RECENT RESEARCH ON THE GROWTH PLATE

Advances in fibroblast growth factor signaling in growth plate development and disorders

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Abstract

Skeletons are formed through two distinct developmental actions, intramembranous ossification and endochondral ossification. During embryonic development, most bone is formed by endochondral ossification. The growth plate is the developmental center for endochondral ossification. Multiple signaling pathways participate in the regulation of endochondral ossification. Fibroblast growth factor (FGF)/FGF receptor (FGFR) signaling has been found to play a vital role in the development and maintenance of growth plates. Missense mutations in FGFs and FGFRs can cause multiple genetic skeletal diseases with disordered endochondral ossification. Clarifying the molecular mechanisms of FGFs/FGFRs signaling in skeletal development and genetic skeletal disorders will have implications for the development of therapies for FGF-signaling-related skeletal dysplasias and growth plate injuries. In this review, we summarize the recent advances in elucidating the role of FGFs/FGFRs signaling in growth plate development, genetic skeletal disorders, and the promising therapies for those genetic skeletal diseases resulting from FGFs/FGFRs dysfunction. Finally, we also examine the potential important research in this field in the future.

Key Words
- FGF signaling
- growth plate
- disorder
- mouse model
- review

Introduction on growth plate development

The skeleton is formed through endochondral or intramembranous ossification (Olsen et al. 2000, Long & Ornitz 2013). The skull and the inner clavicles are formed through intramembranous ossification. The majority of bone is formed by endochondral ossification, including the ribs, limb bones, and vertebrae (Fig. 1). Endochondral ossification is a highly regulated process, starting from initiation of mesenchyme condensation. Mesenchyme differentiates into chondrocytes and forms the cartilage anlage. During endochondral ossification the chondrocytes undergo an orderly sequence of events: proliferation, hypertrophy, mineralization, and apoptosis, leaving the mineralized cartilaginous templates, which will be replaced by bone tissues through osteogenesis. Osteogenesis can be triggered by multiple factors released from both prehypertrophic and hypertrophic chondrocytes, such as Indian hedgehog (IHH) and vascular endothelial growth factor (VEGF), which induce the differentiation of perichondrial cells into osteoblasts, and also the invasion of blood vessels into the mineralized cartilage, bringing...
The osteoclasts and osteoprogenitors to the cartilaginous templates to replace the growth plate cartilage with bone through their bone resorption and formation function respectively (Karsenty & Wagner 2002, Provot & Schipani 2005, Maes et al. 2010, Long & Ornitz 2013).

The growth plate has been described as the developmental center for endochondral ossification. The chondrocytes exhibit distinct and observable stages during this process (Wuelling & Vortkamp 2010). The growth plate can easily be subdivided into four zones on the basis of the shape and function of the chondrocytes (Burdan et al. 2009). The resting zone is constituted by small round chondrocytes adjacent to the articular surface. These cells undergo differentiation into proliferative flat chondrocytes to form proliferative columns. The resting and proliferating chondrocytes secrete collagen type II (COL2), aggrecan, and other matrix proteins to form cartilage matrix. The proliferative chondrocytes differentiate into prehypertrophic and thereafter hypertrophic chondrocytes, which secrete collagen type X toward the diaphysis. Prehypertrophic chondrocytes are essential for controlling the pace of endochondral ossification.

A variety of molecules regulate growth plate development by controlling prehypertrophic differentiation (Maes et al. 2010). Hypertrophic chondrocytes remodel the cartilage matrix into a calcifying matrix (Ortega et al. 2004). The terminal hypertrophic chondrocytes will finally undergo apoptosis accompanied by resorption of mineralized cartilage and vascularization (Shapiro et al. 2005). The proliferation and differentiation of chondrocytes drives the elongation of skeletal elements (Long & Ornitz 2013).

Endochondral ossification is spatially and temporally governed by integrated networks of molecules, especially the lineage-specific transcription factors such as SOX9 and RUNX2 (Karsenty et al. 2009). SOX9 plays an essential role in multiple steps of chondrocyte differentiation together with L-SOX5 and SOX6 (Chang et al. 2004). SOX9 is highly expressed in the mesenchymal condensations and then in proliferative chondrocytes, with a maximal expression in prehypertrophic chondrocytes, but abruptly disappears from the hypertrophic zone (Chang et al. 2004, Hattori et al. 2010). SOX9-expressing precursors during mouse embryogenesis give rise to all osteo-chondroprogenitor cells (Dell’Accio et al. 2001). SOX9 activates multiple genes expressed in proliferating chondrocytes, including the extracellular matrix (ECM) genes COL2A1 (van Rijn et al. 2002, Trebicz-Geffen et al. 2003) and Aggrecan (Keats et al. 2003). Some studies have indicated that SOX9 expressed in the prehypertrophic zone may inhibit COL10A1 expression through MEF2C, a MADS box transcription factor (Leung et al. 2011). SOX9 has been proposed to be
necessary for chondrocyte survival and hypertrophy to delay terminal maturation (Cowan et al. 2003, Hattori et al. 2010). Furthermore, SOX9 can inhibit the expression of RUNX2, a runt-domain transcription factor, and induce the degradation of RUNX2 in chondrocytes (Mangion et al. 1999, Amizuka et al. 2000). RUNX2 plays a pivotal role in the promotion of chondrocyte hypertrophy. RUNX2 is initially expressed in the chondrogenic mesenchyme, subsequent to SOX9 (Dell’Accio et al. 2001). RUNX2 is restrictedly located to the perichondrial cells and osteoblasts after cartilage anlage is formed, and then is expressed in the prehypertrophic and early hypertrophic chondrocytes (Provot & Schipani 2005). Several lines of evidence have supported the role of RUNX2 as an important positive regulator of the hypertrophic program (Vidrich et al. 2009, Hafner et al. 2010, Shinde et al. 2013). RUNX2 initiates chondrocyte hypertrophy, and loss of RUNX2 leads to severely delayed chondrocyte maturation in developing bones (Dooley et al. 2007, Shinde et al. 2013). Furthermore, RUNX2 expressed in the perichondrium can regulate the perichondrial expression of fibroblast growth factor 18 (FGF18) to modulate growth plate development through indirect mechanisms (Park et al. 2007). RUNX2 stimulates the expression of IHH and VEGF (Tanaka et al. 2006) in hypertrophic chondrocytes (Gunhaga et al. 2003). RUNX2 is also well known for its essential role in osteoblast differentiation (Fortin et al. 2005, Shroff 2013) and its promotion of expression of osteoblast-specific genes (Ogata et al. 2008, Lin & Melero-Martin 2012).

The development of the growth plate is tightly regulated by various systemic and local molecules such as growth hormone, thyroid hormone, bone morphogenetic proteins (BMPs), Wnt/β-catenin, FGFs, and transforming and growth factor (TGF) β (Long & Ornitz 2013). Previous work revealed that parathyroid hormone-related protein (PTH(R)/IHH feedback loop plays a major role during chondrogenesis (Kobayashi et al. 2002). PTH(R) is synthesized in the periarticular region, activates the PTH/PTH(R) receptor (PTR) expressed in prehypertrophic zone, then stimulates cell proliferation, and delays the hypertrophic differentiation (Esvarakumar et al. 2005). Prehypertrophic and hypertrophic chondrocytes release IHH, which stimulates chondrocyte proliferation and PTH(R) synthesis. PTH(R) suppresses chondrocyte maturation. IHH also determines the location of bone collar formation (Chung et al. 2001). Many other pathways, including FGF signaling, can regulate chondrocyte proliferation and maturation indirectly through their interactions with the PTH(R)/IHH feedback loop (Grimsrud et al. 2001, Minina et al. 2001, Guo et al. 2009, Yano et al. 2013). Any disturbances of these signaling pathways will interfere with growth plate development, and finally result in a variety of skeletal dysplasias (Maes et al. 2010, Kerkhofs et al. 2012, Michigami 2013).

