Role of fibroblast growth factors in bone regeneration

Pornkawee Charoenlarp, Arun Kumar Rajendran and Sachiko Iseki*

Abstract

Bone is a metabolically active organ that undergoes continuous remodeling throughout life. However, many complex skeletal defects such as large traumatic bone defects or extensive bone loss after tumor resection may cause failure of bone healing. Effective therapies for these conditions typically employ combinations of cells, scaffolds, and bioactive factors. In this review, we pay attention to one of the three factors required for regeneration of bone, bioactive factors, especially the fibroblast growth factor (FGF) family. This family is composed of 22 members and associated with various biological functions including skeletal formation. Based on the phenotypes of genetically modified mice and spatio-temporal expression levels during bone fracture healing, FGF2, FGF9, and FGF18 are regarded as possible candidates useful for bone regeneration. The role of these candidate FGFs in bone regeneration is also discussed in this review.

Keywords: Bone regeneration, FGFs, FGF2, FGF9, FGF18, Osteogenesis, Tissue engineering

Background

Tissue engineering is an interdisciplinary field of research and clinical applications, which focuses on restoration of impaired function and morphology of tissues and organs by repair, replacement, or regeneration. It uses a combination of several technological approaches beyond traditional transplantation and replacement therapies. The key components of these approaches are using of cells, scaffolds, and bioactive factors.

Bone is a specialized connective tissue that is being continuously remodeled throughout life. However, many complex clinical conditions such as large traumatic bone defects, osteomyelitis, tumor resection, or skeletal abnormalities can impair normal bone healing. Bone tissue engineering is required for regenerating tissue from these conditions. Studies on the mechanisms of physiological, pathological skeletal development and fracture healing have provided a wealth of information towards potential methods for regulating osteoblast proliferation and differentiation to regenerate bone.

Here, we focus on one of the main components of tissue engineering, bioactive factors, especially fibroblast growth factors (FGFs) and their roles in bone regeneration.

FGF signaling in skeletal formation has been demonstrated by identification of gain-of-function mutations in human FGF receptor (FGFR) genes in craniosynostosis and dwarfism patients and skeletal phenotypes in genetically modified mice for FGFs and FGFRs [1]. FGFRs are transmembrane tyrosine kinase receptors that belong to the immunoglobulin (Ig) superfamily consisting of extracellular, transmembrane, and intracellular tyrosine kinase domains. Binding of FGFs to FGFRs activates intracellular downstream signaling pathways such as RAS-MAP and PI3K-AKT [2]. The FGF family consists of four members, FGFR1 to FGFR4. Among the four FGFRs, skeletal mutations have been found in FGFRs1–3 expressed in the osteoblast cell lineage. Most of the mutations are point mutations, and distinct mutation sites result in different syndromes [1]. Some of the mutations have been introduced into mice and confirmed to affect skeletal development.

FGFs and bone regeneration

The mammalian FGF family contains 22 members. Some of them are intracellular FGFs (iFGFs), FGFs 11–14, which are expected to function without binding to FGFRs. FGF19 (FGF15 for mice), FGF21, and FGF23 are hormone-like FGFs which act in an endocrine manner.
in postnatal life. All other FGFs have high affinity to heparin and act in a paracrine manner by binding to the four receptors with different levels of affinities [3–5]. The roles of various FGFs are compiled in Table 1. Skeletal phenotypes after deletion of FGFs in mice are found in FGFs 2, 8, 9, 10, 18, and 23 [6], which confirm the indispensable function of FGF/FGFR signaling in the process of osteogenesis. It is of note that FGF/FGFR signaling does not directly induce osteoblast differentiation but is known to modulate osteoblast differentiation. However, the exact mechanism of FGF/FGFR signaling in bone healing or regeneration has not been elucidated. Schmid et al. [7] reported expression levels of different FGFs by reverse transcriptase polymerase chain reaction (RT-PCR) during normal healing of tibial fracture in mice. Throughout the healing process, FGFs 2, 5, and 6 were upregulated with different levels. FGF9 was highly expressed at the early stage of healing. FGFs 16 and 18 were transcribed at the late stage. Upregulation of FGFs 1 and 17 was delayed after callus formation. This study also identified concordance between the expression of the particular FGFs and their known receptors during different stages of fracture repair. Among three FGFRs expressed in the osteoblast cell lineage, FGFR3 showed the greatest change in expression levels. This study provided the idea of how FGFs work at the different stages of healing, which could be applied to bone regenerative therapy.

