

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 diseases 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

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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

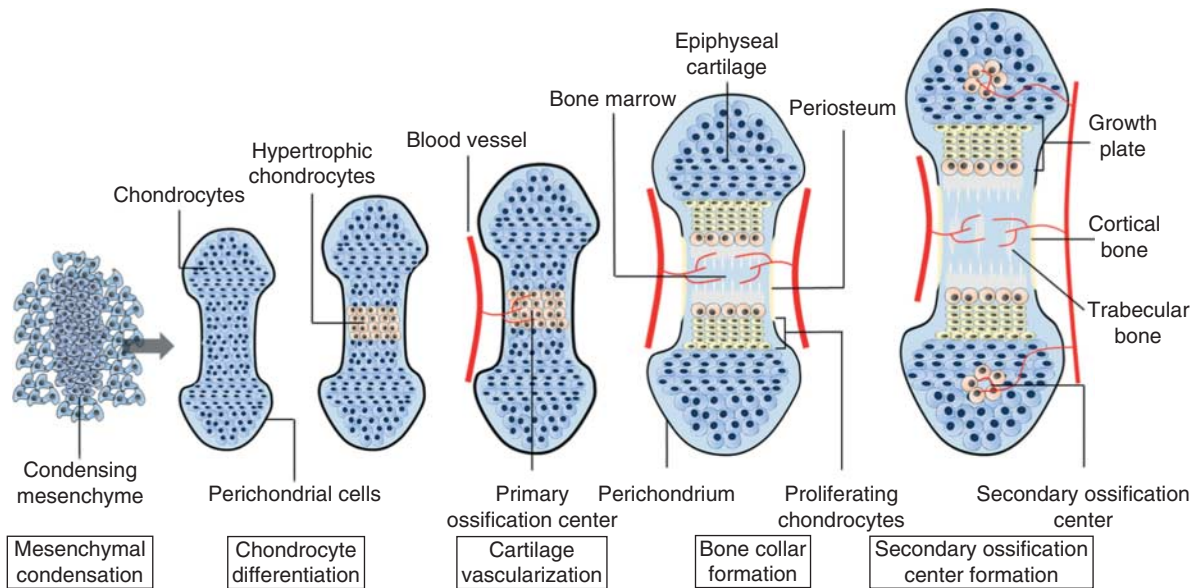


Figure 1

Endochondral bone development. After condensation, mesenchyme gradually differentiates into chondrocytes and forms a cartilage template. Then the chondrocytes will go through hypertrophy, mineralization, and finally apoptosis, leaving the mineralized cartilaginous template. With

invasion of blood vessels into the mineralized cartilage, osteoprogenitors and osteoclasts are brought into replace the cartilaginous template with bone tissue through their bone formation and resorption function (Karsenty & Wagner 2002, Long & Ornitz 2013).

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 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).

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).

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 Rhijn *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 (PTHrP)/IHH feedback loop plays a major role during chondrogenesis (Kobayashi *et al.* 2002). PTHrP is synthesized in the periarticular region, activates the PTH/PTHrP receptor (PTR) expressed in prehypertrophic zone, then stimulates cell proliferation, and delays the hypertrophic differentiation (Eswarakumar *et al.* 2005). Prehypertrophic and hypertrophic chondrocytes release IHH, which stimulates chondrocyte proliferation and PTHrP synthesis. PTHrP 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 PTHrP/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 PTHrP/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 FGFR contains an extracellular ligand-binding domain, a hydrophobic transmembrane region, and an intracellular tyrosine kinase domain (Burdan *et al.* 2009). FGFRs have two major FGFR isoforms generated by alternative splicing of the third extracellular immunoglobulin loop in FGFR transcripts, IIIb and IIIc (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 FGFR 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 (PLC γ)/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

