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ROLES OF FGF SIGNALING IN SKELETAL DEVELOPMENT AND HUMAN GENETIC DISEASES

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1. ABSTRACT

Fibroblast growth factor receptors (FGFRs) exist as a gene family of 4 membrane bound receptor tyrosine kinases (FGFR1-4) that mediate signals of at least 22 fibroblast growth factors (FGF1-22). FGFs/FGFRs play important roles in multiple biological processes, including mesoderm induction and patterning, cell growth and migration, organ formation and bone growth. Furthermore, it has been shown that missense mutations of FGFR1-3 in human result in, at least, 14 congential bone diseases that are broadly classified into two groups: chondrodysplasia syndromes and craniosynostosis syndromes. The chondrodysplasia affects primarily the skeleton formed through endochondral ossification, resulting short-limbed dwarfisms, while the craniosynostosis affects mainly bones formed through intramembraneous ossification, leading to premature fusion of the craniofacial sutures. Using gene targeting, mouse models mimicking some of these human diseases have been created. Analysis of these mutant mice revealed essential functions of FGFs/FGFRs in skeletal development and maintenance. These models may be beneficial in future studies aimed at developing novel therapeutic strategies for FGFR-related skeletal dysplasias. In this review, we discuss the results of recent studies on FGF receptors to illustrate mechanisms through which the abnormally activated FGF/FGFR signaling results in these diseases.

2. INTRODUCTION: FGFS AND FGF RECEPTORS

Fibroblast growth factor (FGF) family is currently composed of 22 genes that encode structurally related secreted proteins (1-3). In vertebrates, FGFs, with molecular masses ranging from 17 to 34 kDa, are highly conserved in gene structure and amino-acid sequence. FGFs are expressed in spatial and temporal patterns during embryonic developmental stages and adult life. Some FGFs, such as FGF3, 4, 8, 15, 17 and 19 are only expressed during embryonic development, while others, such as FGF1, 2, 9, 18, and 22 are expressed throughout lifespan. FGFs exert their biological activities, through interacting with 2 types of receptors, i.e. low affinity receptors (heparan sulfate proteoglycans) and high affinity receptors (FGFRs). Heparin/Heparan sulfate, FGF, and FGF receptor form a trimolecular complex (4). Different FGFRs share 55% and 72% homology at the amino acid level (5). As membrane bound receptor tyrosine kinase, a typical FGFR contains a hydrophobic leader sequence, three immunoglobulin (Ig)-like domains, an acidic box, a transmembrane domain, and a divided tyrosine kinase domain. Classically, FGFR1, 2, 3 have two major isoforms (IIIb, and IIIc) generated through alternative splicing (6,7). For FGFR2, the carboxy-terminal half of IgIII is either encodes by exon 8 (IIIb, FGFR2b) or exon 9 (IIIc, FGFR2c). FGFR2b is expressed in epithelial lineages, while FGFR2c expression is restricted to mesenchyme

(8,9). Interestingly, FGF ligands and FGF receptor isoforms interact with each other in a paracrine fashion, i.e. the mesenchyme-based ligands (FGF7, and 10) activate only FGFR2b that is expressed in the overlaying epithelium, whereas the epithelium-based ligands (FGF2, 4, 6, 8 and 9) mainly bind to mesenchyme-expressed FGFR2c (8-10). In general, each FGFR or its isoform can bind multiple FGF ligands with varying affinity and specificity, and vice versa. For example, FGF9 can either bind to FGFR3c with high affinity, or bind to FGFR2c with lower affinity.

FGF signaling is essential for the embryonic development and adult homeostasis of almost all the tissues/organs. FGFs transmit their signals into cells through a series of tyrosine phosphorylation mediated by FGFRs and other intermediate signaling molecules. Like other receptor tyrosine kinases (RTK), FGFRs normally exist as an inactivated monomer. The binding of FGFs to the extracellular IgII and IgIII of FGFRs leads to dimerization between FGFR monomers (11,12), which physically bring the intracellular domains of FGFRs together, leading to the phosphorylation of several specific tyrosine residues located in the intracellular domains of FGFRs. Phosphorylated tyrosine residues, in turn, recruit SH2 domain-containing signaling molecules and propagate the signal through multiple pathways [reviewed in (13)]. In general, FGFRs mediate extracellular FGF signals by at least two independent pathways. First, FGFRs directly or indirectly bind to SH2-containing targets enzymes, such as PLC-γ, CRK and SRC (13). Secondly, FGFR is linked to SNT-1/FRS2 (FGFR substracte) (14,15) through an interaction at the juxtamembrane domain. By binding to adaptor protein GRB2/SOS, FRS2 further links FGFRs to RAS-RAF-MAPK (mitogen-activated protein kinases) pathways. Since FRS2 is constitutively associated with FGFR1 without receptor activation (16), this pathway seems to function independently of receptor phosphorylation.

As a family, FGFRs share high degree of homology at the amino acid level (5). They also contain similar structural and functional motifs, suggesting that the signaling pathways mediated by different FGFRs are probably similar. Raffini *et al.* made chimeric receptors composed of the extracellular domain of the human platelet-derived growth factor (PDGF)α receptor and the transmembrane and intracellular domains of FGFR1, or 3 (17). They found that all chimeras could activate phospholipase C-γ, SHC, FRS2, ERK1 and ERK2 after being transfected into PC12 cells. It was therefore proposed that the principal difference between FGFR1, 3, and 4 is the strength (or intensity) of the tyrosine kinase activity, but not the qualitative differences in signaling capacity, i.e. FGFR1, 3, and 4 might have similar signaling pathways, primarily FRS2 and, perhaps, PLC-γ (17). Consistent with this, both receptor kinase domains of FGFR1 and FGFR3 appear to have similar activities and cause similar skeletal phenotypes when expressed in proliferating chondrocytes *in vivo* (18).

Signaling pathways triggered by FGFR activation are involved in almost all kinds of cellular events including cell fate specification, proliferation, differentiation, migration, survival and apoptosis. By regulating these events, FGF signaling plays diverse indispensable roles in the developmental processes and adult homeostasis of living organisms. Thus, FGF signaling must be tightly regulated. Recently, both positive and negative feedback loops have been found. SPRY (Sprouty) was the first feedback regulator of the FGF signaling. First found through genetic screen in Drosophila (19), SPRY acts as an antagonist by binding, through its tyrosine residue phophorylated by FGF signaling, to GRB2 and thus prevents GRB2 binding to FRS2 (20). Another possible mechanism for the antagonist action of SPRY is that its binding to RAF interferes with the activation of the MAPK pathway (21).