Considering clinical applications, studies involving modification of FGF signaling by ligands are more practical compared to those involving modulating FGFRs. Animal studies revealed that the expression of FGFs 8 and 10 is required for the early stage of limb development, which suggests that they are not directly involved in osteogenesis. Among FGFs which change their expression levels during bone fracture healing, FGF1 protects the osteoblast cell lineage from cell death [8]. FGF5 is associated with the hair follicle cycle. FGF6 is involved in muscle regeneration and those events that occur during the healing process. Therefore, in this review, we chose FGFs 2, 9, and 18 to discuss about their properties and applications for bone regeneration.

**FGF2**
FGF2 is the most common FGF ligand that is being used in the regenerative medicine field including bone regeneration.

---

### Table 1: List of FGFs and their various functions

| Subfamily | FGFs | Manner of action | Prime functions | References |
|-----------|------|-----------------|-----------------|------------|
| FGF1/2    | FGF1 | Paracrine | Patterning of optical vesicle | [36] |
|           | FGF2 | Paracrine | Neuronal, skeletal, vascular tone; heart repair | [37–39] |
| FGF4/5/6  | FGF4 | Paracrine | Proliferation of inner cell mass | [40] |
|           | FGF5 | Paracrine | Hair growth cycle regulator | [41] |
|           | FGF6 | Paracrine | Regulation of muscle regeneration | [42] |
| FGF3/7/10/22 | FGF3  | Paracrine | Inner ear formation, regulation of tooth morphogenesis | [43, 44] |
|           | FGF7 | Paracrine | Modulation of hair growth, kidney development | [45, 46] |
|           | FGF10 | Paracrine | Regulator of development of many organs such as brain, limb, lung, pancreas | [47, 48] |
|           | FGF22 | Paracrine | Presynaptic organization in brain development, hair development | [49, 50] |
| FGF9/16/20 | FGF9 | Paracrine | Lung development, maintenance of stemness in nephrons, bone repair, mammalian sex determination | [51–53] |
|           | FGF16 | Paracrine | Heart development | [54] |
|           | FGF20 | Paracrine | Inner ear development, maintenance of stemness in nephrons | [52, 55] |
| FGF8/17/18 | FGF8  | Paracrine | Development of brain, limbs, cardiovascular system, craniofacial region | [56–59] |
|           | FGF17 | Paracrine | Brain development | [60] |
|           | FGF18 | Paracrine | Bone and cartilage development, lung development | [22, 61] |
| FGF11/12/13/14 | FGF11 | Intracrine | Signalling functions during tooth development | [62] |
|           | FGF12 | Intracrine | Unclear | |
|           | FGF13 | Intracrine | Signalling functions during tooth development | [62] |
|           | FGF14 | Intracrine | Regulation of neurotransmission of motor functions | [63] |
| FGF15/19/21/23 | FGF15/19 | Endocrine | Regulates hepatic glucose metabolism | [64] |
|           | FGF21 | Endocrine | Lipid metabolism regulator | [65] |
|           | FGF23 | Endocrine | Phosphate and vitamin D metabolism | [66] |

The table shows the subfamilies of various FGFs, FGFs under each subfamily, the manner of action of each FGF, and their prime functions.
It has been well known that FGF2 is a critical component of maintenance of many kinds of stem cell cultures [9]. Stabilization of FGF2 levels in a culture medium using polyesters of glycolic and lactic acid (PLGA) microspheres as a FGF2 release controller successfully improved the expression of stem cell markers, increased stem cell numbers, and decreased spontaneous differentiation [10].

