

REVIEW

Fibroblast growth factor signaling in skeletal development and disease

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Fibroblast growth factor (FGF) signaling pathways are essential regulators of vertebrate skeletal development. FGF signaling regulates development of the limb bud and formation of the mesenchymal condensation and has key roles in regulating chondrogenesis, osteogenesis, and bone and mineral homeostasis. This review updates our review on FGFs in skeletal development published in *Genes & Development* in 2002, examines progress made on understanding the functions of the FGF signaling pathway during critical stages of skeletogenesis, and explores the mechanisms by which mutations in FGF signaling molecules cause skeletal malformations in humans. Links between FGF signaling pathways and other interacting pathways that are critical for skeletal development and could be exploited to treat genetic diseases and repair bone are also explored.

Skeletal development: an overview

Development of the vertebrate skeleton occurs through the processes of endochondral and intramembranous bone formation. Endochondral-derived bones comprise the appendicular skeleton, facial bones, vertebrae, and medial clavicles, while intramembranous bones comprise the cranium and lateral clavicles. Endochondral ossification initiates with the condensation of mesenchyme, followed by the formation of a cartilaginous template that patterns the developing skeleton and the segmentation of this template into cartilaginous joints that are stabilized by a flexible fibrous capsule and mineralized cortical and trabecular (spongy) bone. Intramembranous bone forms from a mesenchymal condensation that directly gives rise to bone.

Chondrogenesis

Mesenchymal condensations are the first histologically visible features of the developing appendicular skeleton

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(Fig. 1A). Mesenchymal condensations form in the developing limb bud and are characterized by the aggregation of loose mesenchymal cells and the expression of extracellular matrix proteins, cell adhesion molecules, transcription factors, and receptors for signaling molecules. The transcription factor Sox9 is required for the differentiation of the chondrogenic lineage, and bone morphogenetic protein (BMP) receptors 1a and 1b are required for chondrogenesis and the maintenance of Sox9 expression (Kosher et al. 1986; Nah et al. 1988; Bi et al. 1999; Akiyama et al. 2002; Yoon et al. 2005; Yeung Tsang et al. 2014; Lim et al. 2015). Proliferating cells within the forming mesenchymal condensation express type II collagen (*Col2a1*) centrally in chondroprogenitors and type I collagen (*Col1a1*) peripherally in osteoprogenitors (Kosher et al. 1986).

Proliferating chondrocytes form columns oriented along the longitudinal axis of the developing bone. Midway along the longitudinal axis of the mesenchymal condensation, proliferating chondrocytes exit the cell cycle and differentiate into prehypertrophic chondrocytes (Fig. 1B,C) that are marked by expression of Indian Hedgehog (IHH), parathyroid hormone (PTH)-related peptide receptor (PTH1R), and type X collagen (*Col10a1*). Several transcription factors are required for the initiation and progression of chondrocyte hypertrophy. Sox9 is required to maintain chondrocyte identity and prepare chondrocytes for subsequent hypertrophy but must be down-regulated for progression to hypertrophy (Ikegami et al. 2011; Dy et al. 2012). *Runx2* (*Cbfa1*) and *Runx3* (*Cbfa3*) are required for chondrocyte maturation and indirectly function to enhance chondrocyte proliferation through promoting *Ihh* expression (Yoshida et al. 2004). The balance between chondrocyte proliferation and hypertrophy is regulated by interactions between several signaling pathways, including IHH, PTH-related peptide (PTH), BMP, Wnt, and fibroblast growth factor (FGF) (Long and Ornitz 2013; Kozhemyakina et al. 2015). The activity of

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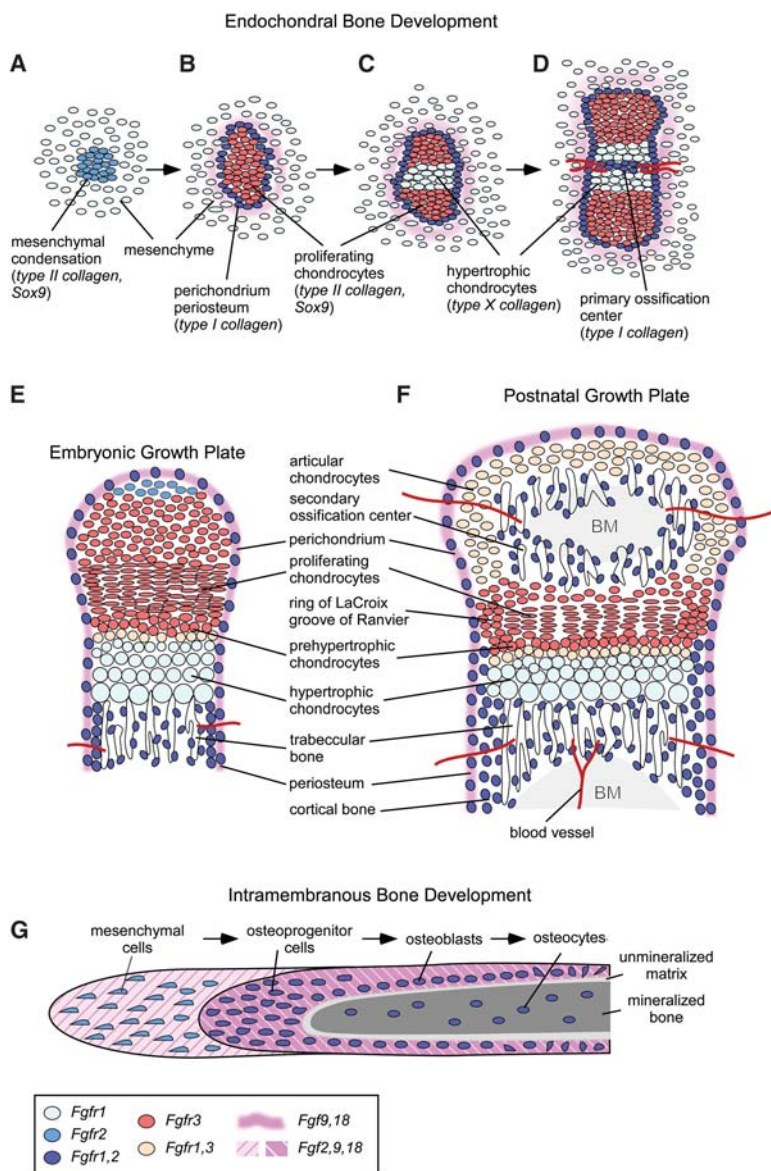


Figure 1. Fibroblast growth factor (FGF) and FGF receptor (FGFR) expression patterns during endochondral bone development. (A–D) Progression of endochondral bone development from the mesenchymal condensation to formation of the primary ossification center. (E) Embryonic growth plate. (F) Postnatal growth plate after formation of the secondary ossification center. (G) Developmental progression of intramembranous bone development. Cells and tissues are color-coded for expression domains of FGFs and FGFRs. (BM) Bone marrow.

Wnt and PTHLH signals regulates the level of calcium/calmodulin-dependent protein kinase II (CAMK2), which induces chondrocyte hypertrophy in part through increasing RUNX2 and β -catenin activity (Li et al. 2011; Long and Ornitz 2013). Chondrocyte hypertrophy leads to an expansive force that is required for bone elongation (Hunziker et al. 1987; Hunziker and Schenk 1989; Noonan et al. 1998). Hypertrophic chondrocytes can also begin to mineralize their extracellular matrix and have the capacity to differentiate into osteoblasts within the primary spongiosa (Yang et al. 2014; Zhou et al. 2014).

Endochondral bone formation

Mineralized bone originates with the establishment of a primary ossification center (Fig. 1D) that initiates with the differentiation of type I collagen-producing osteoblasts and the formation of a bone collar (the precursor

of cortical and trabecular bone) surrounding the central hypertrophic chondrocyte zone. Lineage tracing studies show that osteoprogenitor cells (Osterix lineage) in the perichondrium/periosteum give rise to trabecular osteoblasts, while more mature osteoblasts (Collagen I lineage) remain in the perichondrium and populate cortical bone (Maes et al. 2010). Vascular endothelial growth factor (VEGF-A), expressed in hypertrophic chondrocytes, supports the invasion of endothelial cells, osteoprogenitor cells, and osteoclasts, which remodel the chondrogenic matrix and organize to form the primary ossification center (Liu and Olsen 2014). Bidirectional expansion of the primary ossification center along the longitudinal axis replaces growth plate chondrocytes with trabecular bone (primary spongiosa). After formation of a secondary ossification center within the distal resting chondrocyte zone, the definitive growth plate can be identified by well-demarcated zones of cells (Fig. 1E,F). Toward the epiphysis

of a developing bone, slowly growing resting chondrocytes and cells within the adjacent groove of Ranvier and ring of LaCroix serve as a pool of progenitor cells that populate the rapidly dividing cells within the proliferating chondrocyte zone, which differentiate into prehypertrophic and then hypertrophic chondrocytes (Robinson et al. 1999; Abad et al. 2002; Karlsson et al. 2009). At the distal end of the epiphyseal growth plate (toward the midpoint of a long bone), hypertrophic chondrocytes either die by apoptosis or differentiate into trabecular osteoblasts (Yeung Tsang et al. 2014). Continued growth of the vascular network and influx of hematopoietic-derived cells, including osteoclasts, remodels the primary spongiosa to form the bone marrow cavity.

Intramembranous bone formation

During development, intramembranous ossification begins with the condensation of mesenchymal cells and their progressive differentiation into osteoblasts to form a mineralized matrix within ossification centers (Hall and Miyake 2000). In the skull, these centers expand during development but do not fuse at the junction with other cranial bones, allowing skull expansion during growth (Opperman 2000). The junction between cranial bones, called a suture, is responsible for the maintenance of separation between two membrane bones. Most cells surrounding the suture are mesenchymal cells, a minority of which differentiate into preosteoblasts and then into mature osteoblasts, which are found along the developing bone trabeculae. At the end of the bone formation period, osteoblasts die by apoptosis or become embedded in the matrix as osteocytes, which then eventually also undergo apoptosis (Fig. 1G; Rice et al. 1999; Fromiguet et al. 2005). The early commitment of mesenchymal stem cells into osteoblasts requires expression of *Runx2*, a master transcription factor that regulates several genes in osteoblasts, such as type I collagen, bone sialoprotein (BSP), osteopontin (OP), transforming growth factor (TGF β), and osteocalcin (OC) (Marie 2008). Transcription factors, such as *MSX2* and *DLX5*, play important roles in osteoblast differentiation during cranial bone formation in part by interacting with *RUNX2* (Dailey et al. 2005; Rice and Rice 2008). The regulation of intramembranous bone formation involves several regulatory factors, including TGF β s, BMPs, FGFs, and Wnt signaling, all of which were shown to regulate cell differentiation and survival in a spatiotemporal manner (Opperman 2000; Dailey et al. 2005; Lenton et al. 2005; Rice and Rice 2008).