FGFR1 and 2 also interact with SEF, a single-pass transmembrane protein with high homology with IL-17 receptor. Ectopic expression of SEF results in blocking of FRS2, and suppressing of phosphorylation of ERK, MEK and AKT, suggesting SEF acts at the level of FGFR to downregulate major downstream signaling pathways of FGFRs [Reviewed in (22)]. On the other hand, XFLRT3, a transmembrane protein found in Xenopus, acts as a positive regulator of FGF signaling during Xenopus development. Overexpression of XFLRT3 in animal explants causes activation of RAS-MAPK pathway, while dominant-negative FGFR1 blocks this activation, suggesting XFLRT3 is specific for RAS-MAPK pathway (23). In addition, other proteins, like PEA3, MKP1, 2, etc, are also involved in the regulation of FGF signaling at various levels. Working together, these molecules act at different levels of the signaling transduction cascade to precisely regulate FGF signaling activities (22).

3. FGFS/FGFRS SIGNALING IN THE DEVELOPING LIMB BUD

The normal limb development initially emerges, at the flank region of the embryo, as a bud of mesenchymal cells from the lateral-plate mesoderm covered by a layer of ectoderm. The outgrowth and patterning of the developing limbs are dependent on three functionally distinct anatomic structures of the limb bud—the apical ectodermal ridge (AER), the zone of polarizing activity (ZPA) and the distal mesenchyme (also termed progress zone, PZ). They work coordinately together to establish over time the proximal-distal (P-D), and anterior-posterior (A-P) axes of the limb [Reviewed in (24-27)].

The AER, which is induced by signals from the rapidly proliferating mesodermal cells, is a specialized thickening structure of ectoderm (epithelium) covering the tip of the limb bud. The AER is essential for the sustained outgrowth and the patterning of the limb through its interaction with the underlying mesenchyme (28,29). The ZPA is a region of mesenchyme at the posterior margin of the limb bud, where sonic hedgehog (SHH) provides the spatial cues for the growing of the limb bud along the A-P axis. The PZ is composed of undifferentiated mesenchymal cells lying beneath the AER. According to a model proposed by Summerbell *et al.* Skeletal pattern of

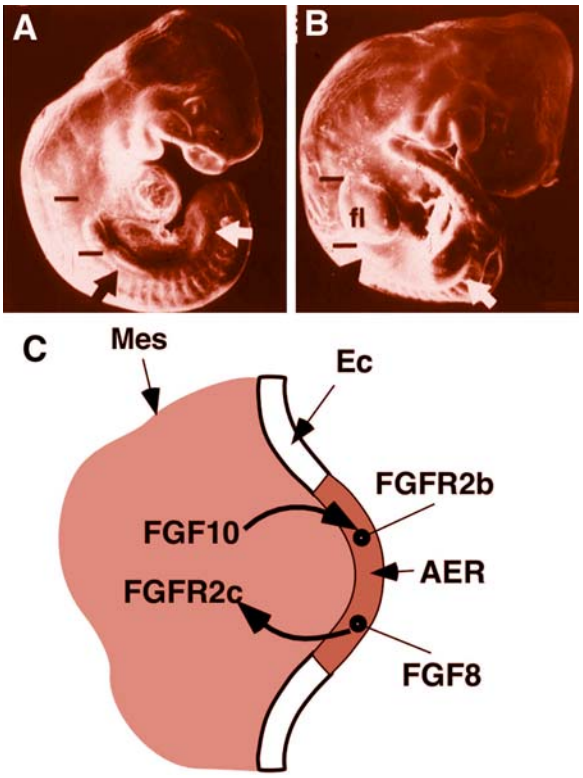

Figure 1. Abence of FGFR2 results in the failure of limb bud initiation. (A, B) Scanning electron microscopy of E10.5 embryos. Both forelimb and hindlimb buds are clearly visible in the control embryo (B), but are absent in mutant embryo (A). The presumptive forelimb (fl) field is marked by two lines and the hindlimb field is indicated by a white arrow. Black arrows points to the lateral ridge that is slightly bigger in mutant. (C) A reciprocal signaling model showing the essential role of FGFR2 in epithelial-mesenchymal interactions during limb bud initiation. *FGF10* transcripts (dark shading) are detected in the mesenchyme (Mes). *FGFR2b* and *FGFR2c* are differentially expressed in the surface ectoderm (Ec) and the underlying mesenchyme, respectively. FGF10 induces *FGF8* expression in the overlying surface ectoderm and ARE through the activation of the ectodermally expressed FGFR2b, and initiates outgrowth of the limb bud. Once it is induced, the FGF8 in the ectoderm interacts with the mesodermally expressed FGFR2c to maintain *FGF10* expression and promote continuous proliferation of the underlying mesenchyme.

developing limb along the P-D axis, including the number, type and position of bones, are determined by the length of time that cells spend in the PZ (30). FGFs/FGFRs are expressed in the limb bud and play essential roles in the initiation, outgrowth and patterning of the limb.

FGF2, 4, 8, 9 and 19, and FGFR2b are expressed in the AER, while FGF10 and FGFR2c are expressed in the mesenchymal cells underlying the AER (9,26,31,32). It has been shown that placing of beads soaked with FGF (FGF2, 4, 8 or 10) on the flank of embryo could induce the formation of a limb, while truncation of the limb caused by removal of the AER can be overcome by implanting beads soaked with FGF2 and FGF4 in the limb mesenchyme (33,34). Furthermore, targeted deletion of FGF4 and FGF8 in the AER generated limbless embryos at birth (25,35). Notably, FGF4 and FGF8 double mutant limb buds were abnormally small despite having initiated normally. Increased apoptosis was found in limb mesenchymal cells, suggesting that AER-FGF serves as a surviving factor that regulates number of precursor cells of nascent limb mesenchyme (25,35). Interestingly, apoptotic cells in the mesenchyme were distance away from the AER, and the alteration of AER-FGF frequently resulted in abnormal formation or complete absence of the proximal skeletal elements (25,35,36). These findings led to a new model for limb development and patterning along the P-D axis (25,36). According to this model, the components of the skeleton (the autopod, zugopod and stylopod) are specified much earlier than assumed by the PZ model and may be independent of drop-out time, and limb outgrowth is associated with expansion and sequential differentiation of these elements (25,36).