FGF2-deleted mice showed a significant decrease in bone mass and bone formation without gross abnormalities. Bone marrow stromal cells (BMSCs) from the FGF2−/− mice demonstrated decreased osteoblast differentiation, which can be partially rescued by addition of exogenous FGF2 in vitro [11]. Furthermore, FGF2−/− BMSC-derived osteoblasts displayed a marked reduction in inactive phosphorylated glycogen synthase kinase-3 (GSK-3) as well as a significant decrease in Dkk2 mRNA, which plays important roles in osteoblast differentiation. These results suggested that FGF2 is an endogenous, positive regulator of bone mass [12]. In contrast, non-specific overexpression of FGF2 (Tg-FGF2) in mice exhibits a dwarf phenotype with impaired bone mineralization and osteopenia [13]. Addition of FGF2 into a culture medium of a mouse osteoblast-like cell line, MC3T3-E1, activated cell proliferation and suppressed mineralization [14]. In this study, treatment of the cells with FGF10 as an experimental control did not show any effects. These observations suggested that FGF2 could work in both directions for osteogenesis promotion and inhibition. It is important to elucidate conditions for positive and negative osteogeneses.

FGF9

FGF9−/− mice showed disproportionate shortening of the proximal skeletal elements (rhzomelia), which suggests that FGF9 promotes chondrocyte hypertrophy and vascularization of the cartilage anlagen [15]. A missense mutation of FGF9 in mice resulted in decreased heparin binding, which caused elbow-knee synostosis [16]. A similar mutation was also found in humans [17]. FGF9−/− mice did not seem to have a particular phenotype. However, bone healing of a 1-mm unicortical defect was impaired with decreased levels of neovascularization and osteoblast recruitment. This condition was rescued by exogenous addition of FGF9 (2 μg) with collagen sponge but not by exogenous FGF2 application [18]. These reports elucidated the specific functions of FGF9 in bone healing.

Bone healing of a 1-mm unicortical defect in diabetic model mice (db/db) was significantly delayed with decreased levels of osteogenesis marker expressions. Treatment of FGF9 with collagen sponge to the defect in the db/db mice induced better bone healing [19]. Treatment with FGF9-soaked collagen sponge to mouse circular calvarial bone defects of a diameter of 2 mm showed sufficient bone regeneration in postnatal day 7 (P7) mice but not in postnatal day 60 (P60) mice [20]. Addition of FGF9 with various concentrations into dexamethasone-containing media for inducing osteogenesis of BMSCs and dental pulp stem cells resulted in stimulation of proliferation but not differentiation [21].

FGF18

Deletion of FGF18 in mice resulted in delayed suture formation, reduced osteoblast lineage cell proliferation, delayed osteoblast differentiation, and perinatal death. The long bones of FGF18−/− mice showed reduced osteoblast differentiation but increased chondrocyte proliferation and differentiation. These results suggested that FGF18 demonstrated a positive effect on osteogenesis by enhancing cell proliferation and differentiation but a negative effect on chondrogenesis [22, 23]. However, it was also proposed that FGF18 transduced the signal through FGR3 to enhance cartilage formation [24].

In vitro analysis on mesenchymal stem cells (MSCs) derived from the bone marrow suggested that FGF18 enhanced osteoblast differentiation by activation of FGFR1 or FGFR2 signaling [25]. They also showed that overexpression of FGF18 by lentiviral infection or direct addition of FGF18 into the culture medium could increase the expression of osteoblast marker genes in C3H10T1/2 fibroblastic cells. Treatment of FGF18 on rat-derived MSCs under a differentiation-inducing condition showed elevated expression of osteoblast differentiation markers and mineralization [26]. Low-dose FGF18 treatment with bone morphogenetic protein 2 (BMP2)-dependent osteogenic induction of MC3T3-E1 cells enhanced mineralization whereas high-dose treatment inhibited the process (unpublished observation of Sachiko Iseki). FGF18-soaked heparin-coated acrylic beads accelerated osteoblast differentiation in mouse fetuses by upregulating the expression of BMP2 in osteoblast cell lineage cells [27]. In accordance with the above reports, FGF18 application with BMP2 in cholesteryl group- and acryloyl group-bearing pullulan (CHPOA) nanogels stabilized BMP2-dependent bone regeneration of critical-sized bone defects on mouse calvarium [28].