A brief introduction to FGFs/FGF receptors signaling

In addition to PTH(R)/IHH feedback loop, FGF signaling also remarkably regulate growth plate development (Amizuka et al. 2004). FGFs are a family of 22 members binding to their high-affinity receptors, FGF receptor 1–4 (FGFR1–4). FGFs mediate their cellular responses through distinct binding affinity with individual FGFRs. FGFs can be divided into three subfamilies: canonical FGFs (FGF1–10, 16–18, 20, 22), hormone-like FGFs (FGF15/19, 21,23), and intracellular FGFs (FGF11–14) (Itoh & Ornitz 2008). Like other receptor tyrosine kinases (RTKs), a prototypical FGF receptor contains an extracellular ligand-binding domain, a hydrophobic transmembrane region, and an intracellular tyrosine kinase domain (Burdan et al. 2009). FGFRs have two major FGF isoforms generated by alternative splicing of the third extracellular immunoglobulin loop in FGF receptor transcripts, IIb and IIc (Gong 2014)). FGFs bind to the extracellular domain of FGFRs, cause receptor dimerization, and induce phosphorylation of tyrosine residues in their intracellular domain. The activated FGF recruits target proteins to its cytoplasmic tail and modifies them mainly by phosphorylation (Powers et al. 2000). There are several important downstream pathways of FGF signaling, such as the STAT, MAPK, phosphatidylinositol-3-kinase (PI3K)/AKT, and phospholipase C-gamma (PLCγ)/protein kinase C (PKC) pathways. These pathways are associated with the phosphorylation of specific tyrosine residues and regulate a variety of cell functions, such as cell proliferation, differentiation, survival, and matrix production (Powers et al. 2000, Dailey et al. 2005).

Expression of FGFs and FGFRs during skeletal development

The spatiotemporal expression patterns of FGFs and FGFRs have been characterized (Fig. 2; Ornitz 2005, Du et al. 2012, Long & Ornitz 2013).

FGFR1 is expressed diffusely in mesenchyme of limb buds and somites, in prehypertrophic, and hypertrophic chondrocytes, and perichondrium of the epiphyseal growth plates, and more in differentiated osteoblasts, as
Figure 2
Expression of FGFs/FGFRs during endochondral bone formation. Expression patterns of FGFRs are shown in the upper panel and FGFs in the lower panel. The cells are color coded for FGFRs (Liu et al. 2002, Ohbayashi et al. 2002, Minina et al. 2005, Hung et al. 2007, Yu & Ornitz 2008).
well as osteocytes (Xiao et al. 2004, Jacob et al. 2006, Lazarus et al. 2007, Kyono et al. 2012). FGFR2 has been detected in condensing mesenchyme of early limb bud (Peters et al. 1992, Orr-Urtreger et al. 1993, Delezoide et al. 1998) and appears as the first marker of prechondrogenic condensation. FGFR2 is predominantly localized to perichondrial and periosteal tissues and expressed weakly in endosteam and trabecular bone during later limb development (Yu et al. 2003). In addition, expression of FGFR2 has been observed in cartilage, especially in the resting zone (Rice et al. 2000, 2003, Wilkie 2005, Lazarus et al. 2007, Yin et al. 2008). In cranial sutures, FGFR2 has been detected in osteoprogenitor cells and osteoblasts (Iseki et al. 1999). FGFR3 expression has been observed in chondrocytes located in the central core of the mesenchymal condensation (Peters et al. 1993). As the epiphysial growth plate is formed, FGFR3 is expressed in all chondrocytes except hypertrophic chondrocytes (Peters et al. 1993, Szebenyi et al. 1995, Colvin et al. 1996, Delezoide et al. 1998, Ornitz 2005, Jacob et al. 2006). Expression of FGFR3 has also been found in osteoblasts and osteocytes (Valverde-Franco et al. 2004, Su et al. 2010). In addition, FGFR3 has been detected in head periostea and sutural osteogenic fronts (Delezoide et al. 1998, Rice et al. 2000). Very low amounts of FGFR4 are present in the osteoblasts of osteogenic fronts (Delezoide et al. 1998, Peter et al. 2002). FGFR4 has also been found in osteoblasts and osteocytes (Iseki et al. 2003). As the epiphysial growth plate is formed, FGFR4 is expressed in resting and proliferative zones of growth plates (Partanen et al. 1991, Cool et al. 2002, Lazarus et al. 2007).

FGF1 has been found in proliferating and hypertrophic chondrocytes (Krejci et al. 2007). FGF2 is expressed in limb bud, chondrocytes, and osteoblasts (Fallon et al. 1994, Montero et al. 2000, Lazarus et al. 2007, Fei & Hurley 2012). Four FGFs are expressed in the mouse apical ectodermal ridge (AER): FGF4, FGF8, FGF9, and FGF17 (Moon et al. 2000, Mariani et al. 2008). FGFI, 2, 4, 8, 9, and particularly FGF18, which is produced by the perichondrium, bind to and activate FGFR3 (Moon & Capecchi 2000). FGF9 is expressed in the mesenchyme surrounding the cartilaginous condensations, chondrocytes, and primary spongiosa as well as perichondrium/periosteum (Hung et al. 2007). FGF10 is found in the presumptive limb field (Ohuchi et al. 1997, Martin 1998, Xu et al. 1998), and its expression persists in the mesenchyme under AER after initial limb bud formation (Xu et al. 1998). FGFI8 is expressed apparently in the perichondrium, mesenchymal cells, and osteoblasts during bone development (Liu et al. 2002, Ohbayashi et al. 2002). FGFI, 2, 4, 6, 8, 19, 21, and 22 are expressed in perichondrium, while FGF2, 7, 18, and 22 are expressed in growth plates in rats (Lazarus et al. 2007). The transcripts for FGF1, 2, 5, 8–14, 16–19, and 21 have also been found in growth plates, while only FGF1, 2, 17, and 19 are detectable at the protein level (Krejci et al. 2007). FGF23 is mainly synthesized by osteocytes and osteoblasts (Bonenwald & Wacker 2013). Recently, FGF23 expression has been found in resting and hypertrophic chondrocytes (Raimann et al. 2013).

Roles of FGFs/FGFRs in skeletal development: clues from human genetic skeletal syndromes and mouse models

Numerous studies have demonstrated the important role of FGFs/FGFRs in bone development. Mutations in FGFs/FGFRs are responsible for a diverse group of skeletal genetic disorders. For FGFRs, mutations in FGFR1 and FGFR2 mainly cause syndromes involving craniosynostoses, whereas the dwarfing syndromes are largely associated with FGFR3 mutations.

