Review Article

Functional Diversity of Fibroblast Growth Factors in Bone Formation

Yuichiro Takei, Tomoko Minamizaki, and Yuji Yoshiko
Department of Calcified Tissue Biology, Hiroshima University Institute of Biomedical & Health Sciences, 1-2-3 Kasumi Minami-ku, Hiroshima 734-8553, Japan
Correspondence should be addressed to Yuji Yoshiko; yyuji@hiroshima-u.ac.jp
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The functional significance of fibroblast growth factor (FGF) signaling in bone formation has been demonstrated through genetic loss-of-function and gain-of-function approaches. FGFs, comprising 22 family members, are classified into three subfamilies: canonical, hormone-like, and intracellular. The former two subfamilies activate their signaling pathways through FGF receptors (FGFRs). Currently, intracellular FGFs appear to be primarily involved in the nervous system. Canonical FGFs such as FGF2 play significant roles in bone formation, and precise spatiotemporal control of FGFs and FGFRs at the transcriptional and posttranscriptional levels may allow for the functional diversity of FGFs during bone formation. Recently, several research groups, including ours, have shown that FGF23, a member of the hormone-like FGF subfamily, is primarily expressed in osteocytes/osteoblasts. This polypeptide decreases serum phosphate levels by inhibiting renal phosphate reabsorption and vitamin D₃ activation, resulting in mineralization defects in the bone. Thus, FGFs are involved in the positive and negative regulation of bone formation. In this review, we focus on the reciprocal roles of FGFs in bone formation in relation to their local versus systemic effects.

1. Introduction

Bone is a connective tissue with a mineralized extracellular matrix that provides support to the body and affects calcium (Ca)/phosphate (inorganic phosphate; Pi) metabolism. Osteoblasts are involved in bone formation via secretion of the organic matrix "osteoid" and the subsequent facilitation of hydroxyapatite crystal formation. Large multinucleated osteoclasts play an active role in bone resorption. Bone formation and resorption, that is, bone metabolism, are regulated by local versus systemic factors. The former includes growth factors and receptor activator of nuclear factor κ-β ligand (RANKL) and its receptor RANK. Representatives of the latter include parathyroid hormone (PTH), 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), and calcitonin [1]. Growing evidence suggests that additional interactions between bone and extraskeletal organs affect, during development, aging and pathogenesis. For example, undercarboxylated osteocalcin secreted by osteoblasts acts on pancreatic β-cells to promote insulin production, which is involved in the regulation of energy metabolism [2]. Osteoblast lineage cells compose hematopoietic [3, 4] and cancer stem cell niches [5], thereby affecting the fates of their stem cells. The adipocyte-derived hormone leptin acts on its specific receptors in the hypothalamus, increases sympathetic activity in bone, and exerts antosteogenic effects [6]. Serotonin (5-HT) secreted by enterochromaffin cells binds to its receptor 5-HT₃BR in preosteoblasts and inhibits their proliferation [7]. Further studies in this field are of significance with regard to understanding the precise functions of bone.

Fibroblast growth factors (FGFs) are pleiotropic growth factors that regulate cell proliferation, migration, and differentiation in many organs including bone. Twenty-two family members of FGFs (FGF1–23, wherein FGF15 is the mouse ortholog of human FGF19) have been identified in mammals so far. FGFs can be divided into three subfamilies: canonical, hormone-like, and intracellular [8]. Numerous studies have shown that canonical FGFs, such as FGF2, act in bone. Hormone-like FGF family members are the most recently identified FGFs, and the discovery of these, especially
the clinical and experimental studies of FGFR23, led us to explore the additional roles of FGFs in bone. Not only FGFR23 but also FGF2 is exclusively expressed in osteoblast lineage cells and shares specific receptors (FGF receptors, FGFRs) to transduce intracellular signals, although the effects of these FGFs are variable. The intracellular FGFs, FGFI–14, have been well studied in neurons but not in bone and, therefore, are not discussed here. This review, therefore, provides new insights into the roles of FGFs during bone formation and compares canonical versus hormone-like FGFs.

2. The FGF and FGFR Family Members and Their Signaling Pathways

Canonical FGFs, including FGF2, comprise the most common subfamily that transduces signals through FGFR tyrosine kinases. A heparin-binding domain is conserved among most FGFs, and heparan sulfate (HS) is an integral component for the acquisition of the binding affinity of FGFs to FGFRs. Therefore, these polypeptides can be retained in the extracellular matrix in the vicinity of their secretory cells. Thus, canonical FGFs act as autocrine and/or paracrine factors [10, 11]. The hormone-like subfamily members, FGF15/19, FGF21, and FGF23, contain extra structural features at the C-terminus and require the membrane protein αKlotho/βKlotho as cofactors rather than HS to bind to FGFRs [8, 12]. This hallmark difference may pertain to the dynamic properties of the two subfamilies. Both canonical and hormone-like FGFs show their biological activities by activating four distinct FGFRs (also known as the existence of splicing variants “b” and “c” of FGFR1–3) with different binding affinities. For information on the binding affinity of individual FGFs to FGFRs, refer to other reviews and papers (see, e.g., [13]). Many studies have found that tyrosine phosphorylation of the intracellular domain of FGFRs activates the Ras-mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK)/AKT, Ras, and c-Jun N-terminal kinase (JNK), the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, and the phospholipase C (PLC)-γ protein kinase C (PKC) pathway (Figure 1) (see, e.g., [14]). Overall, the spatiotemporal dynamics of FGFs and FGFRs may determine how the FGF family members exert their proper activities in particular cells and tissues.

It is also worth noting that negative and positive modulators expressed in a wide range of cells and tissues play precise roles in FGF signaling, and this may further complicate the functional profiles of FGFs. The sprouty (SPRY) family is a highly conserved group of negative feedback loop modulators of growth factor-mediated MAPK activation that was originally described in Drosophila [15]; thereafter, four mammalian orthologs (SPRY1–4) have been identified. Either FGF3 or FGF8 upregulates both mRNA and protein levels of Spry4, while increased Spry4 inhibits both FGF3 and FGF8 signaling by interfering with the downstream activation of FGFR1 in zebrafish blastomeres. Similar expression of Fgf genes (Sef) and FGFRs (Spry) encodes a conserved putative transmembrane protein that has sequence similarity with the intracellular domain of the interleukin-17 receptor. This modulator acts as a feedback-induced antagonist of FGF8/Ras/Raf/MAPK signaling in the development of zebrafish embryos [17]. In contrast, Canopy1 (CNPY1) was identified as a positive feedback regulator for FGF-induced signaling [18]. This positive feedback loop between the polypeptide and FGF8/FGFR1 is involved in the cluster formation of dorsal forerunner cells during gastrulation in zebrafish [19]; however, its underlying mechanism in mammals remains to be elucidated.