Application of FGFs in bone regeneration

The above discussions suggest that although FGFs do not have osteoinductive property, they function as an accelerator of osteogenesis under the appropriate conditions. It is possible that FGF2 and FGF9 work on proliferation of osteoblast cell lineage as well as induction of angiogenesis, and FGF18 functions in promotion of osteoblast differentiation. Tables 2 and 3 show some of the in vivo experiments in which FGFs were applied to non-critical- and critical-sized bone defects for bone healing, respectively. Further applications of FGFs have been elaborated by Du et al. and Gothard et al. [29, 30].
Systemic or subcutaneous injections of FGF2 could enhance osteogenesis. However, it was shown that systemic injections of FGF2 caused adverse extraskeletal effects [31]. Therefore, local administration has been chosen as a more preferable method for applying bioactive factors. FGF2 has been used for inducing angiogenesis and enhancing osteogenesis in non-critical-sized bone defects by activating proliferation of osteoblast cell lineages. FGF9 is also suggested to be involved in angiogenesis by controlling VEGFa expression [18]. As long as osteogenesis is

### Table 2 Application of different FGFs in non-critical-sized bone defect in vivo models

| Growth factor | Dose       | In vivo model                        | Carrier                      | Investigations                                      | Effect                                      | References |
|---------------|------------|--------------------------------------|------------------------------|-----------------------------------------------------|---------------------------------------------|------------|
| FGF2          | 200 μg     | Monkey ulna fracture                 | Injectable gelatin hydrogel  | Bone mineral content and mechanical properties      | Accelerates fracture healing and prevents nonunion | [67]       |
| FGF2          | 2.5 μg     | Rat periodontal defect (2 x 2 x 1.7 mm) | Injectable calcium phosphate cement | Histology and histomorphometry of bone | Increased periodontal regeneration | [68]       |
| FGF2          | 50 μg      | Rat calvarial defect (4-mm diameter) | PLGA/β-TCP                  | Histomorphometry of bone | Enhanced bone regeneration | [69]       |
| FGF2          | 50 μg/ml   | Rat calvarial defect (5-mm diameter) | Collagen and nano-bioactive glass hybrid membrane | Histomorphometry of bone | Accelerated bone regeneration | [70]       |
| FGF2          | 45 μg      | Rabbit femoral condyle (4-mm diameter and 6 mm long) | Hydrogel polymer | Bone mass and microarchitecture | Enhanced bone regeneration | [32]       |
| Melatonin     | 100 mg/kg i.p. | Rat tibia (2-mm diameter, 4 mm long) | Titanium implant | Bone histomorphometry | Synergistically enhanced new bone formation | [71]       |
| FGF2          | 200, 400, or 800 μg | Human tibia (high tibial osteotomy) | Gelatin hydrogel | Radiographic assessment of bone | Dose dependently accelerated bone union | [72]       |
| FGF2          | 100 μg     | Rabbit femoral condyle (10 mm² x 5 mm depth) | Interconnected porous calcium hydroxyapatite ceramic | Bone histomorphometry | Decreases lamellar bone formation, increases vascularization and osseointegration | [73]       |
| FGF2          | 0, 25, or 250 ng | Rat calvarial defect (3.5-mm diameter) | PLGA/gelatin | Radiological, histological, and biochemical examination | Low-dose administration enhanced the degree of calcification and ALP activity | [74]       |
| BMP2          | 0.1 mg/ml  | Mouse calvarial defect (2-mm diameter) | Collagen sponge | Bone histomorphometry | Enhances angiogenesis and bone regeneration | [19]       |
| FGF9          | 2 μg       | Mouse tibia (1-mm defect) | Collagen sponge | Bone histomorphometry | Enhanced bone regeneration | [32]       |

The table shows the various growth factors and their combinations used for regeneration of non-critical-sized defects, their dose, the site of application, the carrier used for the application, and the investigations through which the effects of bone healing have been studied i.p. intraperitoneal injection