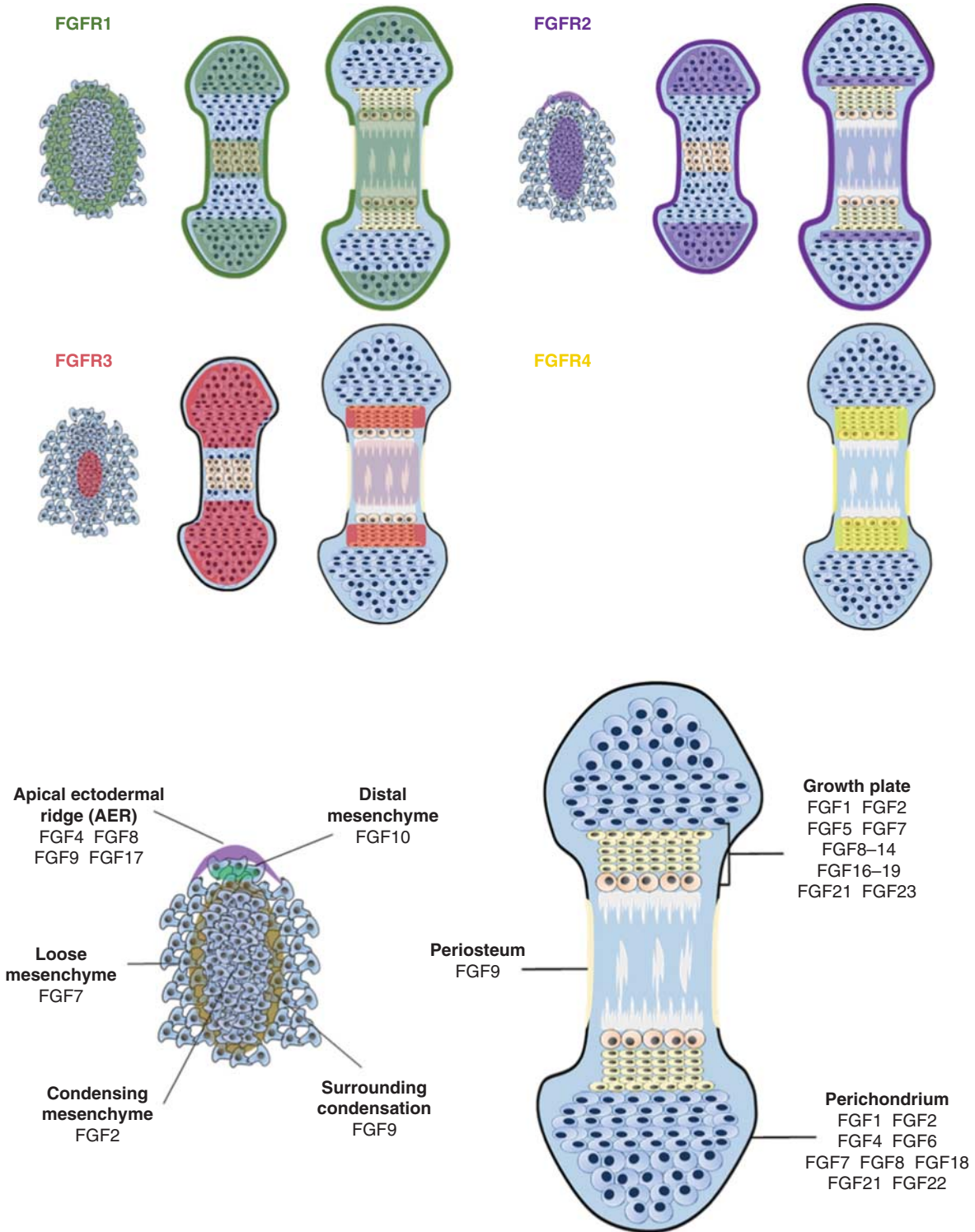


Figure 2

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 endosteum 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 epiphyseal growth plate is formed, FGFR3 is expressed in all chondrocytes except hypertrophic chondrocytes (Peters *et al.* 1993, Szébenyi *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 periosteal and sutural osteogenic fronts (Delezoide *et al.* 1998, Rice *et al.* 2000). Very low amounts of FGFR4 are present in the osteoblasts of calvarial bone and 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). FGF1, 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). FGF18 is expressed apparently in the perichondrium, mesenchymal cells, and osteoblasts during bone development (Liu *et al.* 2002, Ohbayashi *et al.* 2002). FGF1, 2, 6, 7, 9, 18, 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 (Bonewald & 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-ossifying 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).

FGFR3 P250R and P252R mutations cause Muenke syndrome (Meyers *et al.* 1995, Wilkes *et al.* 1996, Muenke *et al.* 1997, Arnaud-Lopez *et al.* 2007, Agochukwu *et al.* 2012). Furthermore, some TD patients show joint fusion and craniosynostoses (Tavormina *et al.* 1995). A p.R621H substitution in the tyrosine kinase domain, leading to partial loss of *FGFR3* function, causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome (Toydemir *et al.* 2006). Until now, no mutation in *FGFR4* has been found to be responsible for genetic skeletal disorders in humans (Valverde-Franco *et al.* 2004, Marie *et al.* 2005).