3. Roles of Canonical FGFs on Bone Formation

In addition to our previous data on FGFRs [9], here we show the expression profile of Fgfs in a well-established fetal rat calvaria cell model (Figure 2). Among these, Fgf9 and hormone-like Fgf23 are abundant and vary in expression levels during osteoblast development. Table 1 summarizes the primary roles of FGFs in bone formation in multiple models. Human calvaria cell cultures describe, in detail, the roles of FGFR2 in osteoblastogenesis [20]. When treated at early developmental stages, FGFR2 inhibits alkaline phosphatase (ALP) activity, collagen synthesis, and matrix mineralization and increases cell proliferation; however, when treated at late developmental stages, it has no obvious effects. Because the in vivo effects of FGF2 on bone formation are apparent, its potential therapeutic benefit in pediatric surgery and
periodontal disease is under consideration [21, 22]. The significant anabolic actions of FGF2 in bone have been widely demonstrated in several animal models; see, for example, growth plate and trabecular bone in growing rats that received daily intravenous injections of FGF2 [23]. Local injections of FGF2 over the calvaria increase new bone formation in mice [24], and those into osteotomized sites of the tibia accelerate surgical fracture repair in rabbits [21]. FGF2 also has an ability to prevent trabecular bone loss in the vertebrae of ovariectomized rats possibly by increasing osteoadipogenic cell proliferation [25]. Fgf2-null (Fgf2−/−) mice exhibit a significant decrease of femoral trabecular bone volume and bone formation rate [26]. This can be explained by a downregulation of BMP-2 in Fgf2−/−
osteoblasts, resulting in a decrease in ALP activity and nuclear accumulation of the master transcription factor of osteoblastogenesis Runx2 [27]. Furthermore, an inverse correlation between adipogenesis and osteogenesis is observed in Fgf1−/− mice, and FGF2 blocks adipocyte formation and increases ALP-positive colony formation in bone marrow cell cultures independent of FGF2 [28]. In FGF2, most attention has been dedicated to the smallest 18-kDa variant (LMW). In addition, genetic manipulation of LMW FGF2 in skeletal tissues contributes to bone phenotypes in vivo [29]. However, there are several higher molecular weight (HMW) variants of the polypeptide. Additional information on the representative roles of the HMW variants in bone is shown below.

Compared with FGF2, other canonical FGFs have not been studied in detail (Table 1). Although Fgfl expression was not obvious in our model, its transcript appears to act in normal rats and tibial metaphysis in ovariectomized rats [30]. However, Fgfl−/− mice do not display any gross phenotypic defects [31]. Because deficiency of FGF1 in mice exacerbated high-fat diet-induced diabetic phenotypes, such as insulin resistance and effects in adipose remodeling in gonadal white adipose tissue, FGF1, may directly and/or indirectly act on bone. FGF4 is more specific to mesenchymal cells, but its subcutaneous injections increase trabecular bone mineral density in the mouse femur [32]. Much less is known about the roles of FGF6 [33], FGF7 [34], and FGF8 [35] in bone; the expression of Fgf7 but not of Fgf6 and Fgf8 is detected in our calvaria cell model, and FGF6 shows catabolic effects on osteoblastic cells, but others have anabolic function in vitro. Histological evidence for chondrogenesis with the upregulation of the Sox9 and Col2α1 genes is seen in cranial mesenchymal cells of transgenic mice overexpressing FGF9, suggesting that FGF9 converts intramembranous ossification to endochondral ossification [36]. FGF9 also shows supportive effects on FGF2-dependent trabecular bone formation [37]. Among Fgfs expressed in our model, Fgfl is abundant during the late developmental stages, along with Fgf23 levels (Figure 2). Notably, both mRNA levels are upregulated by 1,25(OH)2D3, while only Fgf9 levels are suppressed by pretreatment of cycloheximide, a protein synthesis inhibitor, as well as the transcriptional inhibitor actinomycin D (ActD) (Figure 3). Thus, 1,25(OH)2D3-dependent expression of Fgfl but not Fgf23 may result from de novo protein synthesis. Additional role(s) and the precise regulatory mechanism of FGF9 in osteoblast functions remain to be elucidated. Functional anomalies in Fgfl0 cells may be involved in craniosynostosis [38], but there are no obvious effects of FGF10 in our rat (unpublished data) and mouse calvaria cells [39]. Treatment of mouse calvaria cells with FGF18 promotes proliferation and decreases matrix mineralization [39], the effects of this polypeptide on bone formation appear to be similar to those of FGF2.

4. Physiological and Pathological Importance of FGFRs in Bone

The dynamics of FGFRs are also an important determinant of FGF-mediated bone formation. Indeed, mutations in FGFR1 and FGFR2 account for the craniosynostosis and chondrodysplasia syndromes in humans [41–44], suggesting that both FGFRs are important for endochondral and intramembranous bone formation. Because Fgfr1−/− mice are embryonic lethal shortly after gastrulation [45], osteochondrocyte lineage- and osteoblast-specific FGFR1 knockout mice were generated under the control of the proα1(I) collagen (Col2) and proα1(I) collagen (Col1) promoters, respectively. Col2-mediated FGFR1 inactivation delays chondrocyte and osteoblast maturation, while Coll-dependent FGFR1 deficiency accelerates osteoblast differentiation with stimulated mineral deposition and reduces osteoclast activity [46]. Gain-of-function missense mutations in Fgfr2 (S252W and P253R) cause craniosynostosis syndromes, including Crouzon and Apert syndromes [47, 48]. Indeed, heterozygous Fgfr2 (S252W) mutant mice show midline suture.
Table 1: Roles of FGFs in bone.