A gain-of-function (GOF) missense mutation in FGFR1 (Pro252Arg) causes Pfeiffer syndrome (PS) which result in broad toes and split thumbs (Muenke et al. 1994). Several activating FGFR1 mutations, such as N330I and C379R, cause osteoglophonic dysplasia (OD), and the patients displayed craniosynostosis, prominent supraorbital ridges, and depressed nasal bridge, as well as rhizomelic dwarfism and non-opposing bone lesions (White et al. 2005). In contrast, loss-of-function (LOF) mutations in FGFR1 such as C277Y, R622X, P772S, G97D, and A167S are responsible for autosomal-dominant Kallmann syndrome (KS), characterized by hypogonadism and anosmia. Some KS patients have skeletal abnormalities, i.e. butterfly vertebra and oligodactyly of the feet, indicating that FGFR1 can regulate endochondral ossification (Jarzabek et al. 2012). GOF mutations in FGFR2 can cause multiple types of craniosynostoses, such as Apert syndrome (AS), Crouzon syndrome (CS), and PS, as well as Beare–Stevenson cutis gyrata syndrome (BSS) (Wilkie 2005, Cunningham et al. 2007, Park et al. 2012, Sharma et al. 2012, Wilkinson et al. 2012). Several de novo FGFR2 mutations have been demonstrated to be responsible for a perinatal lethal skeletal dysplasia designated as bent bone dysplasia (BBD)-FGFR2 type (Merrill et al. 2012). GOF mutations in FGFR3 lead to hypochondroplasia (HCH), achondroplasia (ACH), and thanatophoric dysplasia (TD) with dysregulated endochondral ossification (He et al. 2012, Krejci 2014). GOF mutations in FGFR3 have also been found to cause premature suture fusion, leading to craniosynostoses. FGFR3, p.Ala334Thr, has been found to be responsible for mild craniosynostosis (Barroso et al. 2011). FGFR3 A391E mutation in the transmembrane region is responsible for Crouzon dermoskeletal syndrome (Wilkes et al. 1996).

Mutations of FGFs have also been found in multiple genetic skeletal disorders. Genotyping of SNPs in FGF genes revealed associations between cleft palate and SNPs in FGFR3, FGFR7, FGFR10, FGFR18, and FGFR1 (Riley et al. 2007). In humans, constitutionally increased dosage of the FGF3 and FGFR4 genes plays an important role in the onset of craniosynostosis (Grillo et al. 2014). FGFR8 mutations may be associated with craniofacial defects and acrocephalosyndactyly (Whitehead et al. 2004, Bouillon et al. 2008). A mutation (S99N) in FGFR9 is associated with multiple synostosis syndromes (SYNS) in humans (Wu et al. 2009). LOF mutations in FGFR10 cause lacrimal-auriculo-dento-digital syndrome (Milunsky et al. 2006, Rohmann et al. 2006, Shams et al. 2007), which is an autosomal-dominant multiple congenital anomaly disorder characterized by lacrimal duct aplasia, malformed ears and deafness, dental, and digital anomalies. FGFR10 is also thought to be a candidate gene for cleft palate (King et al. 1991, Perry et al. 1991, Schwartz et al. 1992, Turnquist et al. 1992). FGFR2 plays a crucial role in phosphate homeostasis (Yu & White 2005, Fukumoto & Yamashita 2007). X-linked hypophosphatemic rickets (XLH) and autosomal recessive hypophosphatemic rickets/osteomalacia are caused by mutations in the phosphate-regulating endopeptidase (PHEX) and dentin matrix protein 1 (DMP1) genes respectively and patients show elevated FGF23 levels (Jonsson et al. 2003, Fukumoto & Yamashita 2007, Yoshiko et al. 2007). Excess levels of FGF23 in patients with these hypophosphatemic disorders lead to renal phosphate wasting and suppression of circulating 1,25(OH)2D3 levels (Wohrle et al. 2013). Autosomal dominant hypophosphatemic rickets (ADHR) are caused by missense mutations in the FGF23 gene, leading to the resistance of the mutant FGF23 to degradation (White et al. 2000). Furthermore, patients afflicted with tumor-induced osteomalacia (Shimada et al. 2001) also show elevated FGF23 serum levels.

Accumulating evidence from mouse model studies (Table 1) and cell lines have confirmed the roles of FGFs/FGFRs and their underlying mechanisms in skeletal development and human skeletal dysplasias.

Fgfr1 P252R transgenic mice mimicking human PS exhibit premature suture closure and de novo digit I polydactyly in the hind limb with upregulation of Wnt5a and downregulation of Dkk1, which encodes a secreted Wnt inhibitor (Hajihosseini et al. 2004). Fgfr1 exists in hypertrophic chondrocytes and perichondrium of the epiphyseal growth plates, but knowledge regarding its role in growth plate development is limited. Zhou found that FGFs/FGFR1 signals cause increased expression of RUNX2 and lead to premature fusion of cranial sutures in mice carrying the Fgfr1 P250R mutation (Zhou et al. 2000). Fgfr1-deficient (Fgfr11⁄−⁄) mouse embryos display severe growth retardation and die before or during gastrulation (Deng et al. 1994, Yamaguchi et al. 1994). We found that disruption of Fgfr1 in adult mouse articular chondrocytes (via Col2a1Cre) inhibits the progression of cartilage degeneration in aging-related and induced osteoarthritis (Weng et al. 2012). Jacob et al. (2006) revealed that FGFR1 inhibits proliferation of mesenchymal progenitor cells but promotes their differentiation into preosteoblasts and suppresses the maturation and mineralization of osteoblasts. Our preliminary data indicate that conditional deletion of Fgfr1 in osteoblasts leads to an increase in bone mass in adult and aging mice (Nan Su & Lin Chen 2013 unpublished observations). In addition, Fgfr1 may positively regulate osteoclasts (Lu et al. 2009). FGFR1 also participates in phosphorus metabolism through regulating the transcription of FGF23 (Wohrle et al. 2011, 2013, Donate-Correa et al. 2012).