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 *FGF3*, *FGF7*, *FGF10*, *FGF18*, and *FGFR1* (Riley *et al.* 2007). In humans, constitutionally increased dosage of the *FGF3* and *FGF4* genes plays an important role in the onset of craniosynostosis (Grillo *et al.* 2014). *FGF8* mutations may be associated with craniofacial defects and acrocephalosyndactyly (Whitehead *et al.* 2004, Bouillon *et al.* 2008). A mutation (S99N) in *FGF9* is associated with multiple synostosis syndromes (SYNS) in humans (Wu *et al.* 2009). LOF mutations in *FGF10* cause lacrimo-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. *FGF10* 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). *FGF23* 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 (*Fgfr1*^{-/-}) 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 periosteum. 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 (Larbuison *et al.* 2013). Targeted disruption of *Fgfr2IIIC* 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 1 Mouse models and related human diseases of FGF signaling in skeletal development and homeostasis

Gene	Mouse model		Survival/phenotypes	Related human syndromes (mutation)	References
	Generation methods	Mutation position			
FGFR1	GOF (KI)	Exon 7 (P252R)	Viable/premature suture closure, increased bone formation at suture	PS (P250R)	Zhou et al. (2000)
	OE (TG)	BAC-P252R	Viable/premature suture closure, <i>de novo</i> digit I polydactyly in the hind limb	PS (P250R)	Hajhosseini et al. (2004)
FGFR2	cKO	Heterozygotic abrogation of exon 9 (Ilic)	Die within 9 days/coronal synostosis, ocular proptosis, precocious sternal fusion, and disturbed secondary branching morphogenesis	CS/PS	Hajhosseini et al. (2001)
	GOF (KI)	Exon 7 (S252W)	Viable/smaller body size, brachycephaly, midface hypoplasia	AS (S252W)	Chen et al. (2003)
	GOF (KI)	Exon 7 (S252W)	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	AS (S252W)	Wang et al. (2005)
	GOF (KI)	Exon 7 (P253R)	Viable/smaller body size, brachycephaly and syndactyly, premature closure of cranial sutures coronal suture, shortened cranial base and growth plates in long bone	AS (P253R)	Yin et al. (2008)
FGFR3	GOF (KI)	Exon 9 (C342Y)	Viable/shortened face, protruding eyes, premature fusion of cranial sutures	CS/PS (C342Y)	Eswarakumar et al. (2004)
	GOF (KI)	Exon 10 (Y394C)	Postnatal lethality/epidermal hyperplasia and premature closure of cranial sutures	BSS (Y394C)	Wang et al. (2012)
	KO	Exon 5	Viable/bone overgrowth, decreased bone mass	CATSHL syndrome	Deng et al. (1996)
	KO	From Ig-like domain II to the transmembrane domain	Viable/bone overgrowth, decreased bone mass with defective mineralization, early onset of arthritis, deafness	CATSHL syndrome	Colvin et al. (1996)
	GOF (KI)	Exon 7 (P244R)	Viable/abnormal craniofacial morphology, decreased cortical thickness and bone mineral densities	MS (P250R)	Franco et al. (2004)
	GOF (KI)	Exon 9 (Y367C)	Viable (die at 6–8 weeks after birth)/smaller body size, hearing loss with inner ear defect	TDI (Y373C)	Twigg et al. (2009)
	GOF (KI)	Exon 10 (G369C)	Viable/macrocephaly and shortened limbs, premature closure of cranial base synchondroses	ACH (G375R)	Pannier et al. (2009)
	GOF (KI)	Exon 10 (G374R)	Viable/small size, short tail, macrocephaly and dome-shaped heads, narrower epiphyseal growth plates	ACH (G380R)	Chen et al. (1999)
	GOF (KI)	K644E cDNA knock in	Viable/retardation of bone growth, macrocephaly, and shortening of the long bones resembling ACH patients	Similar to ACH	Wang et al. (1999, 2004)
	GOF (KI)	Exon 15 (K644E)	Neonatal lethality (die within 1 day after birth)/shorter and curved long bones with decreased ossification and disorganized growth plates	TDII (K650E)	Li et al. (1999)
GOF (KI)	Exon 15 (K644M)	Viable/acanthosis nigricans and anomalies in CNS in addition to severe skeletal dysplasia	SADDAN (K650M)	Iwata et al. (2000)	
OE (TG)	Col2-G374R	Viable/shortening of both the axial and appendicular skeleton, with proportional shortening of proximal and distal bones of the limbs	Similar to ACH	Naski et al. (1998)	
OE (TG)	FGFR3-hG380R	Viable/disproportionate dwarfism similar to human ACH	ACH	Segev et al. (2000)	