| FGFs | Models | Outcomes | Animals | Ref. |
|------|--------|----------|---------|------|
| **FGFs** | **In vivo** | **Ex vivo** | **In vitro** | Recombinant proteins and so forth | | |
| **Canonical** | | | | | |
| **FGF1** | Systemic deletion | Injections over the calvaria | Osteoblasts | Cell proliferation ↑, but nodule formation and mineralization ↓ | Rats | [30] |
| | | Intravenous injections | Calvaria cells Bone marrow cells | | |
| | Systemic deletion | Bone marrow cells | Trabecular bone ↓ | Mice | [26, 28] |
| | Osteoblast-specific expression of human HMW FGF2 | Bone marrow cells | Dwarfism, osteomalacia, none mineral density ↓, serum phosphate levels, and FGF23 expression ↑ | Mice | [95] |
| | Osteoblast-specific expression of human LMW FGF2 | Bone marrow cells | ALP-positive colonies and mineralized nodules ↑ | Mice | [26] |
| | Systemic deletion of human LMW FGF2 | Bone marrow cells | Increased bone formation ↑, sFRP-1 expression ↓ | Mice | [29] |
| | Intravenous injections Subcutaneously injections Injections over the calvaria Single local injection into the distracted callus Subcutaneous transplantations of human bone marrow cells treated with FGF2 | Bone marrow cells | Reverse effects as above | |
| | | | | | |
| **FGF2** | Systemic deletion | Injections over the calvaria | Osteoblastic cell proliferation and new bone formation ↑ | Mice | [31] |
| | | Intravenous injections | Calvaria cells Bone marrow cells |预防 of the ovariectomized (OVX)-related bone loss | OVX Rats | [24] |
| | | Bone marrow cells | Osteoblasts Cell proliferation ↑, but nodule formation and mineralization ↓ | Rats | |
| | Systemic deletion | Bone marrow cells | ALP-positive colonies and mineralized nodules ↓ | Mice | |
| | Osteoblast-specific expression of human FGF2 | Bone marrow cells | Trabecular bone ↓ | Mice | |
| | Osteoblast-specific expression of human LMW FGF2 | Bone marrow cells | ALP-positive colonies and mineralized nodules ↑ | Mice | |
| | Systemic deletion of human LMW FGF2 | Bone marrow cells | Reverse effects as above | | |
| | Intravenous injections Subcutaneously injections Injections over the calvaria Single local injection into the distracted callus Subcutaneous transplantations of human bone marrow cells treated with FGF2 | Bone marrow cells | | |

Note: ↑ indicates increase; ↓ indicates decrease.
| FGFs                        | Models | In vivo | Ex vivo | Outcomes                                                                                       | Animals | Ref. |
|-----------------------------|--------|---------|---------|-----------------------------------------------------------------------------------------------|---------|------|
| Types                       | Members | Genetic manipulations | Recombinant proteins | Recombinant proteins and so forth | | |
| Bone marrow cells           | Calvaria cells | Osteoblasts from trabecular bone | Cell proliferation and matrix mineralization ↑ | Humans | |
| FGF4                        | Subcutaneous injections | | | Cell proliferation ↑, matrix mineralization ↓ | | |
| FGF6                        | Osteoblasts from trabecular bone | Embryonic stem cells | Cell proliferation ↑, ALP activity, and matrix mineralization ↓ | Humans | [33] |
| FGF7                        | Embryonic stem cells | | Mineralized nodules and osteoblast marker gene expression ↑ | Mice | [34] |
| FGF8                        | Osteogenic ROB-26 cells | | ALP activity and Runx2 expression ↑ | Rats | [35] |
| FGF9                        | Subcutaneous transplantations of human bone marrow cells treated with FGF2 plus FGF9 | | Effect of FGF2 on new bone formation in trabecular bone ↑ | Nude mice | [37] |
| FGF10                       | Systemic deletion in Fgfr2 mutant mice | Bone marrow cells | Effect of FGF2 on cell proliferation and mineralization ↑ | Humans | |
|                             | Calvaria cells | | Rescue of craniosynostosis and skeletal defects | Mice | [38] |
|                             |                      | | No obvious effects | Mice | [39] |
| Types       | Members                     | Genetic manipulations | Recombinant proteins | Ex vovo | In vivo | In vitro | Outcomes                                                                 | Animals | Ref.   |
|------------|-----------------------------|-----------------------|----------------------|---------|---------|----------|------------------------------------------------|---------|--------|
| FGF18      | Systemic deletion           |                       |                      |         |         |          | Skeletal defects, proliferation of osteogenic cells, and maturation of osteoblasts ↓ | Mice    | [40]   |
|            |                             |                       |                      |         |         |          | Cell proliferation ↑, matrix mineralization ↓ | Mice    | [39]   |
| FGF21      | Overexpression              |                       |                      |         |         |          | Trabecular bone ↓                                                                 | Mice    | [92]   |
|            | Systemic deletion           |                       |                      |         |         |          | Reverse effects as above                                                           |         |        |
| Hormone-like | Systemic deletion           |                       |                      |         |         |          | Bone mineralization ↓ with hyperphosphatemia                                          | Mice    | [81]   |
|            | Overexpression              |                       |                      |         |         |          | Bone abnormality with hypophosphatemia and serum PTH levels ↑                      | Mice    | [57]   |
| FGF23      | Osteoblast-specific overexpression of FGF23 |                       |                      |         |         |          | Calvaria cells with adenoviral FGF23 overexpression Osteoblastic MC3T-E1 cells | Rats    | [9]    |
|            |                             |                       |                      |         |         |          | Osteogenic differentiation and matrix mineralization ↓                             |         |        |
|            |                             |                       |                      |         |         |          | Cell proliferation ↑, matrix mineralization ↓                                      | Mice    | [87]   |

↑: increase; ↓: decrease. Ref.: References.
bone defects and craniosynostosis with abnormal osteoblastic proliferation and differentiation [49]. An in vitro study shows that constitutively active FGFR2 (S252W) induces the ERK1/2 and PKC pathways causing osteoblastic differentiation in the murine mesenchymal cell line C3H10T1/2 [50]. Three of the Fgfr3 gain-of-function mutations have been reported to cause chondrodysplasia and craniosynostosis. Achondroplasia, the most common form of human dwarfism, is associated with the G380R mutation [51]. The P250R mutation causes Muenke syndrome, a common syndrome of craniosynostosis [52]. Crouzon syndrome and acanthosis nigricans, a skin pigmentation disorder, result from the A391E mutation [53]. Unlike FGFR1 and FGFR2 deficient mice, systemic Fgfr3 null mice are viable and show progressive osteodysplasia with expanded growth plate cartilage [54]. Taken together, because FGFR9, a preferred ligand for FGFR3, upregulates osteopontin (Opn) in chicken chondrocytes [55], FGFR3 signaling may affect chondrocytes rather than osteoblasts [54]. In contrast to these three FGFRs, there are quite a few reports about the relationship between FGFR4 and bone formation. Cool et al. indicated that FGFR4 is expressed in preosteoblasts and osteoblasts in neonatal mouse calvaria, suggesting that FGFR4 is involved in osteogenesis [56], but its role in bone remains unclear.