FGF signaling

The canonical and endocrine branches of the mammalian FGF family comprise 18 secreted signaling proteins that bind to and activate four receptor tyrosine kinase molecules (FGF receptors [FGFRs]) (Ornitz and Itoh 2015). Fifteen canonical FGFs are differentially expressed in most tissues of the developing embryo, where they function as essential regulators of the earliest stages of devel-

opment and organogenesis. Canonical FGFs are also expressed in postnatal and adult tissues, where they regulate growth and function as factors for tissue maintenance, repair, and regeneration. Three endocrine FGFs have critical postnatal roles in the regulation of phosphate, bile acid, carbohydrate, and lipid metabolism.

Of the four tyrosine kinase FGFRs, FGFR1, FGFR2, and FGFR3 undergo alternative splicing to produce variants that have distinct ligand-binding specificities and that are differentially expressed in epithelial and mesenchymal lineages. In general, FGFRb splice variants are found in epithelial tissues and bind FGFs such as FGF7 and FGF10 that are expressed in mesenchymal tissues. FGFRc splice variants are found in mesenchymal tissues and bind FGF ligands that are expressed in both epithelial and mesenchymal lineages. A fifth nontyrosine kinase FGFR, FGFR5 or FGFR11, is homologous to FGFR1–4 in the extracellular ligand-binding domain but lacks an intracellular tyrosine kinase domain. *Fgfr11* was originally identified in human cartilage (Wiedemann and Trueb 2000).

The biological activity of canonical FGFs is enhanced by heparan sulfate (HS) and other sulfated polysaccharides (Shimokawa et al. 2011; Ornitz and Itoh 2015). A class of cell surface and extracellular matrix proteins primarily consisting of HS proteoglycans (HSPGs) contain HS covalently attached to a protein core. HSPGs include cell surface transmembrane proteins (syndecans), cell surface glycerophosphatidylinositol-anchored proteins (glypicans), and diffusible protein components of the extracellular matrix (perlecan and agrin) (Ornitz and Itoh 2015). Cell surface and extracellular matrix HSPGs affect diffusion of FGFs in tissues and interact with FGFs and FGFRs to increase the affinity of FGF–FGFR heterodimers to enhance receptor activation (Wu et al. 2003; Belov and Mohammadi 2013; Xu and Esko 2014). In contrast to canonical FGFs, endocrine FGFs require a protein cofactor to enhance receptor binding and activation. Members of the Klotho family were found to fulfill this role based on phenotypic similarities of *aKlotho* and *Fgf23* knockout mice (Kurosaki et al. 2006; Urakawa et al. 2006; Kuro-o 2008).

Binding of FGF ligands to FGFRs induces dimerization and juxtaposition of the tyrosine kinase domains to initiate the sequential transphosphorylation of at least six tyrosine residues (Furdui et al. 2006; Goetz and Mohammadi 2013; Ornitz and Itoh 2015). Activation of the FGFR tyrosine kinase domain allows the direct phosphorylation of the docked adaptor protein FGFR substrate 2 α (FRS2 α) and binding of other adaptor proteins, including phospholipase C γ (PLC γ), signal transducer and activator of transcription 1 (STAT1), STAT3, and STAT5 (Ornitz and Itoh 2015). In chondrocytes, FGFR3 has been found to specifically activate STAT1 (Su et al. 1997; Sahni et al. 1999). Phosphorylation of FRS2 α recruits the adaptor protein GRB2, which activates the MAPK pathway through SOS and the PI3K–AKT pathway through GAB1 (Kouhara et al. 1997; Lamothe et al. 2004). Downstream from RAS and PI3K, FGFs can activate several distinct MAPKs, including ERK1/2, JNK, and p38 (Tan et al. 1996; Tsang and Dawid 2004; House et al. 2005; Liao et al. 2007; Kanazawa et al. 2010). Binding of CRKL to the activated FGFR

enhances phosphorylation of FRS2 α , MAPK pathway signaling, and ERK1/2 activation (Moon et al. 2006).

The four known branches of the FGFR intracellular signaling cascades are also regulated by inhibitory molecules that include high-level expression of GRB2 (which interferes with PLC γ binding to the FGFR) and Sprouty (Spry) proteins (which interact with GRB2 to block MAPK and PI3K signaling) (Hanafusa et al. 2002; Timsah et al. 2014). Further downstream, SEF (similar expression to FGF) antagonizes the MAPK pathway through interaction with MEK (Torii et al. 2004), and DUSP6 (dual-specificity phosphatase 6) suppresses MAPK signaling through dephosphorylation of ERK1/2 (Camps et al. 1998). The E3 ubiquitin ligase CBL inhibits FGFR signaling by forming a ternary complex with phospho-FRS2 and GRB2, where it promotes the ubiquitination and degradation of the FGFR and FRS2 (Wong et al. 2002). CBL also interacts with PI3K, leading to its ubiquitination and degradation (Dufour et al. 2008). The adaptor protein Grb14 binds to FGFR phospho-Tyr766 and interferes with phosphorylation and activation of PLC γ (Browaeys-Poly et al. 2010).

FGF signaling during skeletal development

The endochondral mesenchymal condensation

Fgfs, *Fgfrs*, and their co-receptor, HS, are expressed in a time- and space-dependent manner during all stages of skeletal development (Fig. 1). At precondensation stages, distal limb bud mesenchyme expresses both *Fgfr1* and *Fgfr2* (Orr-Urtreger et al. 1991; Sheeba et al. 2010), whereas *Fgfr3* and *Fgfr4* are not detected in undifferentiated distal limb bud mesenchyme (Sheeba et al. 2010). Distal limb bud FGFRs respond to FGFs produced by the apical ectodermal ridge (AER) of the limb bud (Zeller et al. 2009). AER FGFs (primarily FGF4 and FGF8) signal to limb mesenchyme and are required for proximo-distal outgrowth of the limb bud. The function of mesenchymal FGFRs in the limb bud has been investigated through conditional gene inactivation using the Cre/LoxP system and by expressing inhibitory RNAs. Conditional inactivation of *Fgfr1* and *Fgfr2* in limb bud mesenchyme (using *Prx1-Cre*) or inactivation of *Fgfr1* in all mesenchyme [using *T(brachyury)-Cre*] results in severe skeletal hypoplasia (Verheyden et al. 2005; Yu and Ornitz 2008). In contrast, inactivation of *Fgfr1* or *Fgfr2* in distal limb bud mesenchyme (using *AP2-Cre*) results in considerably milder skeletal patterning phenotypes (Coumoul et al. 2005; Li et al. 2005). These experiments collectively demonstrate redundancy between FGFR1 and FGFR2 in distal limb mesenchyme.

In condensing mesenchyme (Fig. 1A) *Fgfr2* expression is increased (compared with the surrounding mesenchyme) and initially overlaps with domains of Sox9 expression (Orr-Urtreger et al. 1991; Peters et al. 1992; Szebenyi et al. 1995; Delezoide et al. 1998; Eswarakumar et al. 2002; Sheeba et al. 2010). *Fgfr1* is more uniformly expressed throughout limb bud mesenchyme. Although *Fgfr3* and *Fgfr4* are excluded from distal limb bud mesen-

chyme, these *Fgfrs* are expressed in more proximal locations in tissues corresponding to developing muscle (Orr-Urtreger et al. 1991; Peters et al. 1992; Szebenyi et al. 1995; Delezoide et al. 1998; Sheeba et al. 2010). Cells in the periphery of the condensation that will form perichondrium and periosteum express both *Fgfr1* and *Fgfr2* (Fig. 1B–D; Delezoide et al. 1998; Eswarakumar et al. 2002). As soon as condensed mesenchyme begins to differentiate into chondrocytes, *Fgfr3* expression is activated (Fig. 1B) along with Sox9 and type II collagen, and *Fgfr2* expression is decreased (Peters et al. 1992, 1993; Purcell et al. 2009). As central chondrocytes begin to hypertrophy, *Fgfr3* expression is decreased, and *Fgfr1* expression is increased (Fig. 1C; Peters et al. 1992, 1993; Deng et al. 1996; Naski et al. 1998; Jacob et al. 2006; Karolak et al. 2015).

The initiation of the mesenchymal condensation and its differentiation to the chondrogenic lineage are at least partially dependent on FGFR signaling (Murakami et al. 2000; Mariani et al. 2008; Yu and Ornitz 2008; Kumar and Lassar 2014). In primary chondrocytes and undifferentiated mesenchymal cell lines, FGF signaling increases Sox9 expression (Murakami et al. 2000; Shung et al. 2012). FGF activation of ERK1/2 also maintains competence of limb bud mesenchyme to differentiate into chondrocytes by blocking Wnt-induced methylation and silencing of the Sox9 promoter (Ten Berge et al. 2008; Kumar and Lassar 2014). *Fgfr3* expression in proliferating chondrocytes requires Sox9, and Sox9-binding sites are found in the *Fgfr3* gene (Oh et al. 2014). FGFR3 signaling is mitogenic for immature proliferating chondrocytes and is likely activated by FGF9 and FGF18, which are expressed in adjacent mesenchyme (Fig. 1; Iwata et al. 2000, 2001; Liu et al. 2002, 2007; Ohbayashi et al. 2002; Hung et al. 2007; Havens et al. 2008).