Table 1 Continued

Gene	Mouse model	Mutation position	Survival/phenotypes	Related human syndromes (mutation)	References
	Generation methods				
<i>FGF2</i>	OE (TG)	Col1a3.6-HMW FGF2-IRES-GFPsaph	Viable/dwarfism, osteomalacia, hypophosphatemia and increased FGF23 level	Similar to XLH	Xiao <i>et al.</i> (2010, 2013)
<i>FGF3/FGF4</i>	OE (TG)	Up-regulation of FGF3/4 caused by retroviral insertion	Viable/facial shortening and precocious closure of several cranial sutures (craniosynostosis)	Similar to CS	Carlton <i>et al.</i> (1998)
<i>FGF9</i>	GOF (spontaneous mutation)	N143T	Lethal/radiohumeral and tibiofemoral synostosis, craniosynostosis, lung hypoplasia	EKS	Harada <i>et al.</i> (2009)
<i>FGF23</i>	OE (TG)	CAG-hFGF23	Viable/rachitic bone and growth retardation, hypophosphatemia and low serum 1,25(OH) ₂ D level	ADHR	Larsson <i>et al.</i> (2004)
	OE (TG)	Col1a-hFGF23	Viable/smaller size, hypophosphatemia, and low serum 1,25(OH) ₂ D level	ADHR	Shimada <i>et al.</i> (2004)
	OE (TG)	Apoe3-hFGF23*R176Q	Viable/rachitic bone, hypophosphatemia, and low serum 1,25(OH) ₂ D level	ADHR	Bai <i>et al.</i> (2004)

GOF, gain of function; KI, knock-in; OE, overexpression; TG, transgenic; cKO, conditional knockout; CS, Crouzon syndrome; PS, Pfeiffer 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.

its role and the underlying mechanisms in osteogenesis. FGFR2 is mainly involved in the regulation of the proliferation of osteogenic cells in sutures (Iseki *et al.* 1997, 1999). Results from some studies have indicated that FGFs/FGFR2 signaling positively regulates the differentiation of osteoblasts (Lomri *et al.* 1998, 2001, Lemonnier *et al.* 2001). The *FGFR2* S252W mutation results in advanced proliferation and differentiation of osteoblastic cells, as well as increased apoptosis of osteogenic cells in mice (Chen *et al.* 2003, Wang *et al.* 2005). On the other hand, results from several studies have indicated that FGFs/FGFR2 signaling blocks the differentiation and mineralization and induces apoptosis of osteoblasts (Mansukhani *et al.* 2000, 2005). Furthermore, the *FGFR2* C342Y mutation (the equivalent mutation in humans causes CS and PS) promotes aberrant cranial osteoblast differentiation and apoptosis, and the mutant bone marrow stromal cells have an autonomous defect in osteoblast differentiation and bone mineralization (Feteih *et al.* 1990, Eswarakumar *et al.* 2004). Studies of the *Fgfr2* W290R mouse model mimicking CS revealed that W290R is a LOF mutation (Gong 2012). Heterozygotic abrogation of *Fgfr2IIIc* in mice leads to a splicing switch, resulting in a GOF mutation, which causes a phenotype similar to those of some AS and PS patients (Hajihosseini *et al.* 2001). Due to the embryonic lethality of conventional *Fgfr2* knockout in mice, conditional knockout of *Fgfr2* has been adopted to explore the role of FGFR2 in osteogenesis. Yu and colleagues found that conditional deletion of *Fgfr2* in mesenchyme (via dermo1-Cre) of mice causes dwarfism and decreased bone mineral density with reduction in diaphyseal thickness, indicating that FGFR2 is required for mineral deposition (Yu *et al.* 2003). Results from a study by Wang and colleagues indicate that FGFR2 may influence transdifferentiation. Cultured cells isolated from the limbs of *Fgfr2* S252W mice can differentiate into chondrocytes in osteogenic medium, and these mice have enhanced chondrogenic markers and ectopic cartilage formation at sagittal sutures (Wang *et al.* 2005). In addition, FGFR1/2 have been found to regulate the markers of mesenchymal stem cells (MSC) and are important in the maintenance of MSC stem cell properties (Coutu *et al.* 2011).