### Table 3 Application of different FGFs in critical-sized bone defect in vivo models

| Growth factor | Dose       | In vivo model                        | Carrier                | Investigations                                      | Effect                                      | References |
|---------------|------------|--------------------------------------|------------------------|-----------------------------------------------------|---------------------------------------------|------------|
| FGF2          | 5 ng       | Mouse calvarial defect (3.5-mm diameter) | Col-HA/PEG hydrogel | Micro CT and histology of bone | Enhanced bone regeneration | [34]       |
| BMP2          | 2 μg       | Mouse calvarial defect (5-mm diameter) | Collagen sponge | Radiological and histological examination | Promotes osteogenesis | [75]       |
| FGF2          | 10 ng, 100 μg, and 1 μg | Rat mandibular defect (5-mm diameter) | Collagen sponge | Bone histomorphometry | Enhances formation of new bone and cementum | [76]       |
| FGF2          | 200 μg     | Beagle dog periodontal defect (6 x 5 mm: vertical x horizontal) | β-TCP | Micro CT and histology of bone | Enhanced bone regeneration | [34]       |
| FGF18         | 0.5 μg     | Mouse calvaria (3-mm diameter) | CHPOA/hydrogel | Micro CT assessment of bone | Synergistically enhanced new bone formation | [28]       |
| BMP2          | 0.5 μg     | Mouse calvaria (3-mm diameter) | CHPOA/hydrogel | Micro CT assessment of bone | Synergistically enhanced new bone formation | [28]       |
| FGF2 or FGF9 or FGF18 | 250 ng (P7 mice) or 2.5 μg (P60 mice) | Mouse calvaria (2-mm diameter) | Collagen sponge | Micro CT assessment of bone | All FGF ligands promote healing rate in P7 mice. Only FGF18 promotes healing rate in P60 mice | [20]       |

The table shows the various growth factors and their combinations used for regeneration of critical-sized defects, their dose, the site of application, the carrier used for the application, and the investigations through which the effects of bone healing have been studied.
taking place to recover the bone defect, FGF2 can support or even enhance the healing. Recent studies suggest that high-dose FGF2 inhibits progression of osteoblast differentiation [20, 32, 33] (also unpublished observation of Sachiko Iseki) and low concentration of FGF2 enhanced osteogenesis [33, 34]. In contrast, it is likely that high-dose FGF18 can promote osteoblast differentiation in vivo [20, 28], while FGF18 treatment in vitro inhibits mineralization [14].

Kang et al. developed a sequential delivery system with fiber scaffolds in which FGF2 was released first and then FGF18 [35]. Applying this scaffold to rat calvarial critical-sized bone defects resulted in better bone volume and density, although the amount of FGFs applied to the defect was not clear. This study suggested that it is critical to control the amount or release speed of soluble factors for the bone regeneration process.

Conclusions
FGFs play an important role in the development and regeneration of various tissues. In this article, we have summarized the prime functions of all FGFs, and further, we have discussed elaborately about FGFs 2, 9 and 18, which play a major role in bone regeneration. We have also discussed about different carrier systems for FGF delivery in different animal models for bone regeneration. With the ongoing advancements in the field of cellular and molecular biology, we could expect that more detailed functioning of FGF/FGFR will be elucidated. Further, with the advent of novel carriers and protein delivery systems, it could be possible that the spatio-temporal release of FGFs can be controlled precisely as needed. This would improve our understanding and help us to clinically translate the use of FGFs to achieve effective bone regeneration.

Abbreviations
BMP2: Bone morphogenetic protein 2; BMSC: Bone marrow stromal cells; CHPOA: Cholesteryl group- and acryloyl group-bearing pullulan; Col-HA/PEG: Collagen-hydroxyapatite/polyethylene glycol; Dkk2: Dickkopf-related protein 2; FGF: Fibroblast growth factor; FGFR: Fibroblast growth factor receptor; GSK-3: Glycogen synthase kinase-3; IgFGF: Intracellular fibroblast growth factor; IgG: Immunoglobulin; MSC: Mesenchymal stem cells; PLGA: Polymers of glycolic and lactic acid; RT-PCR: Reverse transcriptase polymerase chain reaction; Tg-FGF2: Transgenic fibroblast growth factor 2; VEGFA: Vascular endothelial growth factor A; β-TCP: Beta tricalcium phosphate

Acknowledgements
We would like to express our sincere gratitude to all the researchers, collaborators, technical assistants, and secretaries for contributing to the research cited in the present manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Authors’ contributions
All authors contributed equally to drafting the manuscript. All authors read, revised, and approved the final manuscript.