The forming perichondrium, bone collar, and trabecular bone express FGFR1 in mesenchymal progenitors and FGFR2 in differentiating osteoblasts (Fig. 1E,F; Molteni et al. 1999b; Britto et al. 2001; Ohbayashi et al. 2002; Jacob et al. 2006; Coutu et al. 2011). FGFR3 is expressed more intensely in chondroprogenitor cells located in the groove of Ranvier and ring of LaCroix (Robinson et al. 1999), and FGFR1 and FGFR3 are expressed in mouse and human articular chondrocytes (Fig. 1F; Yan et al. 2011; Weng et al. 2012). Immature cultured osteoblasts in vitro expressed relatively higher levels of *Fgfr1*, whereas mature osteoblasts express relatively higher levels of *Fgfr2* (Rice et al. 2003). Differential expression of these *Fgfrs* may reflect a distinct response to exogenous FGFs such as FGF2 (Cowan et al. 2003).

Intramembranous mesenchymal condensations

The spatiotemporal pattern of expression of *Fgfs* and *Fgfrs* that control FGF signaling during membranous bone formation is reviewed in Ornitz and Marie (2002). *Fgf2*, *Fgf4*, *Fgf9*, *Fgf18*, *Fgfr1*, *Fgfr2*, and *Fgfr3* are expressed throughout the early stages of intramembranous bone development (Fig. 1G; Kim et al. 1998; Rice et al. 2000; Britto et al. 2001; Liu et al. 2002; Ohbayashi et al. 2002;

Quarto et al. 2009). The FGFR2c splice variant is expressed in early mesenchymal condensations and then in sites of intramembranous ossification, where it interacts with FGF18 (Eswarakumar et al. 2002). At later stages during cranial bone development, *Fgf18* is expressed in mesenchymal cells and differentiating osteoblasts (Ohbayashi et al. 2002; Reinhold and Naski 2007). *Fgf9* is expressed throughout calvarial mesenchyme during mid to late stages of embryonic development (Kim et al. 1998). Mice lacking both *Fgf9* and *Fgf18* have severely deficient cranial bone formation (IH Hung, CG Schoenwolf, M Lewandoski, and DM Ornitz, in prep.). *Fgfr1* and *Fgfr2*, which are expressed in preosteoblasts and osteoblasts (Rice et al. 2003), are likely receptors for FGF9 and FGF18 in developing membranous bone. (Ornitz and Marie 2002)

The growth plate

Growth plate chondrocytes express very low levels of *Fgfr2* in the resting zone, high levels of *Fgfr3* in the proliferating and prehypertrophic zone, and high levels of *Fgfr1* in hypertrophic chondrocytes (Fig. 1E,F; Peters et al. 1993; Delezoide et al. 1998; Hamada et al. 1999; Eswarakumar et al. 2002; Ornitz and Marie 2002; Yu et al. 2003; Jacob et al. 2006; Lazarus et al. 2007; Karolak et al. 2015). To investigate the potential functions of FGFR2 in skeletal development, the *Fgfr2* gene was conditionally inactivated in limb bud mesenchyme with *Dermo1(Twist2)-Cre* (Yu et al. 2003). Although these mice showed decreased postnatal growth, chondrocyte proliferation and the length of the proliferating chondrocyte zone were not affected, suggesting that FGFR2 does not have a nonredundant function in resting or proliferating chondrocytes. The reduced stature of these mice was attributed to decreased

osteogenesis and increased osteoclast activity at the chondro-osseous junction (Yu et al. 2003).

In early embryonic stages of skeletal development, during establishment of the growth plate, FGFR3 has promitogenic activity in chondrocytes (Iwata et al. 2000, 2001; Havens et al. 2008). However, following formation of the secondary ossification center and throughout postnatal skeletal growth, FGFR3 signaling functions to inhibit chondrogenesis, primarily acting on proliferating chondrocytes and their differentiation to prehypertrophic and hypertrophic chondrocytes (Colvin et al. 1996; Deng et al. 1996). This paradoxical activity of FGFR3 underlies the etiology of chondrodysplastic disorders in which gain-of-function mutations in FGFR3 cause decreased proliferation and differentiation of proliferating chondrocytes during prepubertal skeletal growth, resulting in skeletal dwarfism (Naski et al. 1996, 1998). The mechanisms that regulate FGFR3 expression and signaling during prepubertal skeletal growth are an ongoing and important topic of research. In addition to providing an understanding of the mechanisms regulating bone growth, these studies are providing potential therapeutic avenues for the treatment of achondroplasia and other forms of skeletal dwarfism.

FGFR3 signaling in growth plate chondrocytes Signaling through FGFR3 in growth plate chondrocytes activates STAT1, ERK1/2, and p38 intracellular signals; increases expression of *Snail1* (*Snail1*); down-regulates AKT; activates protein phosphatase 2a (PP2a); and leads to dephosphorylation (activation) of the retinoblastoma family members p107 and p130 (Fig. 2; Su et al. 1997; Laplantine et al. 2002; Raucci et al. 2004; Priore et al. 2006; de Frutos et al. 2007; Kolupaeva et al. 2008; Kurimchak et al. 2013). Ultimately, suppression of chondrocyte proliferation

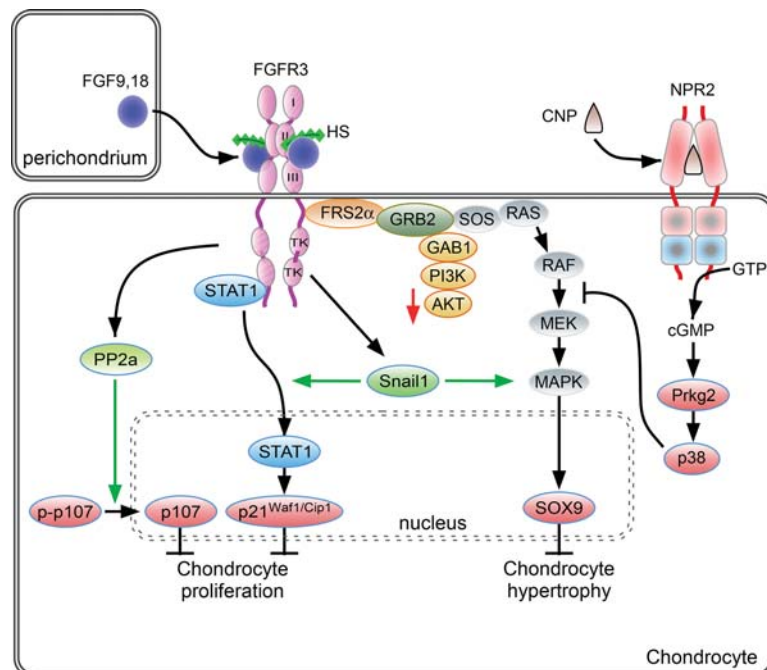


Figure 2. FGF signaling pathways in proliferating chondrocytes. During endochondral bone development, FGF9 and FGF18, derived from the perichondrium and surrounding tissue, signal to chondrocytes. Activity is mediated in part by regulated diffusion through the extracellular matrix through affinity for HS and potentially other sulfated glycosaminoglycans. Activation of FGFR3 in proliferating chondrocytes activates the STAT1 and MAPK signaling pathways. FGFR3 signaling results in increased expression of *Snail1*, which in turn is required for STAT1 and MAPK signaling. FGFR3 signaling can also activate PP2a. Activation of downstream signals, p107, p21^{Waf1/Cip1}, and Sox9 regulates chondrocyte proliferation and differentiation to hypertrophic chondrocytes. C-type natriuretic peptide (CNP) signals through the natriuretic peptide receptor 2 (NPR2), a guanylyl cyclase. cGMP activates cyclic GMP-dependent protein kinase II (cGKII), which, through p38 MAPK activation, functionally antagonizes RAF1 activation of MEK. See the text for more details.

resulting from activation of FGFR3 is effected through increased expression of the cell cycle inhibitor p21^{Waf1/Cip1} and activation of p107 (and p130) (Cobrinik et al. 1996; Su et al. 1997; Aikawa et al. 2001; Laplantine et al. 2002; Dailey et al. 2003; Legeai-Mallet et al. 2004; Kolupaeva et al. 2008, 2013).

SNAIL1 is integral to the FGFR3 signaling pathway in chondrocytes (Fig. 2). *Snail1* expression is induced by FGFR3, and *Snail1* is expressed at high levels in human thantophoric dysplasia bone tissue. Experimentally, ectopic activation of SNAIL1 in mice resulted in an achondroplasia phenotype with decreased chondrocyte proliferation and shortened bones at late embryonic stages (de Frutos et al. 2007). Functionally, SNAIL1 appears to activate both the STAT1 and MAPK branches of the FGFR3 signaling pathway, as ectopic activation of SNAIL1 resulted in nuclear translocation of STAT1 and increased phosphorylation of ERK1/2 (de Frutos et al. 2007). Although the mechanism by which SNAIL1 regulates STAT1 and MAPK is not known, a recent report showed that SNAIL1 may regulate the nuclear localization of p-ERK (Smith et al. 2014). Conversely, activated ERK2 directly phosphorylates SNAIL1, leading to nuclear accumulation and increased protein stability (Zhang et al. 2013). In support of a requirement for SNAIL proteins in regulating chondrogenesis, conditional inactivation of both *Snail1* and *Snail2* resulted in increased p21^{Waf1/Cip1} and decreased chondrocyte proliferation (Chen and Gridley 2013).

Decreased bone growth, caused by activating mutations in FGFR3, results from decreased chondrocyte proliferation and decreased hypertrophy. The underlying mechanisms suggest that these phenotypes are distinct and are regulated by different branches of the FGFR3 signaling pathway. Evidence that the STAT1 branch of the pathway regulates chondrocyte proliferation is derived from in vivo rescue experiments in which chondrocyte proliferation defects in mice with activating mutations in FGFR3 can be rescued by biallelic inactivation of *Stat1* (Murakami et al. 2004). However, in this model, inactivation of *Stat1* still does not rescue the overall achondroplasia phenotype in which impaired chondrocyte hypertrophy leads to decreased bone growth. Further studies showed that, downstream from FGFR3, activation of MAPK signaling suppressed chondrocyte hypertrophy, resulting in skeletal dwarfism (Murakami et al. 2004). The mechanism may involve suppression of SOX9 down-regulation in prehypertrophic chondrocytes by activated FGFR3 signaling (Fig. 2; Murakami et al. 2000; Shung et al. 2012; Zhou et al. 2015). Additionally, activation of the PP2A-B55a holoenzyme by FGFR3, which dephosphorylates and activates p107, may independently contribute to decreased chondrocyte hypertrophy (Fig. 2; Kolupaeva et al. 2013; Kurimchak et al. 2013).

