composite fibrin could improve the relationship between bone resorption and bone formation, promote osteoporotic vertebral tissue to become compact and trabecular bone bulky, and thus improve the healing quality.

In this study, although we showed the effect of BMP-2 composite fibrin in promoting osteoporotic vertebral fracture healing in rats, however, there are several limitations. Firstly, as a basic experiment, whether the clinical efficacy of BMP-2 composite fibrin is consistent with the experimental result needs further research. Secondly, this study has not deeply explored the specific mechanism of BMP-2 in osteoporotic vertebral fracture healing. Therefore, we aim to add clinical experiments in future research and more related basic experiments to explore the specific mechanism of BMP-2 in osteoporotic vertebral fracture healing, so as to supplement and verify our experimental results. In conclusion, BMP-2 compound fibrin can promote the healing speed and improve the healing quality of osteoporotic vertebral fracture in rats.

Authors’ contribution

XO, YD and LY conceived and designed the study. FX, XY and PS performed ELISA. ST, QC and YX contributed to observation indexes analysis. XO wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval

The study was approved by the Ethics Committee of Xuzhou Third Hospital, Affiliated Hospital of Jiangsu University.

References

1. Aspray TJ, Hill TR. Osteoporosis and the Ageing Skeleton. Subcell Biochem 2019;91:453-476.
2. Wong CC, McGirt MJ. Vertebral compression fractures: a review of current management and multimodal therapy. J Multidiscip Healthc 2013;6:205-214
3. Compston J. Glucocorticoid-induced osteoporosis: an update. Endocrine 2018;61:7-16.
4. Khan AH, Jafri L, Ahmed S, Noordin S. Osteoporosis and its perspective in Pakistan: A review of evidence and issues for addressing fragility fractures. Ann Med Surg (Lond) 2018;29:19-25.
5. Qaseem A, Forciea MA, McLean RM, Denberg TD. Clinical Guidelines Committee of the American College of Physicians. Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American College of Physicians. Ann Intern Med 2017;166:818-839.
6. Klein-Nulend J, van Oers RF, Bakker AD, Bacabac RG. Bone cell mechanosensitivity, estrogen deficiency, and osteoporosis. J Biomech 2014;48:855-865.
7. Nancy F, Tojais, Aiqin Cao, Ying-Ju Lai, Lingli Wang, Pin-I Chen, Miguel A. Alejandro Alcazar, Vinicio de Jesus Perez, Rachel K. Hopper, Christopher J. Rhodes, et al. Copependence of bone morphogenetic protein receptor 2 and transforming growth factor-β in elastic fiber assembly and its perturbation in pulmonary arterial hypertension. Arterioscler Thromb Vasc Biol 2017; 37:1559-1569.
8. Diogo IH, Phedy P, Kholinne E, Djaja YP, Fiolin J, Kusnadi Y, Yulisa ND. Autologous mesenchymal stem cell implantation, hydroxyapatite, bone morphogenetic protein-2, and internal fixation for treating critical-
sized defects: a translational study. Int Orthop 2019; 43:1509-1519.
9. James AW, LaChaud G, Shen J, Asatrian G, Nguyen V, Zhang X, Ting K, Soo C. A review of the clinical side effects of bone morphogenetic protein-2. Tissue Eng Part B Rev 2016;22:284-297.
10. Khan SN, Lane JM. The use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in orthopaedic applications. Expert Opin Biol Ther 2004;4:741-748.
11. Cecchi S, Bennet SJ, Arora M. Bone morphogenetic protein-7: Review of signalling and efficacy in fracture healing. J Orthop Translat 2015;4:28-34.
12. Paschalis EP, Fratzi P, Gamsjaeger S, Hassler N, Brozek W, Eriksen EF, Rauch F, Glorieux FH, Shane E, et al. Aging versus postmenopausal osteoporosis: bone composition and maturation kinetics at actively-forming trabecular surfaces of female subjects aged 1 to 84 years. J Bone Miner Res 2015;31:347-357.
13. Gillespie CW, Morin PE. Trends and disparities in osteoporosis screening among women in the United States, 2008-2014. Am J Med 2016;130:306-316.
14. Kuiper BW, Graybill S, Tate JM, Kaufman N, Bersabe D. After the fall: improving osteoporosis treatment following hip fracture. Osteoporos Int 2018;29:1295-1301.
15. Venkatesan J, Bhatnagar I, Manivasagan P, Kang KH, Kim SK. Alginate composites for bone tissue engineering: a review. Int J Biol Macromol 2016;92:183-195.
16. Salazar VS, Gamer LW, Rosen V. BMP signalling in skeletal development, disease and repair. Nat Rev Endocrinol 2016;12:203-221.
17. Gouon-Evans V, Boussemart L, Gadue P, Nierhoff D, Koehler CI, Kubo A, Shafritz DA, Keller G. BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm. Nat Biotechnol 2006;24:1402-1411.
18. Wagner I, Wang H, Weissert PM, Straube WL, Shevchenko A, Gentzel M, Brito G, Tazaki A, Oliveira C, et al. Serum proteases potentiates BMP-induced cell cycle re-entry of dedifferentiating muscle cells during new limb regeneration. Developmental Cell 2017;40:608-617.
19. Kolk A, Boskov M, Haidari S, Tischer T, van Griensven M, Bissinger O, Plank C. Comparative analysis of bone regeneration behavior using recombinant human BMP-2 versus plasmid DNA of BMP-2. J Biomed Mater Res A 2019;107:163-173.
20. Alt V, Borgman B, Eicher A, Heiss C, Kanakaris NK, Giannoudis PV, Song F. Effects of recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) in grade III open tibia fractures treated with unreamed nails - A clinical and health-economic analysis. Injury 2015; 46:2267-2272.
21. Herford AS. The use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillofacial trauma. Chin J Traumatol 2017;20:1-3.
22. Xue D, Zhang W, Chen E, Gao X, Liu L, Ye C, Tan Y, Pan Z, Li H. Local delivery of HMGB1 in gelatin sponge scaffolds combined with mesenchymal stem cell sheets to accelerate fracture healing. Oncotarget 2017;8:42098-42115.
23. Huang K, Wu G, Zou J, Peng S. Combination therapy with BMP 2 and psoralen enhances fracture healing in ovariectomized mice. Exp Ther Med 2018;16:1655-1662.
24. Wang JF, Lee MS, Tsai TL, Leiferman EM, Trask DJ, Squire MW, Li WJ. Bone Morphogenetic Protein-6 Attenuates Type 1 Diabetes Mellitus-Associated Bone Loss. Stem Cells Transl Med 2019;8:522-534.
25. Otsuka F. Modulation of bone morphogenetic protein activity by melatonin in ovarian steroidogenesis. Reprod Med Biol 2018;17:228-233.
26. Riederman BD, Butler BA, Lawton CD, Rosenthal BD, Balderama ES, Bernstein AJ. Recombinant human bone morphogenetic protein-2 versus iliac crest bone graft in anterior cervical disectomy and fusion: Dysphagia and dysphonia rates in the early postoperative period with review of the literature. J Clin Neurosci 2017;44:180-183.
27. Okamoto M, Murai J, Yoshikawa H, Tsumaki N. Bone morphogenetic proteins in bone stimulate osteoclasts and osteoblasts during bone development. J Bone Miner Res 2006;21:1022-1033.
28. Pan HC, Lee S, Ting K, Shen J, Wang C, Nguyen A, Berthiaume EA, Zara JN, Turner AS, et al. Cyst-Like Osteolytic Formations in Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2) Augmented Sheep Spinal Fusion. Am J Pathol 2017;187:1485-1495.