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

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 9 March 2017 Accepted: 25 April 2017
Published online: 01 August 2017

References
1. Wilkie AO. Bad bones, absent smell, selfish testes: the pleiotropic consequences of human FGF receptor mutations. Cytokine Growth Factor Rev. 2005;16:187–203.
2. Goetz R, Mohammadi M. Exploring mechanisms of FGF signalling through the lens of structural biology. Nat Rev Mol Cell Biol. 2013;14:166–80.
3. Itoh N, Ornitz DM. Functional evolutionary history of the mouse Fgf gene family. Dev Dyn. 2008;237:18–27.
4. Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, et al. Receptor specificity of the fibroblast growth factor family. J Biol Chem. 1996;271:15292–7.
5. Zhang X, Ibrahimi OA, Olsen SK, Umemori H, Mohammadi M, Ornitz DM. Receptor specificity of the fibroblast growth factor family: The complete mammalian FGF family. J Biol Chem. 2006;281:15694–700.
6. Itoh N. The Fgf families in humans, mice, and zebrafish: their evolutionary processes and roles in development, metabolism, and disease. Biol Pharm Bull. 2007;30:1819–25.
7. Schmid GJ, Kobayashi C, Sandell LJ, Ornitz DM. Fibroblast growth factor expression during skeletal fracture healing in mice. Dev Dyn. 2009;238:766–74.
8. Keljke S, Reiff D, Prince C, Thompson J. Acidic fibroblast growth factor signaling inhibits peroxynitrite-induced death of osteoblasts and osteoblast precursors. J Bone Miner Res. 2001;16:1917–25.
9. Levenstein ME, Ludwig TE, Xu RH, Llanas RA, VanDenHeuvel-Kramer K, Manning JD, et al. Basic fibroblast growth factor support of human embryonic stem cell self-renewal. Stem Cells. 2006;24:568–74.
10. Lottz S, Goderie S, Tokas N, Hirsch SE, Ahmad F, Cornejo B, et al. Sustained levels of FGF2 maintain undifferentiated stem cell cultures with bimeval feeding. PLoS One. 2013;8:e65289.
11. Montero A, Okada Y, Tomita M, Ito M, Tsurukami H, Nakamura T, et al. Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. J Clin Invest. 2000;105:1085–93.
12. Fei Y, Xiao L, Doetschman T, Coffin DJ, Hurley MMA. Fibroblast growth factor 2 stimulation of osteoblast differentiation and bone formation is mediated by modulation of the Wnt signaling pathway. J Biol Chem. 2011;286:40575–83.
13. Coffin J, Flockiewicz R, Neumann J, Mort-Hopkins T, Gn D, Lightfoot P, et al. Regulation of osteoblast, chondrocyte, and osteoclast functions by basic fibroblast growth factor (FGF-2) in comparison with FGF-2 and FGF-10. J Biol Chem. 2002;277:7493–500.
14. Kuang LI, Liang L, Wang J, Yang D, Wang S, Yang S, et al. FGF18 stimulates osteoblast differentiation and function through a mechanism involving IGF-1. J Cell Biol. 1998;142:1083–93.
15. Hung HH, Yu K, Lavine KJ, Ornitz DM. FGF9 regulates early hypertrophic chondrocyte differentiation and skeletal vasculization in the developing stylopod. Dev Biol. 2007;307:300–13.
16. Harada M, Murakami H, Okawa A, Okimoto N, Hiraoka S, Nakahara T, et al. FGF9 monomer–dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. Nat Genet. 2009;41:289–98.
17. Wu X-l, Gu M-m, Huang L, Liu X-s, Zhang H-x, Ding X-y, et al. Multiple synostoses syndrome is due to a missense mutation in exon 2 of FGF9 gene. Am J Hum Genet. 2009;85:53–63.
18. Behr B, Leucht P, Longaker MT, Quarto N. Fgf9 is required for angiogenesis and osteogenesis in long bone repair. Proc Natl Acad Sci U S A. 2010;107:11853–8.

19. Wallner C, Schira J, Wagner JM, Schulte M, Fischer S, Hirsch T, et al. Application of VEGFA and FGFR enhances angiogenesis, osteogenesis and bone remodeling in type II diabetic long bone repair. PLoS One. 2015;10:e0118823.

20. Behr B, Panetta NJ, Longaker MT, Quarto N. Different endogenous threshold levels of fibroblast growth factor-ligands determine the healing potential of frontal and parietal bones. Bone. 2010;47:281–94.