Further support of the model in which STAT1 activation suppresses chondrocyte proliferation and MAPK signaling regulates matrix production and chondrocyte differentiation is derived from studies that examined interactions between FGFR3 and the C-type natriuretic peptide (CNP) signaling pathways (Fig. 2; Yasoda et al. 2004). CNP expression was found to attenuate the phenotype of

achondroplasia mice through inhibition of MAPK signaling, which restored matrix production and hypertrophic differentiation (see below). The activity of CNP was notably independent of STAT1 activity and did not affect chondrocyte proliferation.

The function of the STAT1 and MAPK branches of the FGFR3 signal pathway may not be completely distinct. In cultured chondrocytes, FGFR3 suppression of chondrocyte proliferation required MAPK signaling and was independent of STAT signaling (Raucci et al. 2004; Krejci et al. 2008a). In support of this observation, mice lacking ERK1 and conditionally lacking ERK2 (*Col2a1-Cre*) in chondrocytes showed increased chondrocyte proliferation at late embryonic stages (Matsushita et al. 2009a; Sebastian et al. 2011). Some of the variability in experimental results may be due to in vitro culture conditions, the developmental stage being examined (embryonic vs. postnatal), and potential indirect effects on proliferating chondrocytes resulting from interactions with other signaling pathways in nearby cells within the groove of Ranvier, ring of LaCroix, and perichondrium.

Indirect mechanisms by which FGFR3 signaling may regulate growth plate development include the regulation of BMP, Wnt, IHH, and PTHLH/PTH1R expression and activity. Comparisons of activating mutations in *Fgfr3* and mice lacking *Fgfr3* (*Fgfr3*^{-/-}) show that FGFR3 signaling suppresses expression of *Bmp4*, *BMPR1a*, *Ihh*, and *Pth1r* in postnatal growth plate chondrocytes (Naski et al. 1998; Chen et al. 2001; Qi et al. 2014). Additionally, in a chondrocyte cell line, overexpression of FGFR3 suppresses expression of both *Pthlh* and its receptor, *Pth1r* (Li et al. 2010), and, in chondrocyte cell lines and micro-mass cultures, FGF signaling activates Wnt/ β -catenin signaling through phosphorylation of LRP6 and functions to suppress hypertrophic differentiation (Krejci et al. 2012; Buchtova et al. 2015).

Regulation of Fgfr3 expression Regulation of FGFR3 expression and activation is essential for chondrogenesis. In cultured cells, thyroid hormone has been shown to inhibit chondrocyte proliferation and promote chondrocyte hypertrophy, activities that are similar to that of signaling through FGFR3. Consistent with this observation, treatment of cultured chondrocytes with thyroid hormone increased *Fgfr3* expression, and mice lacking thyroid hormone receptor had decreased levels of *Fgfr3* in growth plate chondrocytes (Barnard et al. 2005). PTHLH may also regulate FGFR3 expression. Treatment of thantophoric dysplasia mice with intermittent PTH injection partially rescued the lethality and skeletal growth phenotype. In cultured cells, PTH treatment inhibited phosphorylation of FGFR3, suggesting that PTH may function in part through regulation of FGFR3 signaling (Xie et al. 2012). Additionally, analysis of the *Fgfr3* promoter identified a transcriptional regulatory element, CSRh, which was repressed by PTH through a cAMP and protein kinase A (PKA)-dependent mechanism (McEwen et al. 1999). A third mechanism that could regulate FGFR3 is hypoxia, a characteristic feature of growth plate chondrocytes. Although this has not been investigated in chondrocytes,

in bladder cancer cells, *Fgfr3* expression was induced by hypoxia in a transcriptional and HIF-1 α -dependent manner (Blick et al. 2013).

FGFR1 signaling in hypertrophic chondrocytes *Fgfr1* is prominently expressed in prehypertrophic and hypertrophic chondrocytes (Fig. 1) and overlaps with *Fgfr3* in prehypertrophic chondrocytes (Fig. 1). The functions of FGFR1 signaling in hypertrophic chondrocytes are not entirely understood due to imprecise genetic tools and difficulties in distinguishing cell-autonomous functions in hypertrophic chondrocytes from activities in the adjacent perichondrium and other tissues. Activating mutations in *FGFR1* that cause osteoglyphonic dysplasia result in severe dwarfism in humans (White et al. 2005); however, an activating mutation in *Fgfr1* in mice did not affect endochondral ossification (Zhou et al. 2000). Possible functions of FGFR1 signaling have been investigated in mice through targeted inactivation of *Fgfr1* (*Col2a1-Cre* or *Dermo1-Cre*). At early stages of development, *Fgfr1* conditional knockout mice (*Dermo1-Cre*) show impaired chondrocyte hypertrophy (Hung et al. 2007). In contrast, at later stages of development, *Fgfr1* conditional knockout mice (*Col2a1-Cre*) have an expanded hypertrophic chondrocyte zone (Jacob et al. 2006). Both *Dermo1-Cre* and *Col2a1-Cre* efficiently target chondrocytes but also target the osteoblast lineage (Yu et al. 2003; Jacob et al. 2006; Ford-Hutchinson et al. 2007; Wang et al. 2011; Ono et al. 2014). Therefore, defects in osteoblast development could indirectly affect chondrogenesis (Jacob et al. 2006; Karolak et al. 2015), and a definitive assessment of FGFR1 function in hypertrophic chondrocytes will thus require more precise targeting of the chondrocyte lineage.

FGF ligands in endochondral bone growth *Fgf9* and *Fgf18* are expressed in the perichondrium and periosteum, and *Fgf2* is expressed in chondrocytes (Gonzalez et al. 1996; Liu et al. 2002; Ohbayashi et al. 2002; Hung et al. 2007; Reinhold and Naski 2007). Mice lacking *Fgf2* have decreased bone mass but no apparent change in growth plate structure or function (Montero et al. 2000). In contrast, mice lacking *Fgf9* exhibit rhizomelic (affecting proximal skeletal elements) shortening of the appendicular skeleton. FGF9 and FGF18 also have stage-specific effects on chondrogenesis. At early stages of development of the humerus and femur, chondrocyte proliferation is decreased, and chondrocyte hypertrophy is impaired in *Fgf9*^{-/-} mice (Hung et al. 2007). Mice lacking *Fgf18* show decreased chondrocyte proliferation in more distal skeletal elements (Liu et al. 2007). This phenotype is consistent with FGF9 and FGF18 signaling to FGFR3 and the promotogenic properties of FGFR3 on immature chondrocytes (Iwata et al. 2000, 2001; Hung et al. 2007). At later stages of development, *Fgf9*^{-/-} and *Fgf18*^{-/-} mice show an expansion of the femoral hypertrophic chondrocyte zone, a phenotype that is consistent with FGFR3 functioning to suppress chondrocyte proliferation and differentiation in the mature growth plate (Liu et al. 2002; Ohbayashi et al. 2002; Hung et al. 2007). In contrast to mice lacking *Fgf9*, mice lacking *Fgf18* (*Fgf18*^{-/-}) have defects in chondrogenesis

that affect both proximal and distal skeletal elements. *Fgf18*^{-/-} mice also have defects in osteoblast proliferation and differentiation that suggest signaling to FGFR1 and FGFR2 in osteoprogenitor cells (discussed below). Mice lacking both *Fgf9* and *Fgf18* have a severe osteochondrodysplasia that affects proximal and distal skeletal elements (IH Hung, CG Schoenwolf, M Lewandoski, and DM Ornitz, in prep.).

Autosomal dominant mutations in *FGF9* in both mice and humans affect chondrogenesis and formation of joints. The elbow knee synostosis (EKS) mutation in mice and the multiple synostosis syndrome in humans reduce the affinity of FGF9 for its coreceptor, HS. Although reduced affinity for HS impairs receptor binding, the resulting increased diffusion of FGF9 through the extracellular matrix effectively increases its domain of activity in developing chondrocytes and surrounding tissue (Harada et al. 2009; Kalinina et al. 2009; Wu et al. 2009). The joint fusion phenotype is very similar to mice that cell-autonomously overexpress an activated FGFR in developing chondrocytes (Wang et al. 2001).

Cortical, trabecular, and intramembranous bone

FGFR signaling in osteoblasts In cells of the osteoblast lineage, FGFR-mediated activation of ERK1/2, PLC γ /PKC α , and PI3K/Akt signaling results in the modulation of cell proliferation, differentiation, and apoptosis, depending on the stage of cell differentiation (Fig. 3; Dailey et al. 2005; Marie et al. 2005). In early osteoblast precursor cells, activation of ERK1/2 by FGFR signaling primarily leads to increased cell proliferation (Choi et al. 2008; Miraoui et al. 2009). In more mature cells, ERK1/2 activation by FGF2 enhances acetylation and stabilization of *RUNX2*, a key transcription factor involved in osteoblastogenesis and bone formation (Xiao et al. 2002; Park et al. 2010; Yoon et al. 2014). ERK1/2 signaling is also involved in *Runx2* expression and osteoblast differentiation induced by FGF18-mediated activation of FGFR1/FGFR2 in murine osteoblast precursor cells (Hamidouche et al. 2010). PKC δ activation also plays a central role in FGF/FGFR-stimulated expression and transactivation activity of *RUNX2* (Kim et al. 2003; Niger et al. 2013). In turn, *RUNX2* enhances the expression of *Fgfr2*, *Fgf18*, and proteoglycans that are involved in FGF signaling in the perichondrium (Hinoi et al. 2006; Reinhold and Naski 2007; Teplyuk et al. 2009). Finally, in an osteocyte cell line, FGF2-stimulated ERK1/2 activation was found to increase the expression of *Dmp1*, a marker of osteocytes. In vivo, *Dmp1* expression is reduced in limbs of mice lacking ERK1 and conditionally lacking ERK2 (*Prx1-Cre*), which show no osteocytes, indicating that FGF signaling coordinately regulates *Dmp1* expression and osteocyte differentiation (Kyono et al. 2012). Overall, these studies establish that multiple pathways activated by FGF/FGFR signaling control all steps of osteoblastogenesis (Fig. 3).