The importance of FGFRs in limb development is also demonstrated by gene targeting. FGFR2-null embryos are limbless (Figure 1A, B) and no expression of FGF8 in the presumptive limb ectoderm was found in these embryos, while FGF10 diminished later after initial expression in the mesenchyme (9). Based on these findings, Xu *et al.* proposed a model for the roles of FGFs/FGFR2 in the regulation of limb formation (9). According this model, FGF10 secreted by the underlying distal mesenchyme induces expression of FGF8 in the AER by activating FGFR2b expressed in the ectoderm. On the other hand, FGF8, after diffusion to the underlying mesenchyme, maintains the expression of FGF10 by activating the mesenchymally expressed FGFR2c (Figure 1C).

To further distinguish the functional differences between the mesenchyme-expressed FGFR2c and the epithelium-expressed FGFR2b in limb development, mutant mice carrying targeted disruption of FGFR2b or FGFR2c were generated (37,38). FGFR2b-null embryos have limb buds, however, display extensive apoptosis of ectoderm and mesenchyme of the limbs at embryonic (E) day 10, suggesting that FGFR2b is not required for limb bud initiation although it appears to be essential for limb bud maintenance and growth (37). The limb buds of FGFR2b-null embryos still express *Fgf8*, *Fgf10*, *Bmp4*, and *Msx1*, indicating that these genes are not direct downstream targets of FGFR2b. On the other hand, FGFR2c-null mice did not exhibit obvious abnormalities on limb development, suggesting that FGFR2c is not essential for the initiation or the maintenance of the limb (38).

Compare to FGFR2, FGFR1 is mainly expressed in mesenchyme of limb bud. FGFR1-null embryos die at stages prior to limb induction (39,40). To study the role of FGFR1 during limb development, FGFR1-deficient embryonic stem (ES) cells were injected into wild type embryos to form chimeric embryos (41). Analysis of high degree chimeric embryos indicated that these embryos could initiate limb formation, suggesting that lack of

FGFR1 might not block limb bud initiation (41). However, at E10.5, FGFR1-deficient cells were absent from the distal mesenchyme, while preferentially populating the ectoderm and the AER. At E11.5–E12.5, all chimeric limb buds were developmentally retarded with abnormal shape. Further study in embryos deficient for the full-length isoform of FGFR1 confirmed the essential role of FGFR1 in distal limb mesoderm patterning and digit formation [(42) and our unpublished observation].

4. FGFS/FGFRS SIGNALING IN ENDOCHONDRAL OSSIFICATION AND CHONDRODYSPLASIA

4.1. FGFs/FGFRs expression

There are two fundamental mechanisms of bone formation in the vertebrate: endochondral and intramembranous ossification. The former, accounting for the formation of all the long bones of the organism, involves a two-stage process whereby cartilage-forming cells, chondrocytes, form a pre-bone cartilaginous primordia or template in which osteoblasts differentiate and continue the process of ossification. The latter, which will be discussed in the section 5, is principally involved in the formation of the flat bones of the skull in which mesenchymal cells directly develop into osteoblasts which, in turn, secrete the proteins of the bone matrix, ultimately resulting in calcification and bone formation.

FGF2 expression was found both in growth plate chondrocytes and periosteoblasts of the long bones, but FGF2-deficient mice showed only mild decrease in bone mass without obvious abnormality in chondrogenesis (43). FGF9 is also expressed in chondrocytes of growth plates. But FGF9-null mice have apparently normal skeleton development (44). Overexpression of FGF2 or FGF9 in chondrocytes of mice, however, results in dwarfism phenotypes similar to that caused by gain-of-function mutation in FGFR3 (45,46). Other FGFs, including FGF7, 8, 17, and 18, have been found in perichondrium. FGF18-deficient mice showed skeletal phenotypes in bones formed by both endochondral ossification and intramembraneous ossification (47,48). However, disruption of FGF7, 8, or 17 in mice yields no obvious defects in endochondral ossification (49-51).

Distinct expression patterns of FGFR1, 2, and 3 are found during the developmental processes of endochondral ossification. During early limb development, FGFR1 is expressed in mesenchyme and the periphery of mesenchymal condensations, while FGFR2 is mainly expressed in condensing mesenchyme. FGFR3 is first expressed in chondrocytes differentiated initially from the center of the mesenchyme condensation. During late stage of long bone development, FGFR3 is expressed in reserve and proliferating chondrocytes (52-55). FGFR1 and 2 are expressed mainly in the perichondrium and periosteum. FGFR1 expression is also found in prehypertrophic and hypertrophic chondrocytes. The expression patterns of FGFR1, 2, and 3 are consistent with their roles in endochondral bone development.

4.2. Chondrodysplasia caused by mutations of FGFRs

More than a dozen of human skeletal dysplasias have been linked to point mutations in the genes encoding FGFR1, 2 or 3 [Table 1 and reviewed in (27)]. According to the major skeletons affected, these disorders can be broadly classified into two groups: chondrodysplasia syndromes and craniosynostosis syndromes. The chondrodysplasia syndromes include hypochondroplasia (HCH), achondroplasia (ACH), and thanatophoric dysplasia (TD). Interestingly, all these syndromes are caused by mutations in FGFR3, and their major skeletal defects are found in the long bone formed through endochondral ossification. Among the dwarfisms, ACH is the most common form with a frequency of approximately 1 in 20,000 live births. ACH is characterized by rhizomelic dwarfism (shortening of the proximal and, to a lesser extent, distal element of long bones), lumbar lordosis, macrocephaly (frontal bossing), and depressed nasal bridge (56-58). The phenotypes of HCH, ACH, and TD exhibit progressively increasing clinical severity (59,60). HCH is phenotypically similar to, but much milder than ACH.