21. Lu J, Dai J, Wang X, Zhang M, Zhang P, Sun H, et al. Effect of fibroblast growth factor 9 on the osteogenic differentiation of bone marrow stromal stem cells and dental pulp stem cells. Mol Med Rep. 2015;11:1661–8.

22. Ohbayashi N, Shibaikura M, Kurataki Y, Imashiki M, Fujimori T, Itoh N, et al. FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. Genes Dev. 2002;16:870–9.

23. Liu Z, Xu J, Colvin JS, Omitz DM. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. Genes Dev. 2002;16:859–69.

24. Davidson D, Blanc A, Filion D, Wang H, Pluf P, Pfeffer G, et al. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. J BioChem. 2005;280:20509–15.

25. Hamidouche Z, Fromigué O, Nuber U, Vaudin P, Ebert R, Jakob F, et al. Autocrine fibroblast growth factor 18 mediates dexamethasone-induced osteogenic differentiation of murine mesenchymal stem cells. J Cell Physiol. 2010;224:590–16.

26. Jeon E, Yun Y-R, Kang W, Lee S, Koh Y-H, Kim H-W, et al. Investigating the role of FGF18 in the cultivation and osteogenic differentiation of mesenchymal stem cells. PLoS One. 2012;7:e34982.

27. Nagayama T, Okuhara S, Ota MS, Tensho K, Nakaya H, Nawata M, Okabe T, Wakitani S. Low FGF18 expression is associated with low bone mass and fracture risk in postmenopausal women. Bone. 2010;47:281–94.

28. Sekine K, Ohuchi H, Fujiwara M, Yamazaki M, Yoshizawa T, Sato T, et al. Fgf10 is essential for limb and lung formation. Nat Genet. 1999;21:138–41.

29. Du X, Xie Y, Xian CJ, Chen L. Role of FGFs/FGFRs in skeletal development and blood pressure regulation in FGF-2-deficient mice. EMBO J. 2006;25:10321–31.

30. Jeon E, Yun Y-R, Kang W, Lee S, Koh Y-H, Kim H-W, et al. Investigating the role of FGF18 in the cultivation and osteogenic differentiation of mesenchymal stem cells. PLoS One. 2012;7:e34982.

31. Nagayama T, Okuhara S, Ota MS, Tensho K, Nakaya H, Nawata M, Okabe T, Wakitani S. Low FGF18 expression is associated with low bone mass and fracture risk in postmenopausal women. Bone. 2010;47:281–94.

32. Mabilleau G, Aguado E, Stancu IC, Cincu C, Baslé MF, Chappard D. Effects of FGF-2 on chondrocyte differentiation and mineralization in osteoarthritic cartilage. J Cell Physiol. 2010;225:280–90.

33. Nakamura Y, Tensho K, Nakaya H, Nawata M, Okabe T, Wakitani S. Low FGF18 expression is associated with low bone mass and fracture risk in postmenopausal women. Bone. 2010;47:281–94.

34. Nakatake Y, Hoshikawa M, Asaki T, Kassai Y, Itoh N. Identification of a novel fibroblast growth factor, FGF-22, preferentially expressed in the inner root sheath of the hair follicle. BBA Gene Struct Expr. 2001;1517:460–3.

35. Colvin JS, White AC, Pratt SJ, Omitz DM. Lung hypoplasia and neonatal death in Fgf9-null mice identify this gene as an essential regulator of lung mesenchyme. Development. 2001;128:295–106.

36. Barak H, Huh S-H, Chen S, Jeanpierre C, Martinovic J, Parisot M, et al. FGF9 and FGF20 maintain the stemness of neuron progenitors in mice and man. Dev Cell. 2012;22:119–207.

37. Kim Y, Kobayashi A, Sekido R, DiNapoli L, Brennan J, Chaboissier M-C, et al. Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. PLoS Biol. 2006;4:e187.

38. Lu SY, Sheik F, Sheppard PC, Frenozza A, Duckworth ML, Deltilieu KA, et al. Fgf-16 is required for embryonic heart development. Biochim Biophys Res Commun. 2008;373:270–4.

39. Hayashi T, Ray CA, Berningham-McDonogh O. Fgf20 is required for sensory epithelial specification in the developing cochlea. J Neurosci. 2008;28:5991–9.