Mice mimicking human AS show premature closure of cranial base synchondroses and retarded endochondral bone growth with expanded resting zone and narrowed proliferating and hypertrophic zones in growth plates (Chen et al. 2003). The P253R mutation in Fgfr2 leads to retardation of the growth of long bones by directly affecting endochondral ossification (Yin et al. 2008). BBD patients have growth plates with smaller hypertrophic chondrocytes and thicker hypercellular peristeum. The chondrocytes from patients have reduced responsiveness to extracellular FGF18 and deficient plasma-membrane localization of Fgfr2 (Merrill et al. 2012). In zebrafish, fgfr2 is required for mesenchyme condensation and later chondrogenic differentiation (Larbuisson et al. 2013). Targeted disruption of Fgf211IC leads to narrowing of the proliferative and hypertrophic chondrocyte zones with decreased IHH and PTHRP expression and retarded ossification with a decrease in the transcription of secreted phosphoprotein 1 and RUNX2 (Eswarakumar et al. 2002). As Fgfr2 is predominantly expressed in osteogenic cells, a lot of studies have been carried out in order to understand
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mouse model</th>
<th>Mutation position</th>
<th>Survival/phenotypes</th>
<th>Related human syndromes (mutation)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>GOF (KI)</td>
<td>Exon 7 (P252R)</td>
<td>Viable/premature suture closure, increased bone formation at suture closure, de novo digit I polydactyly in the hind limb</td>
<td>PS (P250R)</td>
<td>Zhou et al. (2000)</td>
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<tr>
<td></td>
<td>OE (TG)</td>
<td>BAC-P252R</td>
<td></td>
<td>PS (P250R)</td>
<td>Hajihosseini et al. (2004)</td>
</tr>
<tr>
<td>FGFR2</td>
<td>cKO</td>
<td>Heterozygotic abrogation of exon 9 (IIC)</td>
<td>Die within 9 days/coronal synostosis, ocular proptosis, precocious cranial fusion, and disturbed secondary branching morphogenesis</td>
<td>CS/PS</td>
<td>Hajihosseini et al. (2001)</td>
</tr>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 7 (S252W)</td>
<td>Viable/smaller body size, brachycephaly, midface hypoplasia</td>
<td>AS (S252W)</td>
<td>Chen et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>GOF (KI)</td>
<td>Exon 7 (S252W)</td>
<td>Neonatal lethality/smaller size, midline sutural defect, and craniosynostoses, ectopic cartilage at the midline sagittal suture, and increased cartilage in the basicranium, nasal turbinates and long bones</td>
<td>AS (S252W)</td>
<td>Wang et al. (2005)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 7 (P253R)</td>
<td>Viable/smaller body size, brachycephaly and syndactyly, premature closure of cranial sutures coronal suture, shortened cranial base and growth plates in long bone</td>
<td>AS (P253R)</td>
<td>Yin et al. (2008)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 9 (C342Y)</td>
<td>Viable/shortened face, protruding eyes, premature fusion of cranial sutures</td>
<td>CS/PS (C342Y)</td>
<td>Eswarakumar et al. (2004)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 10 (Y394C)</td>
<td>Postnatal lethality/epidermal hyperplasia and premature closure of cranial sutures</td>
<td>BSS (Y394C)</td>
<td>Wang et al. (2012)</td>
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<td>FGFR3</td>
<td>KO</td>
<td>Exon 5</td>
<td>Viable/bone overgrowth, decreased bone mass</td>
<td>CATSLH syndrome</td>
<td>Deng et al. (1996)</td>
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<tr>
<td></td>
<td>KO</td>
<td>From Ig-like domain II to the transmembrane domain</td>
<td>Viable/bone overgrowth, decreased bone mass with defective mineralization, early onset of arthritis, deafness</td>
<td>CATSLH syndrome</td>
<td>Colvin et al. (1996) and Valverde-Franco et al. (2004)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 7 (P244R)</td>
<td>Viable/abnormal craniofacial morphology, decreased cortical thickness and bone mineral densities</td>
<td>MS (P250R)</td>
<td>Twigg et al. (2009)</td>
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<td>GOF (KI)</td>
<td>Exon 9 (Y367C)</td>
<td>Viable (die at 6–8 weeks after birth)/smaller body size, hearing loss with inner ear defect</td>
<td>TDI (Y373C)</td>
<td>Pannier et al. (2009)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 10 (G369C)</td>
<td>Viable/macrocephaly and shortened limbs, premature closure of cranial base synchondroses</td>
<td>ACH (G375R)</td>
<td>Chen et al. (1999)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 10 (G374R)</td>
<td>Viable/small size, short tail, macrocephaly and dome-shaped heads, narrower epiphyseal growth plates</td>
<td>ACH (G380R)</td>
<td>Wang et al. (1999, 2004)</td>
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<td></td>
<td>GOF (KI)</td>
<td>K644E cDNA knock in</td>
<td>Viable/retardation of bone growth, macrocephaly, and shortening of the long bones resembling ACH patients</td>
<td>Similar to ACH</td>
<td>Li et al. (1999)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 15 (K644E)</td>
<td>Neonatal lethality (die within 1 day after birth)/shorter and curved long bones with decreased ossification and disorganized growth plates</td>
<td>TDII (K650E)</td>
<td>Iwata et al. (2000)</td>
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<td></td>
<td>GOF (KI)</td>
<td>Exon 15 (K644M)</td>
<td>Viable/acanthosis nigricans and anomalies in CNS in addition to severe skeletal dysplasia</td>
<td>SADDAN (K650M)</td>
<td>Iwata et al. (2000)</td>
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<td></td>
<td>OE (TG)</td>
<td>Col2- G374R</td>
<td>Viable/shortening of both the axial and appendicular skeleton, with proportional shortening of proximal and distal bones of the limbs</td>
<td>Similar to ACH</td>
<td>Naski et al. (1998)</td>
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<td></td>
<td>OE (TG)</td>
<td>FGFR3- hG380R</td>
<td>Viable/disproportionate dwarfism similar to human ACH</td>
<td>ACH</td>
<td>Segev et al. (2000)</td>
</tr>
</tbody>
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Table 1 Continued

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Mutation position</th>
<th>Survival/phenotypes</th>
<th>Related human syndromes (mutation)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF2</td>
<td>OE (TG)</td>
<td>Col1a2.6-HMW FGF2-IRE5-GFPsaph</td>
<td>Viable/dwarfism, osteomalacia, hypophosphatemia and increased FGF23 level</td>
<td>Similar to XLH</td>
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<td>FGF23</td>
<td>OE (TG)</td>
<td>Up-regulation of FGF3/4 caused by retroviral insertion</td>
<td>Viable/facial shortening and precocious closure of several cranial sutures (craniosynostosis)</td>
<td>Similar to CS</td>
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<td>FGF9</td>
<td>GOF (spontaneous mutation)</td>
<td>N143T</td>
<td>Lethal/radiohumeral and tibiofemoral synostosis, craniosynostosis, lung hypoplasia</td>
<td>EKS</td>
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<td>FGF23</td>
<td>OE (TG)</td>
<td>Col1a-hFGF23</td>
<td>Viable/rachitic bone and growth retardation, hypophosphatemia and low serum 1,25(OH)2D level</td>
<td>ADHR</td>
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<td>FGF23</td>
<td>OE (TG)</td>
<td>Apoe3-hFGF23-R176Q</td>
<td>Viable/rachitic bone, hypophosphatemia, and low serum 1,25(OH)2D level</td>
<td>ADHR</td>
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<tr>
<td>FGF23</td>
<td>OE (TG)</td>
<td>GOF FGF23</td>
<td>Viable/rachitic bone and growth retardation, hypophosphatemia and low serum 1,25(OH)2D level</td>
<td>ADHR</td>
</tr>
</tbody>
</table>