TD is the most common lethal neonatal skeletal disorder, and is phenotypically similar to homozygous cases of ACH (61,62). TD patients usually die during perinatal or neonatal period presumably as a result of extremely limited respiration caused by impaired thoracic cage development. Based on their characteristic femora and the presence or absence of cloverleaf skull, TD is subgrouped into TD-I (with straight femurs and severe cloverleaf skull) and TD-II (has curved femurs without severe cloverleaf skull). Approximately 90% of ACH is caused by G380R mutation in FGFR3 (58,63,64). Majority of TD-I patients are associated with R248C mutation. Other mutations including S249C, S371C, Stop807G, Stop807R, have been found in TD-I patients (Table 1). Surprisingly, different mutations at K650 in the tyrosine-kinase domain cause distinct diseases. K650E mutation causes all cases of TD-II, whereas K650M mutation is found to cause Severe Achondroplasia with Developmental Delay & Acanthosis Nigricans (SADDAN; Table 1). As the name suggests, SADDAN patients also display acanthosis nigricans, and structural and functional anomalies in central nervous system alongside severe sekeletal dysplasia. Finally K650N mutation causes HCH (Table 1).

Histologically, the major abnormalities of dwarfism occur at the growth plates of the long bone. The growth plates of patients show narrowed zones of proliferating and hypertrophic chondrocytes with shorter and disorganized proliferating chondrocyte columns (65). Consistent with the severity of clinical phenotypes, the growth plates of TD patients are most severely affected.

Since FGFR3 deficient mice showed overgrowth of long bones, a phenotype opposite to that of dwarfism caused by point mutations in FGFR3, it is suggested that the mutations in FGFR3 causing ACH, HCH and TD are gain-of-function mutations. Further studies confirmed that overactivation of FGFR3 caused by those point mutations result in human genetic dwarfing chondrodysplasia (66,67). Although G380R mutation (ACH) increased the basal activity of FGFR3 by approximately 18 fold, the FGFR3 with G380R can be further activated by FGF ligands

Table 1. FGFR-related skeletal syndromes, phenotypes and their corresponding mutations

Craniosynostosis	Genes and mutations	Common features: autosomal dominant craniosynostosis (prenamture fusion of the cranial sutures), tower-shaped skull, spaced protruding eyes, beaked nose, underdevelopped midface
Antley-Bixler-like (ABS)	FGFR2/IgIIIa: Y290C FGFR2/IgIIIc: S351C	Craniofacial and limb abnormalities (no dermatologic abnormalities)
Apert syndrome (AS)	FGFR2/IgIIIa: S252W, P253 De novo insertion of Alu elements is observed.	Severe syndyctaly (cutaneous and bony fusion of the digits). Broad thumbs broad great toes (due to an increase number of precursor cells). Wild midline calvarial defect.
Beare-Stevenson cutis gyrata (BSS)	FGFR2/TM: Y375C FGFR2/linker IgIII-TM: S372C	Cutis gyrata or furrowed (overgrowth) skin with a corrugated appearance and acanthosis nigricans. Digits abnormalities.
Crouson (CS)	FGFR2/IgIIIa: S267P, C278F, W289G, Y290G, HIQ287-289 , T268-TG FGFR2/IgIIIc: Y328C, G338R, Y340H, C342Y,W, R, F or S, A344G or A, S347C, S354C. FGFR3: P250R, A391G	Normal hands and feet
Crouzon and Acanthosis Nigricans syndrome (CAN)	FGFR3/TM: A391E	Acanthosis, nigricans
Jackson-Weiss syndrome (JWS)	FGFR2/IgIIIc: A344G, C342S or R	Hands are usually normal. Foot abnormalities.
Muenke syndrome (MS)	FGFR3/IgIIIa: P250R	Abnormalities of hands and feet (thimble-like middle phalanges, coned epiphyses, and carpal and tarsal fusions).
Non-syn unilateral coronal synostosis	FGFR3/IgIIIa: P250R	Variable, with or without craniosynostosis
Pfeiffer syndrome (PS)	FGFR1/IgIIIa: P252R FGFR2: A314S, D321A, T341P, C342R, W, Y or S (IgIIIc), V359F FGFR3/IgIIIa: P250R	Short fingers and soft-tissue syndactyly (due to increase expression of KGFR)
Saethre-Chotzen-like syndrome(SCS)	FGFR2/IgIIIa: VV269-70del FGFR3/IgIIIa: P250R	Craniofacial and limb abnormalities
Short-limb dwarfism	Mutations in FGFR3	Common features: autosomal dominant, reduced height of vertebral bodies and shortening of limbs. Poor cellular proliferation of growth plate chondrocytes.
Achondroplasia (ACH)	TM: G346E, G375C, G380R	Rhizomelic dwarfism (most pronounced in the proximal portion of the limbs), relative macroencephaly, exaggerated lumbar lordosis. Homozygotes resemble TD patients (see below)
Severe achondroplasia with developmental delay & acanthosis nigricans (SADDAN)	TKII: K650M	Acanthosis nigricans, developmental delay, craniofacial and limb abnormalities
Thanatophoric dysplasia (TDI or TDII)	TDI: R248C, S249C, S371C, Stop807G, Stop807R, Stop807S TDII: K650E, A391E	Most severe and lethal neonatal skeletal dysplasia TDI: curved, short femurs with or without cloverleaf skull TDII: straight, relatively long femurs and severe cloverleaf skull
Hypochondroplasia (HCH)	TKI: N540K TKII: K650N	Similar to but milder than those of ACH and TD

(66,67). *In vitro* receptor activation assay revealed that there is a correlation between the degree of receptor activation and the severity of the dwarfism phenotype. In general, mutations causing TD lead to more obvious receptor activation than those for ACH and HCH (66,67). FGFR3 mutation can also lead to enhanced receptor activity through other mechanisms. For example, it was demonstrated that ACH-causing mutations of FGFR3 disrupted c-Cbl-mediated ubiquitination, allowing diversion of actively signaling receptors from lysosomes to a recycling pathway where their survival is prolonged. Consquently, signaling capacity of the mutant receptors is increased (68).