Mice carrying mutations of *Fgfr3*, mimicking human ACH exhibit smaller body size, dome-shaped skulls, and shortened long bones with disorganized chondrocyte columns in growth plates (Naski *et al.* 1998, Chen *et al.* 1999, Wang *et al.* 1999, 2004, Segev *et al.* 2000, Ornitz & Marie 2002). Mice mimicking human TDII-carrying the *Fgfr3* K644E mutation exhibit enhanced chondrocyte proliferation during the early embryonic skeletal

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 FGF2 treatment and that the expression of P21 is increased in the chondrocytes of achondroplastic 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 PLC γ -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-Trantirkova *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). FGFs/*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 FGFs/*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 *FGFR3* (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 (Twiggs *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).

Fgfr4-deficient mice are developmentally normal, while *Fgfr3/Fgfr4* double-null mice show pronounced dwarfism (Weinstein *et al.* 1998). Further studies revealed that *Fgfr3/Fgfr4* 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 *Fgfr3/Fgfr4* 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, *Fgf2^{lmw}-/-* 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 F_{2α} 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 & Capecchi 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 as 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-Trantirkova *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,

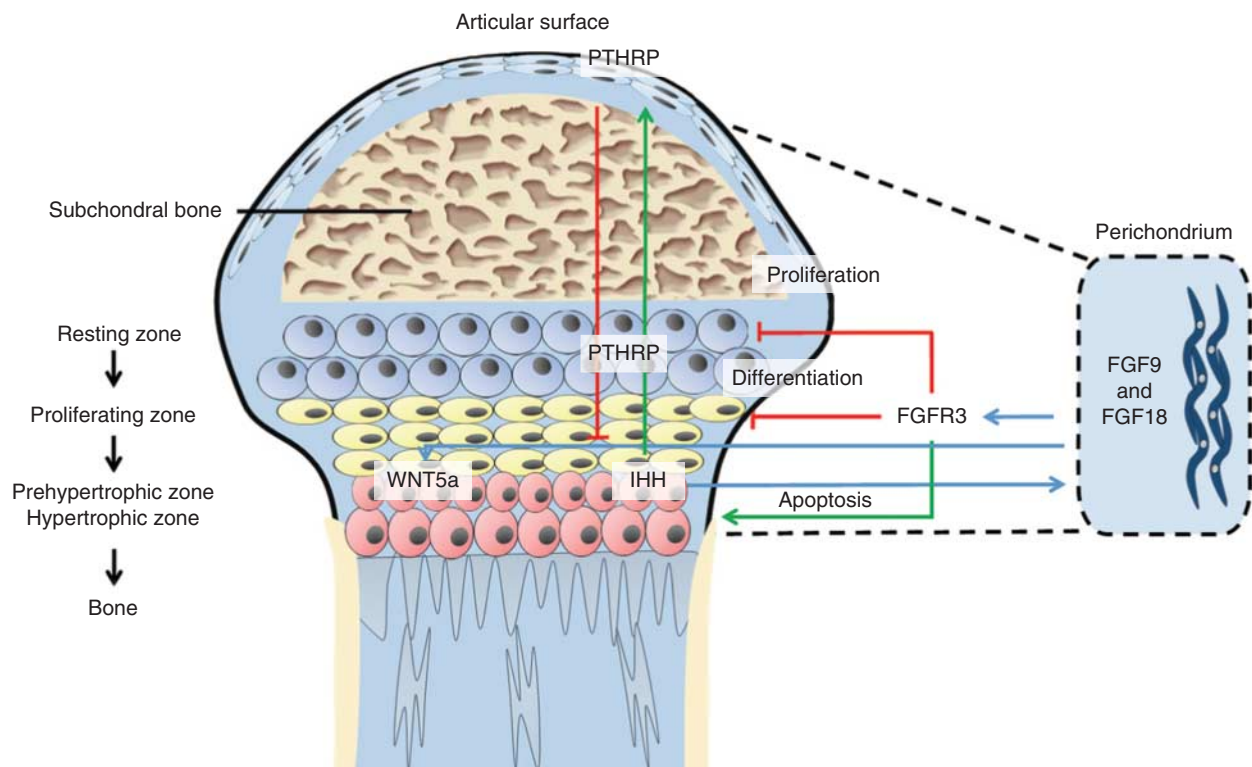


Figure 3

Signals regulating growth plate development. The growth plate is divided into four distinct zones. IHH and PTHRP coordinate chondrocyte proliferation and differentiation through a negative-feedback mechanism. Prehypertrophic and hypertrophic chondrocytes release IHH, which stimulates chondrocyte proliferation and PTHRP synthesis. PTHRP synthesized in periarticular region/resting zone before/after the second ossification

center formation in turn suppresses chondrocyte differentiation associated with IHH expression. FGF9/18 from the perichondrium suppresses chondrocyte proliferation and maturation through FGFR3 in the growth plate during embryonic development and postnatal bone growth. A number of WNTs expressed by growth plate chondrocytes stimulate the proliferation of chondrocytes (data from Long & Ornitz (2013)).