GOF, gain of function; KI, knock-in; OE, overexpression; TG, transgenic; cKO, conditional knockout; CS, Crouzon syndrome; PS, Pfeiffer syndrome; AS, Apert syndrome; BSS, Beare–Stevenson cutis gyrata syndrome; CATSHL, camptodactyly, tall stature, and hearing loss; MS, Muenke craniosynostosis syndrome; TD, thanatophoric dysplasia; ACH, achondroplasia; SADDAN, severe achondroplasia with developmental delay and acanthosis nigricans; XLH, X-linked hypophosphatemic rickets; EKS, elbow-knee synostosis; ADHR, autosomal dominant hypophosphatemic rickets.
development and reduced hypertrophic chondrocytes throughout the embryonic stage (Li et al. 1999, Iwata et al. 2000). The Fgfr3 K644M mutation causes severe dwarfism in mice (Iwata et al. 2001) and the Y367C mutation causes chondrodysplasia, hearing loss, and inner ear defects in mice (Pannier et al. 2009). The above data indicate that FGFR3 negatively regulates the proliferation and differentiation of chondrocytes. Results from several studies have indicated that FGFR3 signaling inhibits chondrocyte proliferation through STAT1 signaling by inducing the expression of P21 (Su et al. 1997, Li et al. 1999, Sahni et al. 1999, Murakami et al. 2004), and promotes chondrocyte terminal hypertrophic differentiation partially through MAPK (Minina et al. 2002, Dailey et al. 2003). The cellular mechanisms underlying the growth arrest of chondrocytes induced by FGFR3 remain unclear. Chen et al. (1999) found that ACH mice exhibits increased staining for cell-cycle inhibitors including P16 and P19 in growth plates. Further studies have shown that P21 and P27 are accumulated upon FG2 treatment and that the expression of P21 is increased in the chondrocytes of achondroplasic children (Krejci et al. 2004, Parafioriti et al. 2009). These results indicate that upregulation of cell-cycle inhibitors contributes to the growth arrest of chondrocytes. Some results indicate that FGFR3 downregulates the IHH signaling pathway in both growth plate chondrocytes and perichondrium (Naski et al. 1998, Iwata et al. 2000). JAK/STAT has been found to mediate the downregulation of PTH/PTHrP signaling by FGFR3 (Chen et al. 2001, Li et al. 2010). FGFR3 inhibits the proliferation of chondrocytes by downregulating telomerase reverse transcriptase expression and reducing telomerase activity (Mendelsohn & Larrick 2012). Results from some studies indicate that FGFR3 can induce chondrocyte apoptosis partially through the PLCγ-STAT1 pathway (Yamanaka et al. 2003, L’Hote & Knowles 2005, Harada et al. 2007, Krejci et al. 2010, Elo et al. 2012). Henderson found that the expression of G380R FGFR3 in CFK2 chondrocytic cells inhibits cell growth but protects them from apoptosis caused by serum starvation (Henderson et al. 2000). Krejci demonstrated that activation of endogenous Fgfr3 in rat chondrosarcoma (RCS) cells (proliferating chondrocytes derived from RCS) leads to a reversible premature senescence phenotype (Krejci et al. 2010). There are also some disputes about the role of FGFR3 in hypertrophic differentiation of chondrocytes. Chen and colleagues found that FGFR3 inhibits the hypertrophic differentiation of chondrocytes in cultured metatarsals (Chen et al. 1999), while Minina et al. (2002) revealed that FGFs/FGFR3 signaling accelerates the onset and the pace of hypertrophic differentiation of chondrocytes in limb culture system. Conversely, Fgfr3 deficiency in mice causes increased bone length due to increased chondrocyte hypertrophy (Colvin et al. 1996, Deng et al. 1996). FGFs/FGFR3 signaling decreases the chondrocyte ECM. On the one hand, FGFR3 signaling inhibits synthesis of chondrocyte ECM through inhibition of the expression of the matrix proteins such as aggrecan and collagen2 (Krejci et al. 2004, Foldynova-Trantikova et al. 2012). On the other hand, FGFR3 can promote the degradation of ECM via upregulating the expression, release, and activation of several MMPs, including MMP3, 9, 10, and 13 (Krejci et al. 2005). Fgfr3-deficient mice show early onset of arthritis, and disruption of Fgfr3 in cartilage at the adult stage leads to early onset of arthritis with elevated expression of MMP13 (Valverde-Franco et al. 2006). FGF18/FGFR3 signaling is involved in autophagy of chondrocytes (Bernheim & Benchetrit 2011). FGFR3 signaling inhibits C-type natriuretic peptide (CNP) signaling, while CNP antagonizes the activation of the MAPK (Ozasa et al. 2005). Snail1 has been found to be a downstream molecule of FGFRs/FGFR3 signaling, regulating both STAT and MAPK in chondrocytes (de Frutos et al. 2007). Results obtained by Shung and colleagues revealed that dysregulation of SOX9 and β-catenin levels and their activity in growth plates might be an important underlying mechanism in skeletal dysplasias caused by mutations in FGF3 (Shung et al. 2012). Furthermore, FGFR3 also regulates osteogenesis (Marie et al. 2012). FGFR3 P244R mice mimicking human Muenke craniosynostosis syndrome show rounded skulls, shortened snouts and decreased cortical thickness and bone mineral densities in long bones, indicating that FGFR3 participates in the regulation of osteogenesis (Twigg et al. 2009). Su et al. (2010), found that FGFR3 inhibits the proliferation of bone marrow stromal cells, but promotes their osteogenic differentiation. Activation of FGFR3 in cartilage induces premature synchondrosis closure and enhances osteoblast differentiation around synchondroses through upregulation of expression of BMPs and down-regulation of expression of BMP antagonists, Noggin, Chordin, and Gremlin expression via MAPK pathway (Matsushita et al. 2009). Mugniery and colleagues found that activating FGFR3 signaling pathways may affect trabecular bone formation by a paracrine mechanism and that FGFR3 has a direct effect on osteoblasts (Mugniery et al. 2012). Conditional deletion of FGFR3 in osteoblasts (via OC-Cre) leads to impaired bone formation and remodeling, indicating the vital role of FGFR3 in the differentiation and function of osteoblasts (Nan Su & Lin Chen 2013 unpublished observations). Both FGFR3 deletion and activation lead to defective bone mineralization and osteopenia with changed osteoclastic activity, indicating an indirect effect of FGFR3 on osteoclasts.
through its regulation of osteoblasts (Valverde-Franco et al. 2004, Su et al. 2010).

Fgf4-deficient mice are developmentally normal, while Fgf3/Fgf4 double-null mice show pronounced dwarfism (Weinstein et al. 1998). Further studies revealed that Fgf3/Fgf4 double-null mice have lower serum phosphorus levels and elevated FGF23 and 1,25(OH)2 vitamin D3 levels compared with WT mice (Gattineni et al. 2011). The supraphysiological levels FGF23 and 1,25(OH)2 vitamin D3 may be responsible for the growth retardation of Fgf3/Fgf4 double-null mice (Larsson et al. 2004, Kawai et al. 2013, Bach et al. 2014). Results also indicated that FGFR1, FGFR3, and FGFR4 function cooperatively to mediate the effects of FGF23 on kidney, and that loss of FGFR function leads to feedback stimulation of FGF23 expression in bone (Li et al. 2011a, Gattineni et al. 2014).