4.3 Animal models

So far, a number of mouse models carrying various mutations in FGFR3 have been generated. Table 2 lists the various mutant mouse strains associated with skeletal dysplasias caused by mutations in FGFRs. Phenotypically, these mice exhibit features mimicking the corresponding human conditions [reviewed in (69)]. The major phenotypes include smaller body size, shortened long bone and body lengths as well as dome-shaped skull. Mutant mice have disorganized growth plates with narrow proliferating and hypertrophic zones. The proliferation chondrocyte columns are irregular and shorter. Mutant mice also have advanced bone collar formation and shorter trabecules. Consistent with observations in human, there is also graded severity of these phenotypes among mutants with different mutations. Mice carrying K644E (equivalent to K650E that causes TD-II in human), show most severe phenotypes, and die within few hours after birth, while mice harboring G369C (equivalent to G375C mutation in human) exhibit milder phenotypes than those carrying S365C (equivalent to human S731C that causes TD-I) and K644M (equivalent to human K650M that causes SADDAN). Even for the same mutation, its expression level determines the severity of phenotype. For example, when expression level of K644E mutation, introduced into mouse FGFR3 using a cDNA knock-in approach, was around 10% of the wide type FGFR3, the mutant mice exhibited mild chondrodysplasia mimicking the human HCH condition (70). However, when the same mutation (K644M) was introduced into FGFR3 using classical genomic DNA knock-in approach, it was expressed at a level comparable to the wild-type allele and led to severe dwarfism phenotypes mimicking human TD-II (71).

4.4. Molecular mechanisms for FGFR-related chondrodysplasia

Significant advances in understanding the mechanisms of FGFR mutation-related human skeletal

genetic diseases have been achieved over the last decade. A combination of human and mouse genetics as well as other molecular techniques have contributed greatly to our endeavor to uncover the role of FGFR3 in endochondral ossification. Long bone development is a tightly regulated process involving the elegant coordination of chondrogenesis and ossification at the growth plates, and osteogenesis at the perisoteum. FGFR3 is mainly expressed at reserve, proliferative and prehypertrophic chondrocytes of growth plates, suggesting that its major role is in chondrogenesis. Chondrogenesis is accomplished by the sequential processes of chondrocyte proliferation, differentiation and apoptosis. FGFR3 affects bone development mainly through its influence on each of these processes. It was shown that FGFR3-null mice exhibited faster and prolonged endochondral bone growth as a result of increased proliferation of chondrocytes at the growth plate (54,55). Conversely, mouse models harboring gain-of-function mutation of FGFR3 showed decreased proliferation of growth plate chondrocytes evidenced by shortened length of long bone and decreased height of proliferation zone of chondrocytes in growth plates. Also, FGF treatment of bone rudiment from wild type mice resulted in decreased chondrocyte proliferation (67). These observations lead to the conclusion that FGFR3 is a negative regulator of bone growth.

Using mouse models to study underlying mechanisms responsible for the decreased chondrocyte proliferation, we have found upregulated expression of cell cycle inhibitors (P21, P16, P18, and P19) and a number of STAT proteins (STAT1, STAT5a, and STAT5b) in chondrocytes of growth plates (70,72). These observations are consistent with the results of *in vitro* studies on cultured cells, which showed that the ectopic overexpression of constitutively activated mutant FGFR3 or treatment of cells with FGF could induce nuclear translocation of STAT1 and expression of p21 (WAF1/CIP1) (73,74). Furthermore, the results of transfection assays indicate that mutations of FGFR3 resulting in milder dwarfism (ACH and HCH) induced weaker activation of STAT1 than mutations resulting in severe chondrodysplasia (TD-II). These data suggest that activated FGFs/FGFRs function through STAT1 mediated pathway. Consistently, FGF2 treatment failed to induce growth inhibition in primary chondrocytes derived from STAT1-null mice (74) as it does in wild type chondrocytes.

By crossing transgenic mice overexpressing FGF2 with STAT1-null mice, it was found that the absence of STAT1 could rescue the dwarfism phenotypes in cultured bones (74) and in adult animals (75), further indicating that STAT1 acts downstream of FGF2 to inhibit chondrocyte proliferation. However, in another study (76), it was found that crossing STAT1-null background into achondroplasia mice harboring G374R mutation only restored the reduced chondrocyte proliferation without rescuing the reduced hypertrophic zone and delayed formation of secondary ossification centers observed in ACH mice. The *STAT1*$^{-/-}$*FGFR3* G374R mice still showed achondroplasia-like phenotype, although of a magnitude slightly milder than that of FGFR3 G374R mice (76). It is therefore proposed that STAT1 mainly mediates the inhibitory effects of FGF signaling on chondrocyte proliferation, while MAPK mediates the inhibition of chondrocyte differentiation (76).

Of note, majority of studies using transgenic mouse models showed that FGFR3 activation inhibits proliferation of chondrocytes (67,70,72,77). However, these investigations studied mice at their perinatal and postnatal stages since these animals did not exhibit obvious skeletal defects until after birth (67,70,72). Studies on mutant mice harboring FGFR3-K644E mutation at embryonic stages by Iwata *et al*. revealed that the mutant embryos displayed enhanced proliferation of growth plate chondrocytes during early stages of endochondral ossification (E14-15). At a later gestational age (E18) however there were no obvious differences between mutant and wild type in their chondrocytes proliferation, suggesting that the inhibitory effects of FGF signaling on chondrocyte proliferation is dependent on the developmental stage (71).

FGFs/FGFRs may initiate multiple signaling pathways to exert their unique action on cells proliferation and differentiation. In chondrocytes, activation of STAT1 and pRB family protein p107 and p130 might mediate the chondrocyte growth arrest induced by FGF signaling (78,79). Additionally, PTHrP and IHH signaling has been known to stimulate chondrocyte proliferation, and reduced expression of IHH and PTHrP-R in ACH mice might also indirectly contribute to the decreased chondrocyte proliferation seen in ACH mice. Also, targeted disruption of PTHrP or PTHrP-R in mice results in lethal osteochondrodysplasia characterized by reduced proliferation (80-82). Notably, ACH and TD mice exhibited marked decrease in the expression of *Ihh* and *PTHrP* receptor, while FGF2 treatment directly led to downregulation of the *Ihh* and *PTHrP-R* prior to appearance of bone abnormality (72). Considering the important roles of IHH and PTHrP in long bone development (83), it has been suggested that part of FGFR3's influence on chondrocyte proliferation is mediated through its regulation of IHH and PTHrP-R (Figure 2) (72).