5. FGF23 and FGF19 Subfamily Members as Hormone-Like Factors

FGF23 is the last member of the FGF family, and its significant roles in Pi and vitamin D metabolism are obvious in genetically engineered mice [57–59] (also see review [60]). FGF23 was originally discovered as the gene responsible for autosomal dominant hypophosphatemic rickets [61] and thereafter as a phosphaturic factor produced by mesenchymal bone defects and craniosynostosis with abnormal osteoblastic proliferation and differentiation [49]. An in vitro study shows that constitutively active FGFR2 (S252W) induces the ERK1/2 and PKC pathways causing osteoblastic differentiation in the murine mesenchymal cell line C3H10T1/2 [50]. Three of the Fgfr3 gain-of-function mutations have been reported to cause chondrodysplasia and craniosynostosis. Achondroplasia, the most common form of human dwarfism, is associated with the G380R mutation [51]. The P250R mutation causes Muenke syndrome, a common syndrome of craniosynostosis [52]. Crouzon syndrome and acanthosis nigricans, a skin pigmentation disorder, result from the A391E mutation [53]. Unlike FGFR1 and FGFR2 deficient mice, systemic Fgfr3 null mice are viable and show progressive osteodysplasia with expanded growth plate cartilage [54]. Taken together, because FGFR9, a preferred ligand for FGFR3, upregulates osteopontin (Opn) in chicken chondrocytes [55], FGFR3 signaling may affect chondrocytes rather than osteoblasts [54]. In contrast to these three FGFRs, there are quite a few reports about the relationship between FGFR4 and bone formation. Cool et al. indicated that FGFR4 is expressed in preosteoblasts and osteoblasts in neonatal mouse calvaria, suggesting that FGFR4 is involved in osteogenesis [56], but its role in bone remains unclear.

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FGF23 and FGF19 Subfamily Members as Hormone-Like Factors

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Both of ectopic (hepatic) overexpression and osteoblast/osteocyte-specific overexpression of the Fg23 transgene result in lower bone mineral density of the femur with hypophosphatemia and high serum levels of PTH [57, 58]. The lack of either FGF23 or αKlotho causes aberrant Ca/Pi and vitamin D metabolism, thus ensuring skeletal anomalies and ectopic calcification [59, 81, 82]. Fg23−/−/Opn−/− double-knockout (DKO) mice mimic hyperphosphatemia in Fg23−/− mice, but the severe osteoidosis in Fg23−/− is markedly reduced [83]. Fg23−/−/Slc34a1−/− DKO mice reverse hyperphosphatemia in keeping with hypomineralization in bone [84]. These observations suggest that skeletal anomalies that involve FGF23 may result not only from serum Pi levels but also from intrinsic anomalies in bone. FGF23 may act independently of the membrane protein αKlotho (Figure 4). For example, overexpression of FGF23 in cultured rat calvaria cells impairs osteoblast differentiation and mineralized matrix formation but not mineralization, via activation of FGFR1 [9]. One plausible explanation is that the existence of the soluble form (circulating αKlotho) shedding from the extracellular domain of αKlotho [85, 86] may act as a cofactor for FGF23. In fact, effects of FGF23 in MC3T3-E1 cells (a mouse osteoblastic cell line) cultured with circulating αKlotho [87] mimic the results observed in rat calvaria cells [9]. In mouse chondrocytes, FGF23 activates FRS2α, FGFR substrate 2 α, and ERK1/2, resulting in a decrease in chondrocyte proliferation in the presence of circulating αKlotho [88]. In contrast, αKlotho is not required for FGF23 action in some cells. For instance, FGF23 can induce the hypertrophy of neonatal rat ventricular cardiomyocytes, in which αKlotho is not detected [89]. In addition, FGF23 decreases PTH secretion in thyroparathyroid organ cultures from parathyroid-specific αKlotho-deficient mice [75]. It is still unknown why FGF23 targets the kidney and parathyroid glands, even in the presence of circulating αKlotho and/or the ubiquitous expression of FGFRs.

\[
\text{FGF23} 
\text{Membrane αKlotho} \\
\text{Circulating αKlotho} \\
\text{Plasma membrane} \\
\text{TKs} \\
\text{FGFRs} \\
\text{HS?} \\
\]

**Figure 4**: Possible klotho-dependent and klotho-independent mechanisms of FGF23 actions. FGF23 may activate FGFR tyrosine kinases with or without membrane and circulating αKlotho. TKs: nonphosphorylated tyrosine kinases.
The roles of two other members of the hormone-like FGF19 subfamily, FGF19 and FGF21, in bone formation remain to be elucidated. Fgf9 transcripts are predominantly expressed in the ileum, while Fgf21 mRNA is expressed in the liver, pancreas, and white adipose tissue [90]. In skeletal tissue under normal conditions, FGF19, but not FGF21, is also detectable at the protein level in human fetal growth plate cartilage [91]. Interestingly, the treatment of mouse bone marrow cells with FGF21 increases βKlotho and Fgf21 mRNA expression, especially in the presence of rosiglitazone [92], an agonist of the master regulator for adipogenesis, PPARγ, possibly affecting bone formation. Thus, genetic FGF21 loss and gain of function in mice increase and decrease bone mass [92], respectively, suggesting that FGF21/βKlotho may act as an inhibitor of bone formation.

6. Local and Systemic Effects of FGFs during Bone Formation, Focusing on FGF2, FGF21, and FGF23

As above, FGF2 and FGF23 may exhibit distinct activities during different stages of osteoblast differentiation, such as cell proliferation versus matrix (osteoid) mineralization. In contrast to osteogenic cell proliferation, differentiation, and associated matrix formation, the molecular mechanism(s) underlying matrix mineralization remains to be fully elucidated. Human FGF2 has multiple isoforms via an alternative initiation of translation at CUG codons from a single FGF2 gene: LMW and high (HMW FGF2, 22-kDa, 22.5-kDa, 24-kDa, and 34-kDa) molecular forms [93]. LMW FGF2—exactly the same FGF2 as described above—is predominantly expressed in osteoblast precursors and activates intracellular signaling via FGFR in an autocrine/paracrine manner. While recent evidence indicates that extracellular LMW FGF2 can translocate to the nucleus after internalization [94], there is little evidence for this process in bone to date. The HMW FGF2 isoforms are not released from the cells and localized to the nucleus and regulate gene expression to exert specific effects. Transgenic mice overexpressing human HMW FGF2 (22-kDa, 23-kDa, and 24-kDa) under the Col1 promoter (Col3.6) exhibit lower bone mineral density with decreased bone formation and increased bone resorption [95]. Interestingly, upregulation of Fgf23 expression and hypophosphatemia are observed in these mice [95]. These observations may lead to the development of an additional framework for understanding the effects of the HMW FGF2 and FGF23 on bone mineralization.