FGFR signaling is down-regulated by negative feedback mechanisms involving receptor internalization and degradation (Schlessinger 2000; Lemmon and Schlessinger

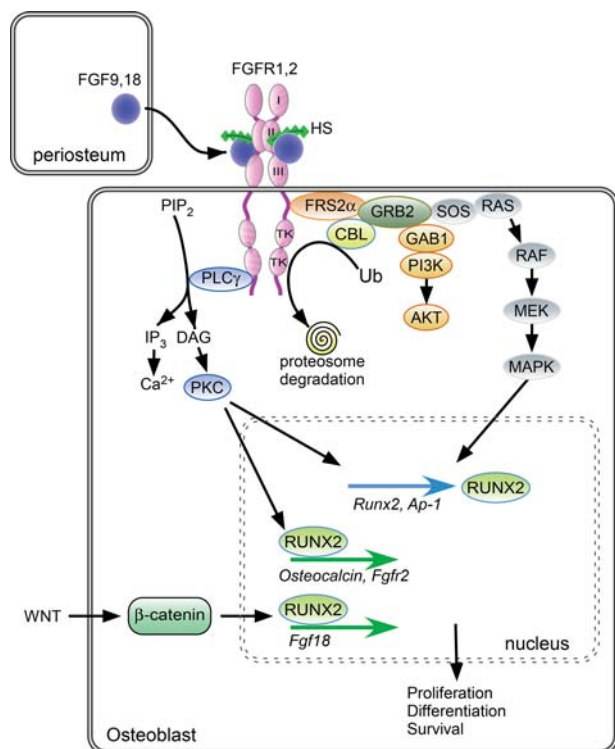


Figure 3. FGF/FGFR signaling in cells of the osteoblast lineage. FGF9 and FGF18, expressed in periosteal and surrounding tissue, interact with HS, FGFR1, and FGFR2. Activation of the FGFR tyrosine kinase domain leads to the recruitment of various substrates and activation of phospholipase C γ /PKC α , ERK MAPKs, and PI3K/AKT, resulting in the modulation of transcription factors that control cell proliferation, differentiation, and survival in cells of the osteoblast lineage. Following FGFR activation, CBL is recruited and mediates FGFR ubiquitination and proteasome degradation, leading to FGFR down-regulation. Activated FGFRs may also be retained in intracellular compartments and degraded following c-Cbl-dependent ubiquitination. FGFR signaling activates expression of the transcription factor RUNX2, which regulates the expression of osteoblast-specific genes. In cooperation with Wnt/ β -catenin signaling, RUNX2 activates *Fgf18* gene expression. See the text for more details.

2010). This down-regulation process involves FGFR interaction with multiple proteins, including the docking protein FRS2 α and the ubiquitin ligase c-CBL, an adaptor protein that mediates FGFR ubiquitination after ligand binding (Schmidt and Dikic 2005). In response to FGF stimulation, tyrosine phosphorylation of FRS2 α forms a ternary complex with CBL and GRB2 (Schlessinger 2003). Increased binding of activated FGFR2 to FRS2 α and c-CBL results in ubiquitination of the FGFR and its subsequent degradation by the proteasome (Schlessinger 2003). Depending on the cell type, a proportion of the constitutively activated FGFRs may be retained in intracellular compartments and degraded following c-Cbl-dependent ubiquitination (Hatch et al. 2006). In osteoblasts, CBL plays an important role in down-regulation of activated FGFR signaling and subsequently in the control of osteoblastogenesis (Severe et al. 2013). FGFR2 ubiquitination

by c-CBL leads to FGFR2 down-regulation and subsequent reduction in osteogenic differentiation capacity, whereas inhibition of c-CBL interaction with FGFR2 promotes osteoblast differentiation and osteogenic differentiation through increased ERK1/2 and PI3K signaling (Dufour et al. 2008). This highlights the essential role of c-CBL-mediated regulation of FGFR signaling in osteoblastogenesis (Severe et al. 2013).

Interactions with other pathways In addition to cell signaling directly generated by activated FGF/FGFRs, FGF interacts with other pathways, such as BMP signaling, to regulate osteoblastogenesis. FGF2 treatment stimulates *Bmp2* gene expression in osteoblasts during cranial bone ossification (Choi et al. 2005; Fakhry et al. 2005). Consistently, BMP2 expression is reduced in bones of *Fgf2*-null mice (Naganawa et al. 2008). In line with this concept, BMP2 and FGF2 have a synergistic stimulatory effect on osteogenic function in human bone cells and in mice (Nakamura et al. 2005; Kuhn et al. 2013). Mechanistically, FGF2 enhances canonical BMP2 signaling in osteoblasts through promoting nuclear localization of RUNX2 and phospho-SMAD1/5/8 (Agas et al. 2013). Moreover, FGF2 isoforms specifically modulate BMP2 function and osteoblast differentiation (Sabbieti et al. 2013), and FGF2, FGF9, and FGF18 inhibit the expression of the BMP antagonist *Noggin* in vitro and in vivo (Warren et al. 2003; Reinhold et al. 2004; Fakhry et al. 2005). Thus, multiple mechanisms mediate FGF and BMP signaling interactions, leading to regulation of osteoblast differentiation.

Genetic and functional evidence indicates that FGF signaling functionally interacts with Wnt/ β -catenin signaling to control mesenchymal stem cell fate and differentiation (Dailey et al. 2005; Miraoui and Marie 2010). During cranial suture development, WNT/ β -catenin signaling controls stem cell renewal, proliferation, and lineage specification by balancing the activity of the FGF and BMP signaling pathways (Maruyama et al. 2010). Mechanistically, WNT/ β -catenin signaling induces *Fgf18* expression through induction of a complex with RUNX2 and TCF/LEF transcription factors on the *Fgf18* promoter (Fig. 3; Reinhold and Naski 2007). In osteoprogenitor cells, FGF2 was found to antagonize WNT/ β -catenin signaling (Mansukhani et al. 2005; Ambrosetti et al. 2008). In vivo, however, Wnt genes are decreased significantly in osteoblasts from *Fgf2*^{-/-} mice, which display reduced osteoblast differentiation (Fei et al. 2011). Consistent with this, exogenous FGF2 can promote β -catenin nuclear accumulation and rescue osteoblast differentiation in *Fgf2*^{-/-} mice, indicating that endogenous FGF2 stimulates cell differentiation in more mature osteoblasts in part by activating WNT/ β -catenin signaling (Fei et al. 2011).

FGF signaling also interacts with the PTH signaling pathway as PTH stimulates *Fgf2*, *Fgfr1*, and *Fgfr2* in osteoblast cells (Hurley et al. 1999). Consistently, the anabolic response to intermittent PTH is impaired in *Fgf2*-null or heterozygous mice (Hurley et al. 1999). Functional studies confirmed that endogenous FGF2 is required for the positive PTH effects on osteoblast proliferation, differentiation, and survival, suggesting that the anabolic PTH

effect is dependent in part on FGF2 expression (Sabbieti et al. 2009). These genetic and functional analyses support the concept that FGF/FGFR signaling by itself and its interactions with other major signaling pathways play essential roles in controlling osteoblastogenesis and bone formation in adult life.

FGF and bone homeostasis

FGF/FGFR signaling in osteoblastogenesis

Bone formation is a complex process that involves the commitment of mesenchymal cells that progressively differentiate into osteoblast precursor cells and then into mature bone-forming cells under the control of transcription factors, systemic hormones, and local growth factors as well as cell–cell and cell–matrix interactions (Lian et al. 2006; Marie 2008; Long 2012). While it is well accepted that FGF/FGFR signaling plays an important role in osteoblastogenesis, it is now clear that the effects in osteogenic cells are complex, as they depend on the type of FGFs and FGFRs expressed, the stage of cell maturation, and the microenvironment (proteoglycans and interacting proteins) that may either enhance or attenuate FGF/FGFR signaling in bone cells. The collective data suggest that FGF2 signaling increases cell proliferation in immature osteoblasts and thereby expands the pool of osteoblast precursor cells that then fully differentiate in the bone environment (Debiais et al. 1998; Shimoaka et al. 2002; Ignelzi et al. 2003; Fakhry et al. 2005). Accordingly, blocking FGF2 biological activity reduces osteogenesis in vivo (Moore et al. 2002). Recent data indicate that FGF18 is also an essential autocrine-positive regulator of the osteogenic differentiation program (Jeon et al. 2012), mediated by FGFR1/FGFR2 activation of ERK1/2 and PI3K signaling (Hamidouche et al. 2010). Genetic studies in mice also demonstrated that both FGF2 and FGF18 are important regulators of skeletal cell proliferation and differentiation during osteogenesis. Mice lacking *Fgf2* display reduced bone formation due to decreased osteoblast differentiation (Montero et al. 2000; Xiao et al. 2010). *Fgf18*-deficient mice show decreased osteogenic mesenchymal cell proliferation, reduced osteoblast differentiation, and delayed ossification (Liu et al. 2002; Ohbayashi et al. 2002). In addition to controlling osteoblast proliferation and differentiation, FGF signaling regulates osteoblast apoptosis. In osteoblast precursor cells, FGF2 induces osteoblast survival through activation of PI3K/AKT signaling (Debiais et al. 1998) and FGFR1-mediated increased Bcl2/Bax ratio (Agas et al. 2008). In more differentiated osteoblasts, FGF treatment or overexpression of FGF2 in transgenic mice induces apoptosis in mouse calvaria (Mansukhani et al. 2000; Ignelzi et al. 2003), which limits the increase in the osteoblast population.

FGF regulation of bone metabolism Recent data indicate that some isoforms of FGF2 have distinct effects on bone metabolism. Overexpression of the low-molecular-weight (18-kDa) isoform of FGF2 in osteoblasts causes increased trabecular and cortical bone mass resulting from in-

creased bone formation, whereas its inactivation results in opposite effects, indicating that low-molecular-weight FGF2 is a critical determinant of bone mass in mice (Xiao et al. 2009). Mechanistic analysis showed that these effects are related to modulation of the WNT/ β -catenin signaling pathway (Xiao et al. 2009). In contrast, overexpression of nuclear-localized high-molecular-weight FGF2 isoforms in osteoblasts causes a phenotype similar to X-linked hypophosphatemia (XLH), a disease characterized by increased expression of FGF23 by osteocytes.