Absence of IHH and PTHrP-R result in expansion of the zone of hypertrophic chondrocytes, suggesting that IHH/PTHrP-R signaling inhibit chondrocyte differentiation (82-84). However, the mice harboring constitutively activated mutant FGFR3, despite the downregulated *Ihh* and *PTHrP-R* in their growth plates, exhibited a much narrower zone of hypertrophic chondrocytes (70,72,85). This observation suggests that FGF/FGF3 signals inhibit chondroctye differention in a manner that is independent of IHH/PTHrP. On the other hand, we have shown that PTHrP treatment could inhibit chondrocyte differentiation in cultured bone rudiments irrespective of their genotypes: wild type, FGFR3-deficient, and FGFR3-activated mutant mice (72). Taken together, these observations indicate that FGF/FGFR3 and IHH/PTHrP signals may function in parallel to inhibit chondrocyte differentiation (Figure 2).

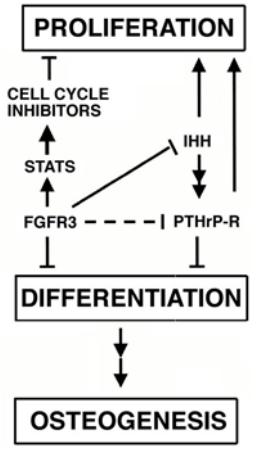

Figure 2. Model of the relations between FGF-FGFR3 and IHH-PTHrP–PTHrP-R signaling in endochondral bone formation. FGF-FGFR3 and IHH-PTHrP–PTHrP-R signals are transmitted by two integrated parallel pathways that mediate both overlapping and distinct functions during the growth of long bones. Both FGFR3 and IHH affect chondrocyte proliferation. However, FGFR3 is a negative regulator of bone growth, whereas IHH positively regulates bone growth. Evidence suggests that FGF-FGFR3 signaling induces activation of STAT proteins, up-regulation of the expression of cell cycle inhibitors, and down-regulation of IHH expression. Both FGF-FGFR3 and PTHrP–PTHrP-R signals inhibit chondrocyte differentiation, and both signals appear to act in a dominant and independent manner.

A number of other genes were also reported to have unexpected roles in modulating FGFR3 function in bone deveopmement. Overexpression of C-type natriuretic peptide (CNP) in chondrocytes rescues achondroplasia phenotype of dwarf mice overexpressing FGFR3 G380R by correcting the decreased extracellular matrix synthesis in the growth plate through inhibition of MAPK pathway of FGF signaling (86). β1-integrins deficient mice showed chondrodysplasia with disorganized proliferation chondrocyte column and decreased proliferation. Surprisingly these abnormalities are accompanied by upregulated expression of FGFR3, P16, P21 and nuclear translocation of STAT1 and STAT5a. This study indicates that β1-integrin may negatively regulate the expression of FGFR3 (87).

Apoptosis of chondrocytes is an essential process for long bone development. Chondrocytes isolated from patients with TD showed increased apoptosis accompanied by increased BAX expression and decreased BCL2 expression (88). Mice overexpressing human FGF2 under the control of a constitutive phosphoglycerate kinase promoter (TgFGF mice) exhibit chondrodysplasia characterized by reduced proliferation and increased apoptosis of growth plate chondrocytes (45,75). These observations suggest that increased apoptosis of chondrocytes may play a leading role in the pathogenesis of chondrodysplasia resulting from activated mutations in FGFR3. Chondrocytes from TD patients show nuclear translocation of STAT1. Targeted ablation of STAT1 (*Stat1*$^{-/-}$) in mice that overexpress human FGF2 (TgFGF2) corrects the reduced proliferation and excessive apoptosis of chondrocytes to near-normal levels (75), suggesting that STAT1 mediates the increased apoptosis and reduced chondrocyte proliferation in mice overexpressing FGF2.

5. FGFs/FGFRs signaling in intramembraneous bone formation and craniosynostosis syndromes

5.1. FGFs/FGFRs expression

FGFs and FGFRs are expressed in cranial bone with distinct spatial and temporal patterns during intramembraneous bone formation. All FGFs, except FGF3 and 4, are identified in coronal suture of E17.5 embryos by RT-PCR (89). FGF2 is expressed in osteogenic mesenchymal cells and osteoblasts (90,91). After birth, FGF2 is produced by mature osteoblasts and stored in the unmineralized bone matrix (92) from where it diffuses into the extracellular environment to form a gradient in the suture. FGF9 is also expressed in mesenchyme of suture in early craniofacial developmental stages (91). During calvarial bone development, FGF18 is expressed strongly in mesenchymal cells and osteoblasts at the sutures and the rims of the calvarial bones as well as the cartilagenous base of the cranium. Expression of FGF20 extends to the rims of all calvarial bones, including the coronal sutures (47,48,89).

Corresponding to the expression patterns of FGFs, FGFRs also have distinct expression patterns during cranial vault formation. FGFR1 is expressed in the calvarial mesenchyme and later in the osteoblasts. FGFR2 is expressed mainly in the proliferating osteoprogenitor cells within the sutures. FGFR3 is expressed primarily in the cranial cartilage, and is also expressed at low levels in the osteogenic front of suture at later developmental stages (93-95). The IIIc splice variants of FGFR1-3 and the IIIb variant of FGFR2 are expressed by differentiating osteoblasts at osteogenic fronts of calvarial bones at E15 (95). FGFR1-3 are all intensely expressed in the cartilages of the cranial base (95,96). Since cranial chondrogenesis in synochondroses might affect suture development indirectly, the expression of FGFR1, 2 and 3 at the cranial base suggests that CS-causing mutations in FGFRs may also manifest as growth defects of the cranial base.

5.2. FGFs/FGFRs signaling in intramembraneous ossification

The distinct expression patterns of FGFs/FGFRs in calvarial bone highlight the important roles of FGF signaling in regulating the calvarial skeletogenesis. Based on the distinct expression patterns of FGFR1 and 2, it was proposed that signaling through FGFR2 regulates stem cell proliferation whereas signaling through FGFR1 is involved in the osteogenic differentiation process but not in maintaining the differentiated state (97). In another study, application of FGF2 or FGF4 soaked beads to developing coronal sutures of mouse resulted in synostotic coronal suture accompanied by induction of apoptosis, collagen type I expression and mineralization (98). In contrast, expression of a dominant-negative FGFR1 gene inhibited suture fusion in rat calvaria (99). Conversely, blocking of endogenous FGF2 activity in chicken using beads soaked with neutralizing antibody against FGF2 prevented cranial

osteogenesis in a dose dependent manner (100). Minor reduction of endogenous FGF2 led to a switch of skeletogenic cells from a differentiative to a proliferative mode whereas further blocking of FGF2 function resulted in abolishing of both proliferation and differentiation.