It is well known that elevated serum FGF23 levels are the most common predictor in patients with chronic kidney disease [96]. Serum FGF23 levels are positively correlated to aortic arterial calcification in hemodialysis patients [97]. Recent studies demonstrate that FGF23 exacerbates left ventricle hypertrophy where αKlotho might not be expressed [89] and elevated plasma FGF23 levels are associated with low body mass index and dyslipidemia in dialysis patients [98]. Thus, systemic actions of FGF23 may reach organs dependently and independently of αKlotho. Although skeletal tissues do not express Fgf21 under normal conditions, circulating FGF21 seems to suppress osteoblastogenesis and induce adipogenesis [92]. Also, FGF21 itself enhances Fgf21 and βKlotho expression in bone marrow-derived adipocytes, and increases in FGF21 and βKlotho have a synergetic effect on its signaling in local area [92]. Comprehensive analyses are needed to determine the local versus systemic effects of FGF21 on bone. Taken all together, FGFs expressed in bone are involved in bone formation directly and indirectly, which indicates that FGFs mediate the interrelationships between bone and other organs under normal and/or clinical situations. The clinical importance of FGF23/21 is now becoming clearer owing to the recent findings in FGF research. However, precise elucidation of FGF mechanisms is still required.

7. Conclusion

The skeleton is a multipotent organ that is fundamental for the survival of vertebrates. Bone and mineral homeostasis are strictly controlled by multiple mechanisms including FGF/FGFR signaling. Canonical and hormone-like FGFs regulate bone formation at different developmental stages in different ways, and these members may compensate for one another in bone and/or extraskeletal tissues. In order to understand these mechanisms, the balance between local and systemic regulation needs to be considered.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] X. Feng and J. M. McDonald, “Disorders of bone remodeling,” Annual Review of Pathology, vol. 6, pp. 121–145, 2011.
[2] M. Ferron, M. D. McKee, R. L. Levine, P. Duy, and G. Karsenty, “Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice,” Bone, vol. 50, no. 2, pp. 568–575, 2012.
[3] L. M. Calvi, G. B. Adams, K. W. Weibrecht et al., “Osteoblastic cells regulate the haematopoietic stem cell niche,” Nature, vol. 425, no. 6960, pp. 841–846, 2003.
[4] J. Zhang, C. Niu, L. Ye et al., “Identification of the haematopoietic stem cell niche and control of the niche size,” Nature, vol. 425, no. 6960, pp. 836–841, 2003.
[5] H. Iwasaki and T. Suda, “Cancer stem cells and their niche,” Cancer Science, vol. 100, no. 7, pp. 1166–1172, 2009.
[6] S. Takeda, F. Elefteriou, R. Levasseur et al., “Leptin regulates bone formation via the sympathetic nervous system,” Cell, vol. 111, no. 3, pp. 305–317, 2002.
[7] C. Collet, C. Schiltz, V. Geoffroy, L. Maroteaux, J.-M. Launay, and M.-C. de Vernejoul, “The serotonin 5-HT1γ receptor controls bone mass via osteoblast recruitment and proliferation,” The FASEB Journal, vol. 22, no. 2, pp. 418–427, 2008.
[8] M. Kuro-o, “Endocrine FGFs and Klothos: emerging concepts,” Trends in Endocrinology & Metabolism, vol. 19, no. 7, pp. 239–245, 2008.
[9] H. Wang, Y. Yoshiko, R. Yamamoto et al., “Overexpression of fibroblast growth factor 23 suppresses osteoblast differentiation
and matrix mineralization in vitro,” *Journal of Bone and Mineral Research*, vol. 23, no. 6, pp. 939–948, 2008.

[10] R. T. Böttcher and C. Niehrs, “Fibroblast growth factor signaling during early vertebrate development,” *Endocrine Reviews*, vol. 26, no. 1, pp. 63–77, 2005.

[11] B. Thisse and C. Thisse, “Functions and regulations of fibroblast growth factor signaling during embryonic development,” *Developmental Biology*, vol. 287, no. 2, pp. 390–402, 2005.

[12] H. Kurosu and M. Kuro-O, “Endocrine fibroblast growth factors as regulators of metabolic homeostasis,” *BioFactors*, vol. 35, no. 1, pp. 52–60, 2009.

[13] M. Mohammadi, S. K. Olsen, and O. A. Ibrahimi, “Structural basis for fibroblast growth factor receptor activation,” *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 107–137, 2005.

[14] L. Dailey, D. Ambrosetti, A. Mansukhani, and C. Basilico, “Mechanisms underlying differential responses to FGF signaling,” *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 233–247, 2005.

[15] N. Hacohen, S. Kramer, D. Sutherland, Y. Hiromi, and M. A. Krasnow, “Sprout encodes a novel antagonist of FGF signaling that patterns apical branching of the Drosophila airways,” *Cell*, vol. 92, no. 3, pp. 253–263, 1998.

[16] M. Fürthauer, F. Reifers, M. Brand, B. Thisse, and C. Thisse, “Sprouty4 acts in vivo as a feedback-induced antagonist of FGF signaling in zebrafish,” *Development*, vol. 128, no. 12, pp. 2175–2186, 2001.

[17] M. Fürthauer, W. Lin, S.-L. Ang, B. Thisse, and C. Thisse, “Sef is a feedback-induced antagonist of RAS/MAPK-mediated FGF signalling,” *Nature Cell Biology*, vol. 4, no. 2, pp. 170–174, 2002.

[18] Y. Hirate and H. Okamoto, “Canopy, a novel regulator of FGF signaling around the midbrain-hindbrain boundary in zebrafish,” *Current Biology*, vol. 16, no. 4, pp. 421–427, 2006.

[19] T. Matsui, S. Thitamadee, T. Murata et al., “Canopy, a positive feedback regulator of FGF signaling, controls progenitor cell clustering during Kupffer’s vesicle organogenesis,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 24, pp. 9881–9886, 2011.