FGF23 is a phosphaturic agent, and its overexpression results in phosphate wasting, hypophosphatemia, and rickets/osteomalacia. High-molecular-weight FGF2 overexpression in osteoblast precursor cells results in the inhibition of osteoblast differentiation and matrix mineralization through FGF23/FGFR/MAPK signaling, independently of phosphate wasting (Xiao et al. 2013). Conversely, genetic inactivation of the FGF2 high-molecular-weight isoforms caused increased bone mass due to increased osteoblast differentiation and decreased osteoclast activity associated with decreased *FGF23* expression, indicating a negative impact of high-molecular-weight FGF2 isoforms on bone cell metabolism and phosphate homeostasis (Homer-Bouthiette et al. 2014). These findings highlight the functional implications of distinct FGF2 isoforms on FGF23 secretion, bone cell activity, and bone matrix mineralization.

Functionally, the expression of FGF23 in osteoblasts and osteocytes is controlled by FGFR1 signaling (Martin et al. 2011), which in turn controls phosphate homeostasis and bone mineralization (Quarles 2012; Feng et al. 2013). Low-molecular-weight FGF2 stimulates FGF23 promoter activity through PLC γ /NFAT and MAPK signaling, whereas high-molecular-weight FGF2 promotes FGF23 promoter activity through cAMP-dependent binding of FGFR1 and CREB to a conserved cAMP response element in the FGF23 promoter (Han et al. 2015). Consistently, conditional deletion of *Fgfr1* in osteocytes of Hyp mice, a mouse model of XLH, reduced the excessive FGF23 levels produced by osteocytes and partially rescued the bone phenotype in these mice (Xiao et al. 2014). Moreover, pharmacological activation of FGFR1 in osteoblasts leads to increased FGF23 secretion and hypophosphatemia in adult mice (Wu et al. 2013), whereas pharmacological FGFR inhibition ameliorates FGF23-mediated hypophosphatemic rickets (Wohrle et al. 2013). These data support an important role for FGF23/FGFR1 signaling in the control of bone mass and mineralization in vivo. The role of FGF23 signaling in nonskeletal tissues and on phosphate metabolism has been extensively reviewed (Quarles 2012; Liao 2013; White et al. 2014).

Stage-specific activity of FGFs in osteoblastogenesis FGFR expression and function control cells of the osteoblast lineage at different stages of differentiation. In mesenchymal osteoprogenitor cells, FGFR1/2 are important for maintaining mesenchymal stem cell stemness by inhibiting cell senescence (Coutu et al. 2011; Di Maggio et al. 2012). During the osteogenic differentiation process, initially high *Fgfr1/Fgfr2* expression is associated with

FGF2 responsiveness and a robust proliferative response (Cowan et al. 2003; Haupt et al. 2009). In more mature osteoblasts, decreased expression of *Fgfr1* and increased expression of *Fgfr2*, *Fgfr3*, and *Fgfr4* are associated with an inability to sustain an FGF2 response (Haupt et al. 2009). In vivo studies confirmed that FGFR1 controls osteoblasts at different stages of maturation (Jacob et al. 2006). Conditional inactivation of *Fgfr1* in osteoprogenitor cells results in increased proliferation and delayed differentiation and matrix mineralization, whereas *Fgfr1* deficiency in differentiated osteoblasts caused enhanced mineralization and increased expression of *Fgfr3* (Jacob et al. 2006). FGFR2 also plays an important role in osteoblastogenesis. In mesenchymal cells, constitutive *Fgfr2* activation results in increased osteoblast differentiation and decreased adipocyte differentiation through ERK1/2 and PKC α signaling, resulting in increased *Runx2* expression (Miraoui et al. 2009, 2010b). Consistently, mice conditionally lacking *Fgfr2* or harboring a mutation in *Fgfr2c*, the mesenchymal splice variant of *Fgfr2*, show decreased *Runx2* expression, retarded ossification, and decreased bone mineral density, suggesting that *Fgfr2* is a positive regulator of osteoblast maturation (Eswarakumar et al. 2002; Yu et al. 2003). Although FGFR3 is mainly expressed in chondrocytes, young adult *Fgfr3*-null mice are osteopenic due to reduced osteoblast maturation and defective trabecular bone mineralization, suggesting a role for FGFR3 in postnatal bone metabolism (Valverde-Franco et al. 2004). Osteoblasts derived from *Stat1*^{-/-} mice have decreased expression of *Fgfr3* and increased expression of *Fgf18*, which acts through FGFR1 or FGFR2, suggesting that changes in the repertoire of *Fgfr* expression affects feedback loops that control FGF-dependent osteoblastogenesis (Xiao et al. 2004).

FGFs bind cell surface HSPGs, which serve as low-affinity coreceptors that enhance FGF-FGFR binding and signaling (Eswarakumar et al. 2005; Ornitz and Itoh 2015). Early studies showed that some HSPGs, such as syndecans, expressed in the bone environment affect the osteoblast response to FGF2 during osteogenesis (Molteni et al. 1999a). In vitro data indicate that both cell surface and secreted HSPGs can further amplify FGF2 signaling and osteoblast differentiation (Jackson et al. 2007). Notably, the HSPG glypican-3 was found to be essential for *Runx2* expression and subsequent osteoblast differentiation in vitro (Haupt et al. 2009). Since RUNX2 was found to enhance the expression of FGFRs and proteoglycans such as syndecans in osteoblasts, RUNX2 and the FGF/proteoglycan axis likely cooperate to control osteoblast proliferation and differentiation (Fromigue et al. 2004; Teplyuk et al. 2009). Thus, the regulation of osteogenesis by FGF/FGFR signaling is dependent on not only the expression of a repertoire of FGFs and FGFRs but also the expression of accessory extracellular molecules that regulate cell signaling and FGF/FGFR biological activity.

FGF signaling in osteoclasts

Early studies suggested that FGF2 regulates bone resorption directly through its effect on osteoclast precursor pro-

liferation and indirectly by promoting prostaglandin G/H synthase-2 (*Ptgs2*) expression and prostaglandin formation (Kawaguchi et al. 1995, 2000; Chikazu et al. 2001). Additionally, FGF2 can act directly on mature osteoclasts to stimulate bone resorption through the activation of FGFR1 and MAPK (Chikazu et al. 2000; Kawaguchi et al. 2000). A role for FGF2 in bone resorption is supported by the finding that osteoclastogenesis in response to PTH is markedly impaired in *Fgf2*-null mice (Okada et al. 2003). FGF18 can also induce osteoclast formation and function indirectly through stimulating receptor activator of the nuclear factor- κ B ligand (RANKL) and cyclooxygenase-2 expression in osteoblasts (Shimoaka et al. 2002). Another study showed that FGF6 increased the formation and resorbing activity of human primary mature osteoclasts in vitro (Bosetti et al. 2010), whereas FGF8a inhibited osteoclastogenesis, but not osteoclast function, in mouse bone marrow cultures.

FGFR1 was detected on isolated mouse osteoclasts (Chikazu et al. 2000). Osteoclasts with FGFR1 deficiency (*LysM-Cre* mice) displayed decreased expression of genes related to osteoclast activity, including TRAP and MMP9, and exhibited abnormal bone remodeling with reduced osteoclast number and impaired osteoclast function (Lu et al. 2009). On the other hand, osteoclast numbers and bone resorption area were increased in cultured bone marrow cells derived from *Fgfr3*^{G369C/+} mice, suggesting that gain-of-function mutations in FGFR3 increase osteoclast activity, which contributes to decreased bone mass in these mice (Su et al. 2010).

Mutations in FGFRs in human skeletal disease

Mutations in FGFRs, first revealed with the discovery of the genetic etiology of achondroplasia as a missense mutation in FGFR3 (Shiang et al. 1994), highlight the importance of FGF signaling in skeletal development. Following this initial discovery, many mutations have been identified in FGFR1–3 in related skeletal dwarfism syndromes, craniosynostosis syndromes, and skeletal overgrowth syndromes (Ornitz and Itoh 2015). The clinical and genetic features of these diseases have been extensively reviewed (Vajo et al. 2000; Ornitz and Marie 2002; Chen and Deng 2005; Marie et al. 2005; Ornitz 2005; Melville et al. 2010; Johnson and Wilkie 2011; Foldynova-Trantirkova et al. 2012; Krejci 2014).

Chondrodysplasia syndromes

Achondroplasia is the most common form of skeletal dwarfism in humans, and >97% of cases result from an autosomal dominant missense mutation (G380R) in *FGFR3* (Shiang et al. 1994; Wilkin et al. 1998; Vajo et al. 2000). Related autosomal dominant chondrodysplasia syndromes include the more severe and usually lethal thanatophoric dysplasia types I and II, which are attributed to two mutations in *FGFR3* (K650E and R248C, respectively), and the milder form of dwarfism, hypochondroplasia, which is caused by an N540K or K650N mutation in *FGFR3* (Bellus

et al. 1995, 2000; Tavormina et al. 1995; Bonaventure et al. 1996). A closely related disease, referred to as severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) (Bellus et al. 1999; Tavormina et al. 1999), and a very rare lethal chondrodysplasia, platyspondylic lethal skeletal dysplasia, San Diego type (Brodie et al. 1999) are both caused by a K650M mutation in *FGFR3*. A dominant mutation (M528I) that causes proportionate short stature was recently identified in *FGFR3* (Kant et al. 2015). Functional studies suggest that this mutation is activating, similar to that of the G380R mutation that causes achondroplasia; however, the mechanisms that determine proportionate versus rhizomelic limb shortening are not known.

The mutations in *FGFR3* associated with dwarfism activate the receptor to varying degrees (Naski et al. 1996; Krejci et al. 2008b). The N540K mutation causing hypochondroplasia is a weak gain-of-function mutation, and mutations causing achondroplasia and thanatophoric dysplasia result in progressively stronger activation and, in the case of the R248C mutation, ligand-independent constitutive activation. *FGFR3* is expressed prominently in proliferating and prehypertrophic chondrocytes in the growth plate, and activation of *FGFR3* results in phenotypes that include decreased chondrocyte proliferation, impaired hypertrophic differentiation, and increased apoptosis (discussed below; Legeai-Mallet et al. 1998, 2004; Naski et al. 1998; Wang et al. 1999, 2001; Krejci et al. 2008b; Pannier et al. 2009).