As a part of a complex signaling network, FGF signaling may interact with many transcription factors in a coordinated way to regulate calvarial bone formation. FGF can upregulate osteocalcin expression in osteoblasts that is inhibited by MSX2 (101). FGF2 also induces Twist expression in mouse calvarial mesenchyme (95). While *Twist* heterozygous mice showed altered FGFR2 expression (95), Funato *et al.* also showed that TWIST could inhibit osteoblast differentiation by downregulating FGFR3 expression (102). This observation suggests that TWIST acts upstream of FGF signaling pathways, and MSX2 may indirectly downregulate TWIST expression through its inhibition of FGF2 (103). Recently, TGF-β family was found to play an important role in suture development (103,104). TGF-β2 and TGF-β3 can regulate osteogenic suture cell proliferation and apoptosis (103,104). Prolonged treatment of FGF2 leads to increased TGF-β2 production in human osteoblasts (105). Conversely, TGF−β regulates FGF2 and FGFRs expression in osteoblastic cells (106). This observation suggests that FGF signaling might affect suture morphogenesis through interaction with TGF-β signaling.

BMP signaling is also very important for skeletal development. BMPs interact with FGF and other signaling pathways to control calvarial bone growth during intramembranous bone development (103,107). Warren *et al.* recently reported that during suture development, FGF2/FGFR2 signaling leads to increased BMP4 activity and suture fusion through its inhibition on the expression of the BMP antagonist, noggin (108), suggesting that FGF signaling can either directly or indirectly control cranial suture fusion through BMP signaling.

In addition to the molecules mentioned above, FGF signaling also interacts with IGF, HGF and VEGF to affect calvarial osteogenesis *in vivo*. For example, FGF2 upregulates IGF-I expression and inhibits the expression of its regulatory binding protein-IGF binding protein-5 in bone cells (109,110). Moreover, FGFs were also found to increase VEGF production of osteoblasts (111).

5.3. FGFs/FGFRs in CS

Craniosynostosis is a clinical condition characterized with precocious closure of one or several calvarial sutures. This relatively common developmental anomaly has been found in over 100 distinct genetic syndromes and has an estimated overall incidence of 1 in 2500-3000 live birth (reviewed in (112-115). Besides malformed skull with varying severity, patients, in some cases, have appendicular skeleton malformation, such as syndactyly of the hands and feet (Apert syndrome) or broad thumbs and big toes (Pfeiffer syndrome), and neurological sequelae of increased intracranial pressure that needs surgical intervention. Some CS patients exhibit other complication such as deafness, blindness and mental retardation. Depending on the sutures affected and the accompanying noncranial complications, CS patients have adopted clinical syndromic designations that include Apert, Crouzon, Pfeiffer and Saethre-Chozen syndromes (Table 1) each with its own characteristic craniofacial features.

Apert is one of the most severe forms of CS caused by S252W and P253R in FGFR2. Mutations in FGFR3 have also been found to cause CS. For example, the P250R mutation of FGFR3 is associated with unicoronal or bicoronal synostosis. Most of the mutations in FGFRs responsible for CS are sporadic, dominant gain-of-function missense mutations. The major histological features of CS patients are premature fusion and excessive bone formation at the suture (Table 1).

5.4. Animal models

Using human material and cultured wild type or mutation-carrying cells, significant achievements have been made in recent years in the elucidation of molecular and cellular mechanism of CS. To study the underlying molecular mechanisms of FGFRs related CS in a better-controlled condition, mouse models for the FGFR-related CS have been generated by gene targeting approach (Table 2). A P250R mutation (corresponding to the P252R mutation found in human Pfeiffer syndrome) was introduced into the mouse FGFR1 to generate mouse model mimicking human PS (116). The mutant mice exhibited craniofacial phenotype mimicking that of Pfeiffer patients, which includes anterior-posteriorly shortened, laterally widened, and vertically heightened neurocraniums, and grossly as well as histologically confirmed premature fusion of the interfrontal, coronal and sagittal suture. Compared with wild type mice, the sutures of mutant mice had more AKP-positive cells and transiently enhanced osteoblast proliferation, and intensified expressions of osteoblast genes including Cbfa1, BSP (Bone Sialoprotein), osteopontin and osteocalcin in sutures. The upregulation of *Cbfa1*/*Runx2* by FGFR1 signaling was further confirmed by transfection of wild type and mutant FGFR1 into cultured cells (116).

These data suggest that P250R mutation promotes the differentiation of sutural osteoblasts through the activation of CBFA1/RUNX2 (116). Zhou *et al.* proposed that premature differentiation of osteoblasts might be responsible for the human Pfeiffer syndrome caused by P252R in FGFR1. Using a similar strategy, we have generated a mouse model mimicking human Apert syndrome by introducing a S252W mutation into mouse FGFR2 (117). Like human Apert patients, the mutant mice showed only premature fusion of the coronal suture, while the sagittal suture was normal. In contrast with results from human samples and cultured cells, mutant mice have neither significant changes in proliferation (BrdU/3H-thymidine incorporation) nor obvious alterations of *Bsp*, *Akp*, *Osteocalcin* and *Cbfa1*/*Runx2* expression, suggesting that the proliferation activity and differentiation of sutural cells are changed. Interestingly, the mutant mice have increased apoptosis and BAX expression in the coronal suture (117). This observation suggests that increased cell death, instead of altered proliferation or differentiation, is the primary reason for the occurrence of Apert syndrome. It