[20] F. Debiais, M. Hott, A. M. Graulet, and P. J. Marie, “The effects of fibroblast growth factor-2 on human neonatal calvarial osteoblastic cells are differentiation stage specific,” *Journal of Bone and Mineral Research*, vol. 13, no. 4, pp. 645–654, 1998.

[21] H. Okazaki, T. Kurokawa, K. Nakamura, T. Matsushita, K. Mamada, and H. Kawaguchi, “Stimulation of bone formation by recombinant fibroblast growth factor-2 in callotasis bone lengthening of rabbits,” *Calcified Tissue International*, vol. 64, no. 6, pp. 542–546, 1999.

[22] M. Kitamura, M. Akamatsu, M. MacHigashira et al., “FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial,” *Journal of Dental Research*, vol. 90, no. 1, pp. 35–40, 2011.

[23] H. Nagai, R. Tsukuda, and H. Mayahara, “Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats,” *Bone*, vol. 16, no. 3, pp. 367–373, 1995.

[24] C. R. Dunstan, R. Boyce, B. F. Boyce et al., “Systemic administration of acidic fibroblast growth factor (FGF-1) prevents bone loss and increases new bone formation in ovariectomized rats,” *Journal of Bone and Mineral Research*, vol. 14, no. 6, pp. 953–959, 1999.

[25] J. I. Aguirre, M. E. Leal, M. F. Rivera, S. M. Vanegas, M. Jorgensen, and T. J. Wronska, “Effects of basic fibroblast growth factor and a prostaglandin E2 receptor subtype 4 agonist on osteoblastogenesis and adipogenesis in aged ovariectomized rats,” *Journal of Bone and Mineral Research*, vol. 22, no. 6, pp. 877–888, 2007.

[26] A. Montero, Y. Okada, M. Tomita et al., “Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation,” *Journal of Clinical Investigation*, vol. 105, no. 8, pp. 1085–1093, 2000.

[27] T. Naganawa, L. Xiao, J. D. Coffin et al., “Reduced expression and function of bone morphogenetic protein-2 in bones of Fgf2 null mice,” *Journal of Cellular Biochemistry*, vol. 103, no. 6, pp. 1975–1988, 2008.

[28] L. Xiao, T. Sobue, A. Edsger et al., “Disruption of the Fgf2 gene activates the adipogenic and suppresses the osteogenic program in mesenchymal marrow stromal stem cells,” *Bone*, vol. 47, no. 2, pp. 360–370, 2010.

[29] L. Xiao, P. Liu, X. Li et al., “Exported 18-kDa isoform of fibroblast growth factor-2 is a critical determinant of bone mass in mice,” *Journal of Biological Chemistry*, vol. 284, no. 5, pp. 3170–3182, 2009.

[30] K. T. Tang, C. Capparelli, J. L. Stein et al., “Acidic fibroblast growth factor inhibits osteoblast differentiation in vitro: altered expression of collagenase, cell growth-related, and mineralization-associated genes,” *Journal of Cellular Biochemistry*, vol. 61, no. 1, pp. 152–166, 1996.

[31] D. L. Miller, S. Ortega, O. Bashayan, R. Basch, and C. Basilico, “Compensation by fibroblast growth factor 1 (FGF1) does not account for the mild phenotypic defects observed in Fgf2 null mice,” *Molecular and Cellular Biology*, vol. 20, no. 6, pp. 2260–2268, 2000.

[32] S. Kuroda, S. Kasugai, S. Oida, T. Iimura, K. Ohya, and T. Ohyama, “Anabolic effect of aminoterminally truncated fibroblast growth factor 4 (FGF4) on bone,” *Bone*, vol. 25, no. 4, pp. 431–437, 1999.

[33] M. Bosetti, M. Leigheb, R. A. Brooks, F. Boccafossi, and M. F. Cannas, “Regulation of osteoblast and osteoclast functions by FGF-6,” *Journal of Cellular Physiology*, vol. 225, no. 2, pp. 466–471, 2010.

[34] Y.-M. Jeon, S.-H. Kook, S.-J. Rho et al., “Fibroblast growth factor-7 facilitates osteogenic differentiation of embryonic stem cells through the activation of ERK/Runx2 signaling,” *Molecular and Cellular Biochemistry*, vol. 382, no. 1-2, pp. 37–45, 2013.

[35] K. Omoteyama and M. Takagi, “FGF8 regulates myogenesis and induces Runx2 expression and osteoblast differentiation in cultured cells,” *Journal of Cellular Biochemistry*, vol. 106, no. 4, pp. 546–552, 2009.

[36] V. Govindarajan and P. A. Overbeek, “FGF9 can induce endochondral ossification in cranial mesenchyme,” *BMC Developmental Biology*, vol. 6, article 7, 2006.

[37] T. Kizhner, D. Ben-David, E. Rom, A. Yayon, and E. Livne, “Effects of FGF2 and FGF9 on osteogenic differentiation of bone marrow-derived progenitors,” *In Vitro Cellular and Developmental Biology—Animal*, vol. 47, no. 4, pp. 294–301, 2011.

[38] M. K. Hajhosseini, R. Duarte, J. Pegrum et al., “Evidence that Fgf10 contributes to the skeletal and visceral defects of an apter syndrome mouse model,” *Developmental Dynamics*, vol. 238, no. 2, pp. 376–385, 2009.

[39] T. Shimoka, T. Ogasawara, A. Yonamine et al., “Regulation of osteoblast, chondrocyte, and osteoclast functions by fibroblast growth factor (FGF)-18 in comparison with FGF-2 and FGF-10,” *The Journal of Biological Chemistry*, vol. 277, no. 9, pp. 7493–7500, 2002.
[40] N. Ohbayashi, M. Shibayama, Y. Kurotaki et al., "FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis," *Genes and Development*, vol. 16, no. 7, pp. 870–879, 2002.

[41] L. Chen and C.-X. Deng, "Roles of FGF signaling in skeletal development and human genetic diseases," *Frontiers in Bioscience*, vol. 10, no. 2, pp. 1961–1976, 2005.

[42] G. M. Morriss-Kay and A. O. M. Wilkie, "Growth of the normal skull vault and its alteration in craniostenosis: insights from human genetics and experimental studies," *Journal of Anatomy*, vol. 207, no. 5, pp. 637–653, 2005.

[43] D. M. Ornitz and P. J. Marie, "FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease," *Genes & Development*, vol. 16, no. 12, pp. 1446–1465, 2002.