The genetic homogeneity and relatively high incidence of the achondroplasia G380R mutation suggested that the associated G1138A transition in *FGFR3* might be one of the most highly mutable nucleotides in the human genome (Wilkin et al. 1998; Vajo et al. 2000). However, the paternal origin of the mutation and advanced paternal age associated with achondroplasia suggested that a germline selection model in which mutant spermatogonial stem cells have a proliferative or survival advantage over unmutated cells could better account for the observed paternal age effect (Dakouane Giudicelli et al. 2008; Shinde et al. 2013). This paternal age effect also occurs with gain-of-function mutations in *FGFR2* that cause craniosynostosis syndromes (Goriely et al. 2003, 2005; Goriely and Wilkie 2012).

Skeletal overgrowth and camptodactyly, tall stature, and hearing loss (CATSHL) syndrome

Inactivation of *FGFR3* in mice results in skeletal overgrowth (Colvin et al. 1996; Deng et al. 1996; Eswarakumar and Schlessinger 2007). Growth plate histology in mice lacking *Fgfr3* (*Fgfr3*^{-/-}) showed an expanded proliferating chondrocyte zone and hypertrophic chondrocyte zone beginning at late stages of embryonic development and persisting to adulthood. Bones of the appendicular skeleton were disproportionately long compared with body weight, and the mice developed elongation of vertebral bodies and scoliosis. *Fgfr3*^{-/-} mice also have primary defects in inner ear development and are consequently deaf (Colvin et al. 1996). The viability of *Fgfr3*^{-/-} mice and the unique

combination of skeletal overgrowth and hearing loss phenotypes suggested that a similar condition resulting from loss of function of *FGFR3* could occur in humans. Analysis of a four-generation family with dominantly inherited CATSHL identified a missense mutation (R621H) in the catalytic loop of the *FGFR3* tyrosine kinase domain (Toydemir et al. 2006). This mutation inactivates the kinase domain and likely confers dominant-negative activity to the receptor. A homozygous mutation in the *FGFR3* tyrosine kinase domain (T546K) has also been identified in two siblings with CATSHL from consanguineous parents (Makrythanasis et al. 2014). Interestingly, a similar recessive mutation in *FGFR3* (V700E) in sheep results in spider lamb syndrome, characterized by long limbs, kyphoscoliosis, malformed ribs and sternbrae, Roman noses, lack of body fat, and muscular atrophy (Beever et al. 2006). Sheep that are heterozygous for this mutation appear normal but show a mild increase in skeletal growth (Smith et al. 2006).

Craniosynostosis syndromes

Mutations in *FGFR1*, *FGFR2*, and *FGFR3* cause skeletal dysplasias (craniosynostosis) in Apert, Crouzon, and other syndromes characterized by premature fusion of one or more cranial sutures (Ornitz and Marie 2002; Johnson and Wilkie 2011). Mutations in *FGFRs* also result in dysmorphology of the appendicular skeleton, such as in bent bone dysplasia, which is caused by a mutation in *FGFR2* (Merrill et al. 2012). The molecular mechanisms that result from *FGFR* mutations are complex and include constitutive (ligand-independent) or ligand-dependent *FGFR* activation, loss of function, and altered cellular trafficking of receptors (Yu et al. 2000; Ibrahim et al. 2001; Yu and Ornitz 2001; Neben et al. 2014). Genetic, cellular, and molecular studies in mice and humans provided clues to the abnormalities induced by gain-of-function *FGFR* mutations (Hajihosseini 2008; Marie et al. 2008; Senarath-Yapa et al. 2012). Specifically, Apert *FGFR2* (S252W) mutations were shown to induce abnormal mesodermal progenitor cell proliferation, differentiation, and cell fate in cranial sutures in various mouse models, although the cellular abnormalities vary among specific sutures and between cells at distinct stages of differentiation in a particular suture (Wang et al. 2005; Holmes et al. 2009; Heuze et al. 2014; Motch Perrine et al. 2014).

At an early stage of development, osteoprogenitor cell proliferation in cranial sutures was unchanged (Chen et al. 2003) or transiently and moderately increased by Apert- and Crouzon-activating *FGFR2* mutations (Mansukhani et al. 2000, 2005). At a later stage, activating *FGFR2* mutations were widely found to cause increased osteoblast marker gene expression and accelerated osteoblast maturation in cranial sutures (Eswarakumar et al. 2004; Yang et al. 2008; Yin et al. 2008; Holmes et al. 2009; Yeh et al. 2012; Liu et al. 2013a,b; Morita et al. 2014). This finding in mice supports the original observation in humans that Apert and Crouzon *FGFR2* mutations cause increased osteoblast maturation and function in postnatal human suture development (Lomri et al. 1998; Lemonnier

et al. 2001b; Tanimoto et al. 2004; Baroni et al. 2005; Marie 2015). *FGFR2* mutations are also associated with increased osteoblast apoptosis in fused sutures in mice (Mansukhani et al. 2005; Chen et al. 2014) and humans (Lemonnier et al. 2001a; Lomri et al. 2001; Kaabeche et al. 2005); however, this appears to be a secondary event that occurs subsequent to osteoblast maturation and not the primary cause of the synostosis (Holmes et al. 2009). Overall, the current literature indicates that the premature cranial ossification induced by *FGFR2* mutations relates to an accelerated maturation of osteoblasts, whereas the moderate and transient increase in osteoprogenitor cell recruitment and the late osteoblast apoptosis do not appear to contribute primarily to the pathogenesis of the synostosis. The mutations in *FGFR2* that causes bent bone dysplasia (M391R or Y381D) reduce ligand-dependent receptor activation and lead to altered trafficking of the mutant receptor to the nucleolus. Increased nucleolar activity of *FGFR2* increases ribosomal RNA expression to promote proliferation and suppresses differentiation of osteoprogenitor cells (Merrill et al. 2012; Neben et al. 2014).

Besides *FGFR1* and *FGFR2* mutations, activating *FGFR3* mutations in achondroplasia may also cause partial premature fusion of the coronal sutures, suggesting a role for *FGFR3* activation in membranous ossification (Twigg et al. 2009; Di Rocco et al. 2014). Indeed, chondrocyte-specific activation of *Fgfr3* in mice was found to induce premature suture closure and indirectly enhance osteoblast differentiation through MAPK activation and BMP signaling (Matsushita et al. 2009b). In long bones, gain-of-function *FGFR3* mutations in chondrocytes, but not in osteoblasts, caused increased osteoclast recruitment, defective mineralization, and decreased trabecular bone mass, indicating that overactive *FGFR3* signaling indirectly affects trabecular bone development (Su et al. 2010; Mugniery et al. 2012).

Intracellular pathways involved in craniosynostosis

Genetic and functional studies indicate that the premature suture fusion induced by *FGFR* mutations occurs as a consequence of activation of signaling pathways and downstream target genes. In several mouse models, Apert *FGFR2*(S252W) mutations activate ERK1/2, p38 MAPK, AKT, β -catenin, and PLC γ in osteogenic cells, which may contribute to premature cranial ossification (Chen et al. 2003; Kim et al. 2003; Shukla et al. 2007; Yin et al. 2008; Wang et al. 2010; Suzuki et al. 2012). Interestingly, reduced dosage of ERF, an inhibitory ETS transcription factor directly bound by ERK1/2 signaling, was found to cause craniosynostosis in humans and mice, implying a role of ERF downstream from ERK signaling in cranial suture ossification (Twigg et al. 2013). In human craniosynostosis, increased osteoblast gene expression and cranial osteogenesis induced by Apert *FGFR2*(S252W) mutation are related to PLC γ and PKC α activation (Lemonnier et al. 2001b). The consequence is increased expression of *Runx2*, which is associated with premature fusion of cranial sutures in mice and humans (Zhou et al. 2000; Eswar-

akumar et al. 2002; Tanimoto et al. 2004; Baroni et al. 2005; Guenou et al. 2005). In both mice and humans, *FGFR2* levels are decreased in response to the Apert *FGFR2*(S252W) mutation as a result of c-CBL-mediated *FGFR2* ubiquitination and degradation (Kaabeche et al. 2004; Holmes et al. 2009). In addition, c-CBL recruitment by Apert *FGFR2*(S252W) in human osteoblasts causes ubiquitination and down-regulation of the *Src* family members *LYN* and *FYN* (Kaabeche et al. 2004) and α 5 β 1 integrin (Kaabeche et al. 2005), resulting in increased osteoblast differentiation and apoptosis, respectively. Increased c-CBL recruitment in Apert osteoblasts also leads to PI3K ubiquitination and degradation, resulting in attenuation of PI3K signaling and reduced osteoblast survival (Dufour et al. 2008). Thus, both *FGFR*-mediated activation of signaling pathways and down-regulation of regulatory molecules contribute to the cellular events in craniosynostosis.

Recent studies indicate that *FGFR* signaling interacts with other pathways to induce craniosynostosis. In human osteoblasts, Apert *FGFR2*(S252W) mutations cause increased expression of platelet-derived growth factor receptor α (*PDGFR α*) and epidermal growth factor receptor (*EGFR*) in osteoblasts via activation of PKC α -mediated AP-1 transcription, which contributes to premature suture ossification (Miraoui et al. 2010a). Consistent with these data, transgenic mice conditionally expressing an constitutively activated *PDGFR α* in neural crest cells display activation of PLC γ , resulting in premature fusion at early postnatal stages (Moenning et al. 2009). *FGFR2* activation caused by Apert *FGFR2*(S252W) mutations also leads to increased c-CBL–Sprout2 interaction and c-CBL sequestration in human osteoblasts, causing increased *EGFR* levels and signaling and increased osteoblast gene transcription (Miraoui et al. 2010a). Thus, both transcriptional and post-transcriptional mechanisms mediate the aberrant signaling pathways induced by *FGFR2* mutations in cells of the osteoblast lineage.

Therapeutic strategies for FGF-related skeletal disease

Therapeutic strategies in achondroplasia

Skeletal dwarfism in hypochondroplasia, achondroplasia, and thanatophoric dysplasia results from increased *FGFR3* signaling in proliferating and hypertrophic chondrocytes (Foldynova-Trantirkova et al. 2012). For achondroplasia, in which affected individuals attain adult heights that are well below the fifth percentile, pharmacological therapy promises to offer an alternative to painful and costly surgical therapies that are currently used to increase height and correct disproportional bone growth.