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, *FGFR2* (sFGFR2IIIcS252W), 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 sFGFR2IIIcS252W 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 sFGFR2IIIcS252W 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 TDII 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 *Fgfr2* 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 (Nowroozi *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 PDO325901 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 Meclozine, a histamine H1 antagonist, facilitates the proliferation and differentiation of chondrocytes by attenuating excessively activated FGFR3 signaling possibly mediated

Table 2 Promising therapeutic methods to alleviate the skeleton phenotypes resulting from FGFs–FGFRs related mutations

Gene	Targets	Molecules/methods	Materials	Effects	References
FGFR2	Itself	sFGFR2IIIcS252W	MG63 osteosarcoma cells	Binds to FGFs acts as a decoy receptor	Tanimoto <i>et al.</i> (2004)
			Calvarial osteoblasts	Downregulates the activity of MEK, ERK, and p38 pathways	Suzuki <i>et al.</i> (2012)
FGFR2	Downstream signaling	FGFR2 S252W RNAi MEK inhibitor, U0126 P38 inhibitor, SB203580	Transgenic mice	Prevents craniosynostosis in the Apert mouse	Morita <i>et al.</i> (2014)
			Apert mice	Rescues Apert-like syndrome Rescues craniosynostosis	Shukla <i>et al.</i> (2007) Wang <i>et al.</i> (2012)
FGFR3	Itself or tyrosine kinase	Tyrosine kinase inhibitor A31	Beare–Stevenson mice	Ameliorates skin and skull abnormalities	Shukla <i>et al.</i> (2007)
			Femur from ACH mice	Restores the expression of cell cycle regulator Rescues differentiation of chondrocytes	Jonquoy <i>et al.</i> (2012)
	Downstream signaling	Peptide P3	Cell lines	Inhibits the tyrosine kinase activity of FGFR3	Jin <i>et al.</i> (2012a)
			TDII mice	Reverses the neonatal lethality of Td2 mice	
			Cell lines	Binds to FGFs as a decoy receptor	Garcia <i>et al.</i> (2013)
			ACH mice	Rescues the phenotypes of Ach mice	
			ACH mice	Ameliorates the reduced chondrocyte proliferation	Murakami <i>et al.</i> (2004)
			ACH mice	Enlarges the spinal canal and promotes bone growth	Sebastian <i>et al.</i> (2011)
			Osteoblasts and osteoclasts	Promotes bone formation and inhibits bone resorption	El-Hoss <i>et al.</i> (2014)
			Mice	Expands the hypertrophic zones of the growth plates	
Other signaling	MAPKs antagonist, CNP CNP analog BMN 111 PTHrP PTH1–34 PTH1–34 BMP	ACH mice	Rescues the impaired skeletal growth of Ach mice	Yasoda & Nakao (2010)	
		ACH mice	Attenuates the dwarfism phenotype of Ach mice	Lorget <i>et al.</i> (2012)	
		Metatarsal bone from ACH mice	Partially reverses the retarded long bone growth	Chen <i>et al.</i> (2001)	
		Femur from Ach mice	Promotes bone growth of Ach mice	Ueda <i>et al.</i> (2007)	
		ACH and TDII mice	Rescues the retarded skeletal development	Xie <i>et al.</i> (2012)	
		Limbs from ACH mice	Rescues the reduced domains of proliferating and hypertrophic chondrocyte	Minina <i>et al.</i> (2002)	
FGF23	Itself	Recombinant FGF23	Cell lines and bone explant	Facilitates the proliferation and differentiation of chondrocytes	Matsushita <i>et al.</i> (2013)
			WT mice	Induces hypophosphatemia with increased renal phosphate clearance	Shimada <i>et al.</i> (2004)
FGF23	Itself	Neutralizing anti-FGF23 MABs	Hyp mice	Ameliorates the rachitic bone phenotypes of Hyp mice	Aono <i>et al.</i> (2011)

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

that applications of neutralizing anti-FGF23 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 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-Trantirkova *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 PLC γ (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 downregulate protein levels of *BMPRI1a* 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 pathophysiological 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.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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