[44] D. M. Ornitz, "FGF signaling in the developing endochondral skeleton," *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 205–213, 2005.

[45] T. P. Yamaguchi, K. Harpal, M. Henkemeyer, and J. Rossant, "Rfgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation," *Genes and Development*, vol. 8, no. 4, pp. 3032–3044, 1994.

[46] A. L. Jacob, C. Smith, J. Partanen, and D. M. Ornitz, "Fibroblast growth factor receptor 1 signaling in the osteo-chondrogenic cell lineage regulates sequential steps of osteoblast maturation," *Developmental Biology*, vol. 296, no. 2, pp. 315–328, 2006.

[47] E. W. Jabs, X. Li, A. F. Scott et al., "Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2," *Nature Genetics*, vol. 8, no. 3, pp. 275–279, 1999.

[48] A. O. M. Wilkie, S. F. Slaney, M. Oldridge et al., "Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome," *Nature Genetics*, vol. 9, no. 2, pp. 165–172, 1995.

[49] Y. Wang, R. Xiao, F. Yang et al., "Abnormalities in cartilage and bone development in the Apert syndrome FGFR2<sup>W/W</sup> mouse," *Development*, vol. 132, no. 5, pp. 3537–3548, 2005.

[50] H. Miriou, K. Oudina, H. Petite, Y. Tanimoto, K. Moriyama, and P. J. Marie, "Fibroblast growth factor receptor 2 promotes osteogenic differentiation in mesenchymal cells via ERK1/2 and protein kinase C signaling," *The Journal of Biological Chemistry*, vol. 284, no. 8, pp. 4897–4904, 2009.

[51] F. Rousseau, J. Bonaventure, L. Leger-Mallet et al., "Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia," *Nature*, vol. 371, no. 6494, pp. 252–254, 1994.

[52] M. Mueneke, K. W. Gripp, D. M. McDonald-McGinn et al., "A unique point mutation in the fibroblast growth factor receptor 3 gene (FGFR3) defines a new craniosynostosis syndrome," *The American Journal of Human Genetics*, vol. 60, no. 3, pp. 555–564, 1997.

[53] G. A. Meyers, S. J. Orlow, I. R. Munro, K. A. Przybyla, and E. W. Jabs, "Fibroblast growth factor receptor 3 (FGFR3) transmembrane mutation in Crouzon syndrome with acanthosis nigricans," *Nature Genetics*, vol. 11, no. 4, pp. 462–464, 1995.

[54] C. Deng, A. Wynshaw-Boris, F. Zhou, A. Kuo, and P. Leder, "Fibroblast growth factor receptor 3 is a negative regulator of bone growth," *Cell*, vol. 84, no. 6, pp. 911–921, 1996.

[55] S. Weizmann, A. Tseng, B. Reich, O. Genina, A. Yaron, and E. Monsonego-Ornan, "FGF upregulates osteopontin in epiphyseal growth plate chondrocytes: implications for endochondral ossification," *Matrix Biology*, vol. 24, no. 8, pp. 520–529, 2005.

[56] S. Cool, R. Jackson, P. Pincus, I. Dickinson, and V. Nurcombe, "Fibroblast growth factor receptor 4 (FGFR4) expression in newborn murine calvaria and primary osteoblast cultures," *International Journal of Developmental Biology*, vol. 46, no. 4, pp. 519–523, 2002.

[57] X. Bai, D. Miao, J. Li, D. Goltzman, and A. C. Karaplis, "Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders," *Endocrinology*, vol. 145, no. 11, pp. 5269–5279, 2004.

[58] T. Larsson, R. Mards, E. Schipani et al., "Transgenic mice expressing fibroblast growth factor 23 under the control of the a1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis," *Endocrinology*, vol. 145, no. 7, pp. 3087–3094, 2004.

[59] T. Shimada, M. Kakitani, Y. Yamazaki et al., "Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism," *The Journal of Clinical Investigation*, vol. 113, no. 4, pp. 561–568, 2004.

[60] L. D. Quarles, "Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism," *Nature Reviews Endocrinology*, vol. 8, no. 5, pp. 276–286, 2012.

[61] K. E. White, W. E. Evans, J. L. H. O'Riordan et al., "Autosomal dominant hypophosphatemic rickets is associated with mutations in FGF23," *Nature Genetics*, vol. 26, no. 3, pp. 345–348, 2000.

[62] T. Shimada, S. Mizutani, T. Muto et al., "Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 11, pp. 6500–6505, 2001.

[63] Y. Yoshihiko, H. Wang, T. Minamizaki et al., "Mineralized tissue cells are a principal source of FGF23," *Bone*, vol. 40, no. 6, pp. 1565–1573, 2007.

[64] M. Mirams, B. G. Robinson, R. S. Mason, and A. E. Nelson, "Bone as a source of FGF23: regulation by phosphate?" *Bone*, vol. 35, no. 5, pp. 1192–1199, 2004.

[65] R. Masuyama, I. Stockmans, S. Torrekens et al., "Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts," *The Journal of Clinical Investigation*, vol. 116, no. 12, pp. 3150–3159, 2006.

[66] S. Liu, W. Tang, J. Zhou, L. Vierthaler, and L. D. Quares, "Distinct roles for intrinsic osteocyte abnormalities and systemic factors in regulation of FGF23 and bone mineralization in Hyp mice," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 293, no. 6, pp. E1636–E1644, 2007.

[67] I. Urakawa, Y. Yamazaki, T. Shimada et al., "Klotho converts canonical FGF receptor into a specific receptor for FGF23," *Nature*, vol. 444, no. 7120, pp. 770–774, 2006.

[68] H. Kurosu, Y. Ogawa, M. Miyoshi et al., "Regulation of fibroblast growth factor-23 signaling by Klotho," *The American Journal of Physiology*, vol. 281, no. 6, pp. F282–F291, 2009.
co-transport activity and 1α,25-dihydroxyvitamin D₃ production,” *The Journal of Biological Chemistry*, vol. 278, no. 4, pp. 2206–2211, 2003.

[72] S. Liu, W. Tang, J. Zhou et al., “Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D,” *Journal of the American Society of Nephrology*, vol. 17, no. 5, pp. 1305–1315, 2006.

[73] R. Yamamoto, T. Minamizaki, Y. Yoshiko et al., “1α,25-dihydroxyvitamin D₃ acts predominately in mature osteoblasts under conditions of high extracellular phosphate to increase fibroblast growth factor 23 production in vitro,” *Journal of Endocrinology*, vol. 206, no. 3, pp. 279–286, 2010.