Several approaches have been proposed to suppress *FGFR3* signaling with the primary goal of enhancing skeletal growth (Laederich and Horton 2012). Therapeutic strategies can target FGF ligands that activate *FGFR3*, the activated receptor, signaling pathways that are downstream from *FGFR3*, signaling pathways that modulate *FGFR3* intracellular pathways, or signaling pathways that are independent of *FGFR3* signaling. Tyrosine kinase

inhibitors that are selective for FGFRs have thus far not been effective in vivo because of difficulties in achieving therapeutic levels of inhibitors in the avascular growth plate and potential side effects of systemic inhibition of FGFRs over the long periods for time that will be required for treatment (Laederich and Horton 2012).

Human growth hormone was evaluated as a therapy; however, no long-term benefits were observed (Kanazawa et al. 2003; Horton et al. 2007). An antihistamine drug, meclozine dihydrochloride, has been shown to inhibit FGFR3 signaling in chondrocytes by suppressing ERK1/2 phosphorylation. Meclozine functioned to enhance bone growth in explant culture and increase bone growth in wild-type and achondroplasia mice (Matsushita et al. 2013, 2015). Inhibition of ligands that activate FGFR3 can be achieved through expression of a soluble extracellular domain of FGFR3 (sFGFR3) that can compete with endogenous FGFR3 by binding FGF ligands such as FGF9 and FGF18 that functionally regulate the growth plate (Liu et al. 2002, 2007; Ohbayashi et al. 2002; Hung et al. 2007; Garcia et al. 2013). Subcutaneous injections of recombinant sFGFR3 in a mouse model for achondroplasia was found to decrease mortality and improve skeletal growth (Garcia et al. 2013). Inhibitory antibodies that specifically target the FGFR3 extracellular domain have been developed as potential cancer therapeutics but have not been evaluated for treatment of achondroplasia (Martinez-Torrecuadrada et al. 2005; Hadari and Schlessinger 2009; Qing et al. 2009). A recent drug screen of induced pluripotent stem cell-derived chondrocytes from patients with achondroplasia and thanatophoric dysplasia identified statin drugs as effective in improving chondrogenic differentiation in vitro and improving the phenotype (limb and body length) of achondroplasia mice in vivo (Yamashita et al. 2014).

The most promising therapy thus far for treatment of achondroplasia is the use of a stabilized form of CNP called BMN-111 (Lorget et al. 2012; Wendt et al. 2015). BMN-111 is currently undergoing clinical trials for the treatment of achondroplasia (<https://clinicaltrials.gov/ct2/show/NCT02055157>). Mice or humans that overexpress CNP or activate natriuretic peptide receptor 2 (*Npr2*; guanylyl cyclase B) have a skeletal overgrowth phenotype (Fig. 2; Yasoda et al. 2004; Bocciardi et al. 2007; Hannema et al. 2013; Miura et al. 2014), while mice that lack CNP (*Nppc*^{-/-}) or humans with heterozygous mutations in *NPR2* exhibit skeletal dwarfism (Tsuji and Kunieda 2005; Nakao et al. 2015).

CNP signals through NPR2 in chondrocytes and inhibits the MAPK signaling pathway at the level of RAF1 (Fig. 2). Several studies suggest a mechanism in which CNP-induced cGMP activates cyclic GMP-dependent protein kinase II (cGKII; encoded by *PRKG2*) and p38 (*MAPK14*), which functionally antagonizes RAF1 activation of MEK (*MAP2K1*), which is a critical pathway regulating chondrocyte hypertrophy (Murakami et al. 2004; Ozasa et al. 2005; Agoston et al. 2007; Hutchison 2012; Peake et al. 2014). Interestingly, FGFR3–MAPK signaling may also inhibit cGMP production in chondrocytes (Ozasa et al. 2005). Thus, signaling by FGF ligands (FGF9 and FGF18)

through FGFR3 functionally antagonizes CNP signaling through NPR2 to regulate the rate of chondrocyte hypertrophy. Consistent with feedback regulation of the FGF and CNP pathways in chondrocytes, patients with activating mutations in *FGFR3* have increased circulating levels of CNP (Olney et al. 2015).

Therapeutic strategies in craniosynostosis

The identification of the aberrant signals induced by *FGFR* mutations provided clues for potential therapeutic nonsurgical interventions in craniosynostosis (Wilkie 2007; Melville et al. 2010). In vitro, overactivated FGFR2 signaling by Apert and Crouzon mutations can be attenuated by specific glycosaminoglycans (McDowell et al. 2006), FGFR or tyrosine kinase inhibitors (Perlyn et al. 2006), or a soluble mutant form of FGFR2 that exerts a dominant-negative effect on FGFR2 (Tanimoto et al. 2004). In vivo, selective uncoupling of the docking protein FRS2 α and the Crouzon-like activated FGFR2c mutant was found to attenuate FGFR signaling and prevent premature suture fusion in mice (Eswarakumar et al. 2006). In mice, treatments with a shRNA targeting the Apert *FGFR2*(S252W) mutation or the use of a ERK1/2 MAPK inhibitor (Shukla et al. 2007) or a soluble mutant form of FGFR2 (Morita et al. 2014; Yokota et al. 2014) were also found to prevent craniosynostosis. The recent finding that FGFR signaling interacts with PDGFR α signaling to induce premature suture fusion in mice and humans may offer alternative therapeutic strategies to indirectly attenuate signals induced by activating *FGFR* mutations (Moening et al. 2009; Miraoui et al. 2010a). These recent studies may thus provide promising therapeutic strategies to efficiently reduce the aberrant FGFR signaling induced by *Fgfr2* mutations in craniosynostosis.

FGF and bone regeneration

Fgf and *Fgfr* expression are regulated during fracture healing (Schmid et al. 2009), and there are accumulating data indicating that FGF/FGFR signaling plays active roles during the fracture repair process (Du et al. 2012; Fei et al. 2013). These studies led to the wide use of FGF2 as a therapeutic agent for promoting bone regeneration in animal models (Fei et al. 2013). Typically, the local implantation of FGF2 impregnated in a gelatin hydrogel or a molecular scaffold or the injection of chemically controlled FGF2-secreting cells was found to promote bone healing in cranial or long bone defects (Chen et al. 2004; Komaki et al. 2006; Kodama et al. 2009; Kawaguchi et al. 2010; Kwan et al. 2011). However, the effects on bone healing are dependent on the dose used, the time of application, and the duration of FGF treatment (Du et al. 2012; Fei et al. 2013). The anabolic effect of locally injected FGF2 results mainly from increased expansion of the pool of osteoblast precursor cells, resulting in their differentiation into osteoblasts (Nakamura et al. 1998; Nakajima et al. 2007). In addition, FGF2 may also increase angiogenesis at the site of injury. A close spatial and temporal relationship exists between

angiogenesis and osteogenesis during bone repair and endochondral bone formation, and the connection between the two processes is essential for bone formation (Dirckx et al. 2013; Saran et al. 2014). Recent genetic studies indicate that FGF/FGFR signaling plays an essential role in both processes. It was shown that FGF9^{-/+} mice display reduced repair of cortical bone defects resulting from decreased osteoblast proliferation and differentiation associated with impaired neovascularization and reduced *Vegf* expression. This phenotype was rescued by exogenous FGF9, indicating that endogenous FGF9 is important for cortical bone repair in part through regulating *Vegf* expression (Behr et al. 2010). In addition to promoting angiogenesis, VEGF controls bone formation by stimulating the differentiation of mesenchymal stem cells to osteoblasts (Liu and Olsen 2014). Accordingly, VEGF alone has a potent bone repair potential by promoting angiogenesis and osteogenesis in vivo (Behr et al. 2012). Since FGF2 promotes *Vegf* expression in osteoblasts (Takai et al. 2007), these two factors may promote bone repair through activation of both angiogenesis and osteogenesis. *Fgf18*^{-/-} mice also showed reduced cortical bone defect healing capacity but due to decreased osteoblast differentiation with normal angiogenesis, showing that FGF18 is required for osteogenesis but not angiogenesis during bone repair (Behr et al. 2011). In a mouse model of skeletal prosthesis insertion, a local injection of FGF18 resulted in increased peri-implant bone formation in both *Fgfr3*^{+/+} and *Fgfr3*^{-/-} mice, indicating that FGF18 can promote bone repair independently of FGFR3 (Carli et al. 2012), presumably through activation of FGFR1 and/or FGFR2. In line with this concept, it was recently found that DJ-1 (*PARK7*), an angiogenic protein, stimulates both osteoblast differentiation and angiogenesis via FGFR1 activation, resulting in enhanced long bone fracture repair in rats, further highlighting the importance of FGFR1 signaling in fracture repair (Kim et al. 2012). Overall, these studies uncover novel roles for FGF/FGFR signaling in bone repair through activation of both angiogenesis and osteogenesis, supporting the concept that FGFs may have therapeutic potential to stimulate bone regeneration.

Conclusion

During the past decade, significant advances have been made in our understanding of the role of FGF/FGFR signaling in skeletal development, bone formation, and bone diseases. Specifically, the analysis of genetic models in mice led to a better understanding of the complex role of FGF/FGFR signaling in endochondral and intramembranous ossification and bone repair. Also, the analysis of the skeletal phenotype induced by *FGFR* mutations in various mouse models and human skeletal dysplasias has improved our knowledge of the pathogenesis of chondrodysplasias and craniosynostosis. Finally, genetic and functional studies led to the identification of aberrant signaling mechanisms involved in skeletal diseases associated with *FGFR* mutations. This knowledge validated the concept that modulating FGFR signaling and downstream

signaling pathways can prevent skeletal dysplasias induced by *Fgfr* mutations in mice, and this important concept may lead to novel therapeutic approaches in human skeletal dysplasias. In the future, further studies are needed to delineate the stage-specific role of FGF/FGFR signaling in cells of the chondrocyte and osteoblast lineage in vivo and characterize FGF interactions with other effectors during normal and aberrant chondrogenesis and osteogenesis. It is expected that a more complete understanding of the role FGF/FGFR signaling in prenatal and postnatal skeletal development will translate into novel therapeutic approaches for human skeletal dysplasias and improved skeletal repair.

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