Fgf2 transgenic mice (TgFgf2), such as the dwarf, show shortened bone and moderate macrocephaly and defective bone formation and mineralization (Coffin et al. 1995). Fgf2 deficiency in mice leads to osteopenia in the adult stage, without observable abnormalities during development (Montero et al. 2000). Results from multiple studies have indicated the role of ectogenic FGF2 in regulation of bone formation (Mayahara et al. 1993, Nakamura et al. 1995). Sobue et al. (2005) proposed that FGF2 negatively regulates postnatal bone growth and remodeling resulting from targeted overexpression of Fgf2 in the chondrocytes and osteoblasts of mice. FGF2 also participates in bone homeostasis and phosphate metabolism. Targeted overexpression of high molecular weight (hmw, 21 and 22 kDa) isoforms of FGF2 in osteoblasts of mice results in dwarfism, decreased BMD, increased FGF23 level, hypophosphatemia, and rickets/osteomalacia, which is similar to XLH (Xiao et al. 2010, 2013). Targeted overexpression of low molecular weight isoform (lmw, 18 kDa) of FGF2 in osteoblasts leads to increased BMD, bone mass, and enhanced mineralization, resulting from the reduced expression of the secreted frizzled receptor 1, a Wnt antagonist (Xiao et al. 2013). In contrast to TgFgf2lmw mice, Fgf2hmw−/− mice show markedly reduced BMD and impaired mineralization (Xiao et al. 2009). Interestingly, endogenous FGF2 has been proved to be required for bone formation and osteoclastogenesis either under basal conditions or after treatment with PTH and BMP2 (Okada et al. 2003, Hurley et al. 2006, Sabbieti et al. 2009). Activating transcription factor 4 (ATF4) (Fei et al. 2011) and prostaglandin F2α also induce osteoblast proliferation and differentiation via endogenous FGF2 (Sabbieti et al. 2010). Insertional mutations at the Fgf3/Fgf4 locus can lead to craniofacial dysmorphology in mice (Carlton et al. 1998). FGF6 has been considered to be a regulator of bone metabolism as shown by its activity in both osteoblasts and osteoclasts in vitro (Huch et al. 2003). Conditional disruption of Fgf8 in the forelimbs leads to aplasia of radius and/or humerus and digit disorders, but mouse limbs lacking other AerFgfs, such as Fgf4, Fgf9, and Fgf17, show normal skeletal patterns (Moon & Capaceti 2000). Fgf8 can effectively predetermine the osteogenic differentiation of mouse bone marrow stromal cells and C2C12 cells and increase bone formation in vitro (Valta et al. 2006, Omoteyama & Takagi 2009). In another study, Fgf8 has been found to promote proliferation of primary rat osteogenic cells and inhibit osteogenic differentiation and mineralization (Lin et al. 2009). FGF8 has also been shown to be a regulator of ectopic cartilage formation by breast cancer cells (Valta et al. 2006). FGF8 can promote the degradation of cartilage leading to exacerbation of osteoarthritis (Uchii et al. 2008). The Fgf9 N143T mutation in mice causes elbow-knee synostosis (EKS) (Murakami et al. 2002, Harada et al. 2009). Fgf9 promotes chondrocyte hypertrophy and vascularization of the growth plates (Hung et al. 2007). Overexpression of Fgf9 in mouse chondrocytes causes rhizomelia similar to ACH, with disturbed proliferation and terminal differentiation in growth plate chondrocytes (Garofalo et al. 1999). Fgf18 deficiency in mice leads to increased chondrocyte proliferation and delayed long bone ossification and calvarial suture closure with reduced expression of the osteogenic markers such as osteopontin and osteocalcin. These results indicate that FGF18 is involved in chondrogenesis of growth plate and osteogenesis in cortical and trabecular bone, as well as the osteogenesis in the calvarial bone (Liu et al. 2002, Ohbayashi et al. 2002). Increased expression of FGF21 during food restriction causes growth attenuation via antagonizing the stimulatory effects of growth hormone on chondrogenesis, while high concentrations of FGF21 may directly suppress chondrocyte proliferation and differentiation in growth plates (Wu et al. 2012). FGF21 can enhance the osteogenic activity of BMP2 via upregulating the Smad signaling pathway in C2C12 cells (Ishida & Haudenschild 2013). FGF23 is a member of the endocrine FGFs and is mainly produced by bone cells, klotho, a single-pass transmembrane protein, is required for the binding of FGF23 to its receptors (Yu et al. 2005). FGF23 targets the kidney to suppress 1,25(OH)2 vitamin D3 synthesis and accelerate phosphate excretion into the urine. Patients with elevated FGF23 have similar clinical phenotypes and serum biochemical profiles, such as short stature, rickets, osteomalacia, lower extremity deformities,
and a low serum phosphate concentration. Transgenic mice overexpressing Fgf23 have phenotypes similar to the clinical characteristics of ADHR, TIO and XLH (Bai et al. 2004, Larsson et al. 2004, Shimada et al. 2004, Yu & White 2005). Conversely, mice with deletions in Fgf23 show hyperphosphatemia, ectopic mineralization, and poorly formed skeletons with an extremely low PTH level and an elevated 1,25(OH)2vitamin D3 level (Liu et al. 2006). Fgf23 decreases the expression of renal NaPi-2a and NaPi-2c and induces hypophosphatemia predominantly via Fgfr1 (Gattineni et al. 2009). Results from further studies indicate that supraphysiological FGF23 and soluble α-klotho may directly affect bone through their suppression on IHH expression, and administration of IHH protein partially rescued the suppressive effect of FGF23 on metatarsal growth (Shalhoub et al. 2011, Kawai et al. 2013).

Although there are controversies about the role of FGFs/FGFRs signaling in skeletal cells, the results described above indicate that FGF signaling regulates skeletal development and homeostasis by affecting all skeletal cells, including MSCs, chondrocytes, osteoblasts, osteocytes, and osteoclasts. FGF signaling affects skeletal cells through their downstream signaling pathways and their interactions with other molecules controlling skeletal development and homeostasis, including IHH, BMPs, PTHRP, WNTs, SOXs, and RUNX2 (Fig. 3; Murakami et al. 2004, Dailey et al. 2005, Mansukhani et al. 2005, Ambrosetti et al. 2008, Yin et al. 2008, Su et al. 2010, Foldynova-Trantikova et al. 2012, Krejci et al. 2012).

**Promising therapeutic methods to alleviate the skeletal phenotypes resulting from dysfunction FGFs/FGFRs**

A better understanding of the underlying mechanisms involved in the skeletal diseases mentioned earlier will allow the development of novel biologic therapies for these diseases. Accumulated studies have been carried out to alleviate the skeletal phenotypes caused by dysfunctional FGFs/FGFRs signaling (Table 2). For GOF mutations,
the major strategy is to reduce their excessive activities, subsequently alleviating the impaired cell functions, while, for LOF mutations or deficiency, supplementation of related factors may be helpful.

To prevent excessive intracellular signaling and rescue the symptoms of FGFs/FGFRs-related genetic disorders, a variety of molecules targeting FGFRs or their tyrosine kinase activity were used. A soluble form of the Apert mutant, FGR2 (sFGFR2IicS252W), lacking the transmembrane and cytoplasmic domains, as a decoy receptor, can compete for ligand binding with FGFRs and enhance osteoblastic differentiation of the MG63 osteosarcoma cell line transfected with the Apert mutant (Tanimoto et al. 2004). Furthermore, calvarial osteoblasts derived from sFGFR2IicS252W mice show lower activation of MEK, ERK, and p38 pathways than that in osteoblasts from Apert mice (Suzuki et al. 2012). Recently, Morita and colleagues found that sFGFR2IicS252W may partially prevent craniosynostosis in the Apert mouse model (Morita et al. 2014). RNA interference targeting the Fgfr2 S252W can completely rescue the Apert-like phenotype in mice (Shukla et al. 2007). There are also an increasing number of works related to Fgfr3-related skeleton disorders. A31, a novel tyrosine kinase inhibitor, can restore normal expression of cell cycle regulators (proliferating cell nuclear antigen, Ki67, cyclin D1, and p57) and allow pre-hypertrophic chondrocytes to properly differentiate into hypertrophic chondrocytes in cultured femurs from ACH mice (Jonquoy et al. 2012). We have described the utility of a novel FGFR3-binding peptide for rescuing the lethal phenotype and partially restoring the structural distortion of growth plates of mice carrying the TDI mutation (Jin et al. 2012a). Garcia et al. (2013) developed a recombinant protein therapeutic approach using a soluble form of human FGFR3 (sFGFR3), as a decoy receptor, and found that it could rescue the phenotypes of ACH transgenic mice with no toxicity. Another approach to target FGFR3 directly is use of an anti-FGFR3 antibody. To date, antibodies targeting FGFR3 have been developed and shown to exhibit antitumor activity for FGFR3-associated multiple myeloma and bladder carcinoma (Trudel et al. 2006, Hadari & Schlessinger 2009, Qing et al. 2009, Kamath et al. 2012). But antibody may carry a risk of an antibody-dependent cell cytotoxic reaction (Yamamoto et al. 2010), which prevents its use in ACH.