[74] I. Z. Ben-Dov, H. Galitzer, V. Lavi-Moshayoff et al., “The parathyroid is a target organ for FGF23 in rats,” *The Journal of Clinical Investigation*, vol. 117, no. 2, pp. 4003–4008, 2007.

[75] H. Olauson, K. Lindberg, R. Amin et al., “Parathyroid-specific deletion of Klotho unravels a novel calcineurin-dependent FGF23 signaling pathway that regulates PTH secretion,” *PLoS Genetics*, vol. 9, no. 12, Article ID e1003975, 2013.

[76] Y. Rhee, N. Bivi, E. Farrow et al., “Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 in vitro and in vivo,” *Bone*, vol. 49, no. 4, pp. 636–643, 2011.

[77] J. Gattineni, K. Twombley, R. Goetz, M. Mohammadi, and M. Baumb, “Regulation of serum 1,25(OH)₂ vitamin D₃ levels by fibroblast growth factor 23 is mediated by FGF receptors 3 and 4,” *The American Journal of Physiology—Renal Physiology*, vol. 301, no. 2, pp. F371–F377, 2011.

[78] N. Arai-Nunota, M. Mizobuchi, H. Ogata et al., “Intravenous phosphate loading increases fibroblast growth factor 23 in uremic rats,” *PLoS ONE*, vol. 9, no. 3, Article ID e910996, 2014.

[79] M. Kawai, S. Kinoshita, S. Shimba, K. Ozono, and T. Michigami, “Sympathetic activation induces skeletal Fgf23 expression in a circadian rhythm-dependent manner,” *The Journal of Biological Chemistry*, vol. 289, no. 3, pp. 1457–1466, 2014.

[80] R. C. Smith, L. M. O’Bryan, E. G. Farrow et al., “Circulating αKlotho influences phosphate handling by controlling FGF23 production,” *Journal of Clinical Investigation*, vol. 122, no. 12, pp. 4710–4715, 2012.

[81] M. Kuro-o, Y. Matsumura, H. Aizawa et al., “Mutation of the mouse klotho gene leads to a syndrome resembling ageing,” *Nature*, vol. 390, no. 6655, pp. 45–51, 1997.

[82] Y. Takei, H. Yamamoto, T. Sato et al., “Stanniocalcin 2 is associated with ectopic calcification in α-klotho mutant mice and inhibits hyperphosphatemia-induced calcification in aortic vascular smooth muscle cells,” *Bone*, vol. 50, no. 4, pp. 998–1005, 2012.

[83] Q. Yuan, Y. Jiang, X. Zhao et al., “Increased osteopontin contributes to inhibition of bone mineralization in FGF23-deficient mice,” *Journal of Bone and Mineral Research*, vol. 29, no. 3, pp. 693–704, 2014.

[84] D. Sitara, S. Kim, M. S. Razzaque et al., “Genetic evidence of serum phosphate-independent functions of FGF-23 on bone,” *PLoS Genetics*, vol. 4, no. 8, Article ID e1000154, 2008.

[85] Y. Matsumura, H. Aizawa, T. Shiraki-Iida, R. Nagai, M. Kuro-o, and Y. I. Nabeshima, “Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein,” *Biochemical and Biophysical Research Communications*, vol. 242, no. 3, pp. 626–630, 1998.

[86] T. Shiraki-Iida, H. Aizawa, Y. Matsumura et al., “Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein,” *FEBS Letters*, vol. 424, no. 1-2, pp. 6–10, 1998.

[87] V. Shahhoub, S. C. Ward, B. Sun et al., “Fibroblast growth factor 23 (FGF23) and α-klotho stimulate osteoblastic MC3T3.E1 cell proliferation and inhibit mineralization,” *Calcified Tissue International*, vol. 89, no. 2, pp. 140–150, 2011.

[88] M. Kawai, S. Kinoshita, A. Kimoto et al., “FGF23 suppresses chondrocyte proliferation in the presence of solublex-klotho both in vitro and in vivo,” *Journal of Biological Chemistry*, vol. 288, no. 4, pp. 2414–2427, 2013.

[89] C. Faul, A. P. Amaral, B. Oskouei et al., “FGF23 induces left ventricular hypertrophy,” *Journal of Clinical Investigation*, vol. 121, no. 11, pp. 4393–4408, 2011.

[90] B. Angelin, T. E. Larsson, and M. Rudling, “Circulating fibroblast growth factors as metabolic regulators—a critical appraisal,” *Cell Metabolism*, vol. 16, no. 6, pp. 693–705, 2012.

[91] P. Krejci, D. Krakow, P. B. Mekikian, and W. R. Wilcox, “Fibroblast growth factors 1, 2, 17, and 19 are the predominant FGF ligands expressed in human fetal growth plate cartilage,” *Pediatric Research*, vol. 61, no. 3, pp. 267–272, 2007.

[92] W. Wei, P. A. Dutchak, X. Wang et al., “Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor γ,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 8, pp. 3143–3148, 2012.

[93] I. Delrieu, “The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism,” *FEBS Letters*, vol. 468, no. 1, pp. 6–10, 2000.

[94] P. Claus, F. Döring, S. Gringel et al., “Differential intranuclear localization of fibroblast growth factor-2 isoforms and specific interaction with the survival of motoneuron protein,” *Journal of Biological Chemistry*, vol. 278, no. 1, pp. 479–485, 2003.

[95] L. Xiao, T. Naganawa, J. Lorenzo, T. O. Carpenter, J. D. Coffin, and M. M. Hurley, “Nuclear isoforms of fibroblast growth factor 2 are novel inducers of hypophosphatemia via modulation of FGF23 and KLOTHO,” *The Journal of Biological Chemistry*, vol. 285, no. 4, pp. 2834–2846, 2010.

[96] M. Wolf, “Update on fibroblast growth factor 23 in chronic kidney disease,” *Kidney International*, vol. 82, no. 7, pp. 737–747, 2012.

[97] Z. Chen, X. Chen, J. Xie et al., “Fibroblast growth factor 23 is a predictor of aortic artery calcification in maintenance hemodialysis patients,” *Renal Failure*, vol. 35, no. 5, pp. 660–666, 2013.

[98] J. R. Montford, M. Chonchol, A. K. Cheung et al., “Low body mass index and dyslipidemia in dialysis patients linked to elevated plasma fibroblast growth factor 23,” *American Journal of Nephrology*, vol. 37, no. 3, pp. 183–190, 2013.