40. Refers F, Bohti H, Walsh EC, Crossley PH, Stainier D, Brand M. Fgf9 is mutated in zebrafish acerebellar lacet mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. Development. 1998;125:2831–95.

41. Crossley PH, Minowada G, MacArthur CA, Martin GR. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell. 1996;84:127–36.

42. Abu-Issa R, Smyth G, Smoak I, Yamamura K-i, Meyers EN. Fgf8 is required for mesenchyme specification in the developing pharyngeal arch and cardiovascular development in the mouse. Development. 2002;129:4613–25.

43. Albertson RC, Yelick PC. Roles for fgf8 signaling in left lateral plate mesoderm. Dev Dyn. 1994;199:355–62.

44. Crossley PH, Minowada G, MacArthur CA, Martin GR. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell. 1996;84:127–36.

45. Kettunen P, Laurikkala J, Itäranta P, Vainio S, Itoh N, Thesleff I. Associations of Fgf2 and Fgf10 with signaling networks regulating tooth morphogenesis. Genes Dev. 2000;14:2133–44.

46. Potthoff MJ, Boney-Montoya J, Choi M, He T, Sunny NE, Satapati S, et al. FGF15/19 regulates hepatic glucose metabolism by inhibiting the CREB-PGC-1α pathway. Cell Metab. 2011;13:729–38.
65. Chui PC, Antonellis PJ, Bina HA, Kharitonenkov A, Flier JS, Maratos-Flier E. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes. 2010;59:2781–9.

66. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, et al. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest. 2004;113:3561–6.

67. Kawaguchi H, Nakamura K, Tabata Y, Ikada Y, Aoyama I, Anzai J, et al. Acceleration of fracture healing in nonhuman primates by fibroblast growth factor-2. J Clin Endocrinol Metab. 2001;86:875–80.

68. Oortgiesen DA, Walboomers XF, Bronckers AL, Meijer GJ, Jansen JA. Periodontal regeneration using an injectable bone cement combined with BMP-2 or FGF-2. J Tissue Eng Regen Med. 2014;8:202–9.

69. Yoshida T, Miyaji H, Otani K, Inoue K, Nakane K, Nishimura H, et al. Bone augmentation using a highly porous PLGA/β-TCP scaffold containing fibroblast growth factor-2. J Periodont Res. 2015;50:265–73.

70. Hong KS, Kim EC, Bang SH, Chung CH, Lee YI, Hyun J, et al. Bone regeneration by bioactive hybrid membrane containing FGF2 within rat calvarium. J Biomed Mater Res A. 2010;94:1187–94.

71. Takechi M, Tatehara S, Satomura K, Fujisawa K, Nagayama M. Effect of FGF-2 and melatonin on implant bone healing: a histomorphometric study. J Mater Sci Mater Med. 2008;19:2949–52.

72. Kawaguchi H, Jingushi S, Izumi T, Fukunaga M, Matsushita T, Nakamura T, et al. Local application of recombinant human fibroblast growth factor-2 on bone repair: a dose-escalation prospective trial on patients with osteotomy. J Orthop Res. 2007;25:480–7.

73. Nakata T, Ishida O, Sunagawa T, Nakamae A, Yokota K, Adachi N, et al. Feasibility of prefabricated vascularized bone graft using the combination of FGF-2 and vascular bundle implantation within hydroxyapatite for osteointegration. J Biomed Mater Res A. 2008;85B:1090–5.

74. Tanaka E, Ishino Y, Sasaki A, Hasegawa T, Watanabe M, Dalla-Bona DA, et al. Fibroblast growth factor-2 augments recombinant human bone morphogenetic protein-2-induced osteoinductive activity. Ann Biomed Eng. 2006;34:717–25.

75. Zellin G, Linde A. Effects of recombinant human fibroblast growth factor-2 on osteogenic cell populations during orthopic osteogenesis in vivo. Bone. 2000;26:161–8.

76. Ishii Y, Fujita T, Okubo N, Ota M, Yamada S, Saito A. Effect of basic fibroblast growth factor (FGF-2) in combination with beta tricalcium phosphate on root coverage in dog. Acta Odontol Scand. 2013;71:325–32.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at www.biomedcentral.com/submit