So far, several approaches aiming to reduce excessive activation of FGF signaling by targeting its downstream pathways have been proposed. Treating Fgrf2 S252W mice mimicking AS in humans with an inhibitor of MEK1/2, U0126, can effectively alleviate craniosynostoses (Shukla et al. 2007). An inhibitor of p38, SB203580, can ameliorate skin and skull abnormalities in Beare-Stevenson mice (Wang et al. 2012). Deletion of STAT1 in Ach mice can restore the reduced chondrocyte proliferation but cannot rescue the Ach phenotype (Murakami et al. 2004). ERK has been found to be responsible for the retarded growth of long bones and premature fusion of the synchondroses caused by aberrant FGFR3 expression (Novroozi et al. 2005, Matsushita et al. 2009). Sebastian and colleagues found that genetic inactivation of ERK1 and ERK2 in chondrocytes can enlarge the spinal canal and promote bone growth (Sebastian et al. 2011). MEK inhibitors PD0325901 and AZD6244 are under clinical investigation for cancer treatment, they can expand the hypertrophic zone of the growth plates in cultured bone (El-Hoss et al. 2014). These results indicate that inhibition of ERK signaling may enlarge the narrowing of the spinal canal, alleviating the neurological complications of ACH. As overexpression of SNAIL1 in mice causes an ACH-like phenotype, and SNAIL1 acts as a transcriptional factor for FGFR3 signaling, targeted inhibition of SNAIL1 may be a therapeutic method for FGFR3-related disorders (Martinez-Frias et al. 2010). CNP rescues the shortened bones due to ACH by ameliorating the decreased synthesis of ECM in chondrocytes through inhibition of MAPKs. Targeted overexpression of CNP in cartilage or systemic administration of CNP can rescue the disturbed growth of ACH mice (Yasoda & Nakao 2010). A 39-amino-acid CNP analog (BMN 111) mimicking CNP pharmacologically has an extended half-life. Treating Ach mice with BMN 111 leads to the attenuation of the dwarfism phenotype (Lorget et al. 2012). Weaker PTH/PTR expression was found in the growth plates of Ach mice, we for the first time, to our knowledge, found that PTHRP can partially reverse the growth retardation of cultured long bone rudiments from ACH mice (Chen et al. 2001). Ueda and colleagues found that the PTH1–34 treatment may counterbalance the effects of FGFR3 with ACH mutation on long bone development (Ueda et al. 2007). We further found that intermittent PTH1–34 injection rescues the retarded skeletal development and postnatal lethality of mice mimicking human ACH and TD dysplasia through the promotion of chondrocyte proliferation (Xie et al. 2012). Minina and colleagues revealed that BMP signaling improves the narrowed proliferative and hypertrophic zone in growth plates of ACH mice (Minina et al. 2002). Recently, Matsushita found that Melczozine, a histamine H1 antagonist, facilitates the proliferation and differentiation of chondrocytes by attenuating excessively activated FGFR3 signaling possibly mediated...
by downregulation of ERK phosphorylation (Matsushita et al. 2013). Shung and colleagues revealed that dysregulation of SOX9 and β-catenin levels and activity in growth plates might be an important underlying mechanism in skeletal dysplasia caused by mutations in FGFR3 (Shung et al. 2012) which indicates that regulating SOX9 or β-catenin could be a therapy for these diseases.

Either GOF- or LOF of FGF23 can cause abnormal growth plate development and several metabolic bone diseases (Lu & Feng 2011). Novel therapies, inhibiting the excessive activity of FGF23 or replacement therapy with recombinant FGF23, may be beneficial for those patients with abnormal growth plate development and several metabolic bone diseases. Aono et al. (2009, 2011) showed

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<th>Gene</th>
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<td>FGFR2</td>
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<td>sFGFR2IICs252W</td>
<td>MG63 osteosarcoma cells</td>
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<td>Calvarial osteoblasts</td>
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<td>Transgenic mice</td>
<td>Prevents craniosynostosis in the Apert mouse</td>
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<td>Downstream signaling</td>
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<td>Tyrosine kinase inhibitor A31</td>
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<td>FGFR3</td>
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<td>FGF23</td>
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<td>Neutralizing anti-FGF23 MABs</td>
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that applications of neutralizing anti-FGFR3 MAbs can ameliorate the rachitic bone phenotypes of Hyp mice mimicking XLH in humans (e.g., impaired longitudinal elongation, defective mineralization, and abnormal cartilage development). Administration of either recombinant WT Fgf23 or the ADHR mutant form of Fgf23 to WT mice induces hypophosphatemia with increased renal phosphate clearance (Shimada et al. 2004, Shimada & Fukumoto 2012).

More studies are needed before clinical application, through close collaboration between laboratory scientist and clinicians. Encouragingly, BMN 111 developed by BioMarin Pharmaceuticals is now being evaluated in a Phase 2 pediatric study in children with ACH (http://www.bmrn.com).

**Perspectives**

Although we have made extensive progresses in understanding the roles of FGF signaling in the skeleton, especially in growth plate development and diseases during the last 20 years by using genetically modified mouse models and patients with mutations in FGFs and FGFRs, we are still far from fully understanding their underlying mechanisms, which prevents us from finding effective cures for those FGFs/FGFRs-related genetic diseases and injuries. Much work need to be done before we can develop successful treatments. We here list several important issues that need further study.

Each individual FGF and FGFR has its own spatio-temporal distribution and unique roles in skeletal development and diseases as mentioned earlier (Ornitz & Itoh 2001, Marie et al. 2012). These results are mainly derived from animal models, especially from mice, which are quite different from human beings. For example, FGF18 is expressed in the adjacent perichondrium but not in the cartilage of mice, acting as a physiological ligand for FGFR3 and regulating FGFR3 activity in growth plate chondrocytes and FGFR2 in perichondrium to coordinate chondrogenesis and osteogenesis in mice (Liu et al. 2002, 2007). However, in humans FGF18 is not found in perichondrium or cartilage, and FGFS secreted from perichondrium are unlikely to efficiently diffuse into the whole growth plate chondrocytes, indicating that other FGFS but not FGF18 expressed in human growth plates such as FGF1, FGF2, and FGF17 may be involved in the regulation of chondrocyte proliferation and differentiation (Krejci et al. 2007, Foldynova-Trantikova et al. 2012). The accurate spatiotemporal expressions of FGFS/FGFRs, especially in the case of diseases, need to be further studied. Using conventional or inducible transgenic Cre mice with genes driven by endogenous FGFS/FGFRs promoters, together with reporter mice, it should be possible to explore the expression patterns of FGFS/FGFRs during skeletal development and genetic or acquired diseases/injuries. Expression patterns founded in animal models need to be further checked in clinical specimens. In addition, there are still some FGFs, such as FGF21 and FGF23, that have been found to be expressed in growth plates (Krejci et al. 2007, Raimann et al. 2013), but for which their function in growth plate development and damage is still largely unknown. In vivo models with spatially and temporally modified expression of FGFs, and FGFRs in mice or other animals need to be generated to explore the roles of each individual FGF and FGFR in growth plate development and diseases/injuries.

Although there are accumulating studies about the role of FGFS/FGFRs signaling in growth plate development, we still have very limited information about how FGFS, and FGFRs themselves are regulated by other molecules. We need to know how FGFS, and FGFRs are transcriptionally regulated, whether they are also subjected to epigenetic modification, such as regulation by methylation, non-coding RNA (microRNA and long non-coding RNA). Post-transcriptional modifications (PTM) such as phosphorylation, acetylation, and small ubiquitin-related protein modification are also very important in regulating protein activity; however, we know very little about the PTM of FGFS and FGFRs. Stability of proteins is essential for the maintenance of protein function. Overexpression of Sprouty 1 in chondrocytes results in decreased FGFR2 ubiquitination, increased FGFR2 stability, and sustained ERK activation (Yang et al. 2008). The detailed mechanisms underlying the degradation of FGFRs after ligand binding are poorly known.

FGF signals are transduced by a series of downstream signaling molecules typically including but not limited to FRS2-RAS-MAPK, PI3K-AKT, DAG-PKC, and PLCγ (Chung et al. 1998). Presently, we do not know whether all FGFS, and FGFRs share similar downstream signaling molecules, or whether each individual FGF and FGFR, in different cell lineages or development stages, exert its functions through a distinctive combination of different downstream molecules. For example, are the differential roles of FGFR3 and FGFR1 in growth plate chondrocytes just the result of the distinct spatiotemporal expression patterns of FGFR1, and FGFR3? Or also related to the distinct downstream signaling molecules of FGFR1, and FGFR3? One important phenomenon in this field is the inhibition of AKT activity and chondrocyte proliferation by FGFR3,
which normally acts as a mitogenic signal in other cell lineages. We need to clarify why just FGFR3, but not other FGFRs, can downregulate AKT activity and cause inhibited chondrocyte proliferation and differentiation (Raucci et al. 2004, Priore et al. 2006). Whether there is interplay or redundancy among FGFs and FGFRs during growth plate development and diseases also needs further study. Like other signaling pathways, FGF signaling also need coordination with other pathways to regulate cartilage development and diseases (Jin et al. 2012b, Marie et al. 2012). For example, in early mouse embryos, FGFs and BMPs are integrated to regulate limb-bud overgrowth (Verheyden & Sun 2008). The balance between BMP and FGF signaling pathway determines the rate of chondrocyte proliferation during chondrogenesis (Minina et al. 2002). Some data indicates that FGFs and BMPs can regulate the expression and stability of their downstream signaling components mutually (Matsushita et al. 2009, Retting et al. 2009). We recently found that FGFR3 can down-regulate protein levels of BMPR1a in a proteasome-dependent way (Qi et al. 2014). However, it is not clear how these two important signaling pathways regulate each other in processes involved in the development and damage of growth plates. Many pathways such as WNT/β-catenin, TGF-β, and PTH play essential roles in growth plate development and diseases and the interplay among FGF signaling, and these important signaling pathways occur in multiple cell types of the skeleton during development and disease development. Clarifying the interactions among FGF signaling and other signaling pathways in skeleton cells will deepen our understanding of the mechanisms underlying growth plate development and diseases, which will provide us with the molecular bases to search for therapies for growth plate maldevelopment and diseases/injuries. Different mutations in the same FGFRs cause distinct clinical syndromes, for example A391E in FGFR3 causes CDS, while G380R leads to ACH, P250R in FGFR1 causes PS, but C379R cause OD syndrome, which indicates that mutations in FGFRs do not just cause just GOF or LOF, studying these mutations may lead to identification of novel functions of FGFRs, but we know little about this at present.

Other RTK-related molecules such as VEGF has been found to have intracrine functions (Lee et al. 2007). The traffic of FGFs/FGFRs inside cells may also play an important role in exerting the physiological and pathophysiologic functions of FGFs/FGFRs (Ueno et al. 2011, Coleman et al. 2014). We need to study the trafficking of FGFs/FGFRs, especially the nuclear FGFs/FGFRs, and their role in growth plate development and diseases.

The development of growth plates involves a series of coordinated cellular events including the proliferation and chondrogenic/osteogenic differentiation of mesenchymal cells, and the hypertrophic differentiation, mineralization, and apoptosis of chondrocytes. To date, the detailed effect of individual FGFs and FGFRs on each of these developmental stages of chondrocytes is not fully understood. For example, there are disputes about the role of FGFR3 in chondrocyte proliferation, differentiation, and apoptosis, a similar dispute also exists about the role of FGFR2 in osteoblast differentiation and apoptosis. Furthermore, we have found that prehypertrophic chondrocytes in Ach mice express osteocalcin, a marker for mature osteoblasts (Chen et al. 1999), but whether activating FGFR3 causes the transdifferentiation of prehypertrophic chondrocytes into osteoblasts has still not been elucidated. The activation FGFR2 has been found to cause chondrogenic differentiation of cultured cells in osteogenic medium, collected from the long bone of Apert mice (Wang et al. 2005). All these results indicate that FGF signaling may also change the fates of chondrocytes and osteoblasts. We need to confirm this and explore the underlying mechanisms. Luckily, emerging new techniques will certainly help with exploration of the mechanisms of growth plate development. These new techniques include, but are not limited to, genomics technology, generation of novel Cre/Cre-ERT2 mouse models (inducible, split Cre-ERT, etc.) and endogenous FGFs/FGFRs reporter mice, cell lineage tracing, in vivo dynamic imaging, cell ablation, etc. Also important is cloning of more specific markers for distinct developmental stages of chondrocytes, which could be used to generate more stage-specific Cre mouse strains and to classify developing chondrocytes into more narrow but distinct stages. In addition, emerging clues indicate that FGF signaling regulates many important cellular events such as autophagy, endoplasmic reticulum stress, and cell senescence under both physiological and pathological conditions, but whether FGF signaling also regulate these events in skeletal development and diseases and the underlying mechanisms are still unknown.

Numerous variants clustered in genomic loci including FGF18, FGFR3, and FGFR4 have been found to affect human height (Lango Allen et al. 2010, Lui et al. 2012). FGFR1 has been found to be associated with normal variation in craniofacial shape (Coussens & van Daal 2005). More clinical studies such as Genome Wide Association Studies (GWAS) and exon sequencing need to be carried out to explore the relationships between single-nucleotide polymorphisms in FGF and FGFR genes and skeletal phenotypes including body height, bone shapes, and bone mineral
density, as well as to find new genetic disorders resulting from mutations in FGFs/FGFRs.

Although some drugs such as BMN111 appears to be promising for ACH, as they are for other genetic diseases, we presently do not have effective biological treatments for almost all FGF signaling-related skeletal dysplasias. While measures such as preimplantation genetic diagnosis and prenatal screening should be taken to prevent these diseases, biological treatment of FGF-related genetic diseases is an important research field. In general, we can modulate FGF signaling at multiple levels. For GOF mutations, we can target mutant FGFS, and FGFRs at their DNA, RNA, and protein level. Genome editing is being increasingly used to correct genetic diseases (Li et al. 2011b, Ma et al. 2013), so far little similar work has been done to treat FGF-signaling-related genetic skeletal diseases. These new techniques include, but are not limited to, genome editing technology (zinc finger nuclease, transcription activator-like (TAL) effector nucleases, and CRISPR/Cas9), RNA interference, and neutralizing antibodies (Li et al. 2011b, Lokody 2014, Ochiai et al. 2014). We can also target the downstream signaling molecules such as MAPK by using CNP (Yasoda & Nakao 2010), or we can regulate other pathways involved in regulation of growth plate development, such as PTH (Xie et al. 2012). As there are a variety of downstream molecules and related pathways regulating growth plate development, we need to screen these molecules carefully to find out which molecules and/or pathways are more suitable and practical for use to alleviate the phenotypes of those skeletal dysplasia. Also importantly, we need to test at which levels (DNA, RNA, and protein), using which strategies (e.g., CRISPR/Cas9 versus TALEN, RNAi versus antisense, neutralizing antibody versus chemical inhibitors, etc.) we can better modulate those pathways. Another important issue is to find better way to target those modulating molecules to chondrocytes, the fact that chondrocytes lack a blood supply makes this task more difficult. Finally, skeletal dysplasia is caused by maldevelopment, indicating that the timing of intervention is very important, the treatment should usually be carried out early.

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Thematic Review

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FGF signaling on growth plate

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