Table 2. Current mouse models for FGF/FGFR-related human skeletal dysplasias

Genes	Human syndromes	Mutations in human genes	Mutations in Mouse genes	References
FGFR1	PS	P252R	P252R	116
		P252R	P252R BAC transgenic	141
FGFR2	AS	S252W	S252W	117
		P253R	S253R	Chen *et al.* unpub. data
	CS/PS	C342Y	C342Y	118
		N/A	Gain of function in IIIc	142
FGFR3	ACH	G380R	G374R	143
		G380R	G374R	77
		G380R	G374R transgenic	85
		G380R	G374R transgenic	144
		G375C	G369C	67
	TDI	S371C	S365C	72
	TDII	K650E	K644E	71
		K650E	K644E cDNA knock-in	70
	SADDAN	K650M	K644M	145
FGF3/FGF 4	Craniosynostosis	N/A	Up-regulation of Fgf3/4 caused by retroviral insertion	146
FGF2	Dwafism	N/A	FGF2 transgenic mice	45
FGF9	Dwarfism	N/A	FGF9 transgenic mice	46

has long been thought that defects in cranial base might inform the pathogenesis of craniosynostosis. However, the FGFR2-S252W and FGFR1-P250R mutant mice did not display histological abnormalities in cranial base, suggesting that the major reasons for the FGFR1, and 2 related craniosynostosis are abnormal morphogenesis in the sutures with very limited role for the cranial base.

Recently, a viable mouse model with a Cys342Tyr mutation in FGFR2 (equivalent to a mutation in human for Crouzon and Pfeiffer syndromes) showed shortened face, protruding eyes, and premature fusion of cranial sutures (118). More importantly, these mice showed enhanced *Cbfa-1* and *Spp1* (osteopontin) expression, indicating increased osteogenesis. Notably, the mutant mice exhibited significantly increased number of osteoblasts in the femur and osteoprogenitor cells in the bone marrow stem cells. There also was an increase in the number of proliferating (osteoprogenitor) cells in the coronal suture at E14.5.

5.5 Molecular mechanisms for FGFR-related CS

Although studies have shown that majority of the mutations corresponding to CS in FGFR1, and 2 lead to constitutive activation of the receptors (2,27,67,119), the underlying mechanisms responsible for the activation seem different. Some mutations in FGFR1 and 2 constitutively activate the receptors by stabilizing intermolecular disulfide bonds, causing ligand-independent dimerization and activation. Many mutations occur in the ligand-binding region, between Ig like domains II and III, and result in loss or gain of a cysteine residue, leading to ligand-independent activation of the receptors. The FGFR2-S252W mutation, however, was not found to result in constitutive activation of the receptor, but instead, receptors with this mutation bind ligands tighter (120). Yu *et al.* reported that the FGFR2-S252W mutation altered the ligand-binding specificity of FGFR2 and allows the mesenchymal splice form (FGFR2c) to bind and be activated by the mesenchyme-expressed FGFs (FGF7 and 10). Furthermore, the epithelial splice form (FGFR2b) binds and is activated by the epithelium-expressed FGFs (FGF2, 6, and 9) resulting in disturbed FGF signaling within the suture (10,121). The C342Y mutation in FGFR2 results in ligand-independent FGFR2 activation and significantly decreased binding of FGF2 to the receptor (122).

Like many anatomic structures, suture development depends on the balance between mesenchymal cell condensation, proliferation, differentiation and apoptosis. Perturbation of any of these processes may lead to suture anomaly and subsequent malformation of the skull. Elevated cell numbers are proposed as a contributory factor for premature closure of cranial suture. FGF4 in cultured calvaria and overexpression of MSX2 in mice led to premature suture fusion with increased cell proliferation (91,123). Recombinant human FGF1 treatment increases the number of osteogenic cells and promotes calvarial osteogenesis of mice (123). Conversely, disruption of FGF2 gene in mice results in decreased calvarial bone formation and bone mass (43). In addition, blocking of endogenous FGF2 activity inhibits cranial osteogenesis (100).

FGFs signaling also regulate differentiation of calvarial cells. Local application of FGF2 induces suture closure accompanied by locally decreased cell proliferation and increased expression of markers for osteoblast differentiation, suggesting that shift from proliferation to differentiation is caused by enhanced FGF signaling (97). It has been suggested that FGF signaling affects osteoblast differentiation at different levels through interacting with CBFA1/RUNX2 and Osteocalcin. CBFA1/RUNX2 can induce expression of a series of osteoblast genes including *Osteopontin* and *Osteocalcin*. Human cleidocranial dysplasia is associated with haploinsufficiency mutation of CBFA1/RUNX2 (124). CBFA1/RUNX2 -ull mice have completely no Osteoblasts (125). C3H10T1/2 cells either transfected with wild-type or Pfeiffer-causing mutant FGFR1 cDNA, or treated with FGF2/FGF8, showed induced expression of *Cbfa1/Runx2* and its downstream transcription targets such as osteocalcin and bone sialoprotein (116), strongly suggesting the induction of *Cbfa1/Runx2* even in non-osteoblasts by FGF activity.

As a downstream target gene regulated by CBFA1/RUNX2, *Osteocalcin* is so far the most specific marker for matured osteoblast. Osteocalcin-deficient mice

have increased osteoblast function resulting in high bone mass phenotype (126). As mentioned earlier, FGFR1-P250R mice have increased expression of *Osteocalcin* in suture, and FGFR3 activated mutant mice also have enhanced expression of *Osteocalcin* in long bone, suggesting the involvement of FGF/FGFR signaling in regulation of osteocalcin. Boudreaux *et al.* found that the promoter of rat *Osteocalcin* has a bipartite FGF-responsive element conferring FGF2 responsiveness (127,128), suggesting direct regulation of *Osteocalcin* expression by FGF signaling. FGF signaling also regulate *Osteocalcin* transcription through CBFA1/RUNX2. FGF2 treatment enhances the binding of CBFA1/RUNX2 to CBFA1-binding consensus sequence in the *Osteocalcin* promoter (129), indicating that CBFA1/RUNX2 mediates the regulatory effects of FGF on *Osteocalcin*.

It was demonstrated recently that FGF-stimulated phosphorylation and transcriptional activity of *Cbfa1/Runx2* may be mediated by ERK and PKC pathways (129,130). PKC and SRC-kinase pathway mediated the induction of N-cadherin expression in human neonatal calvaria osteoblasts by FGF-2 (131). PKC is also involved in the stimulation of sodium-dependent phosphate transport induced by FGF2 in MC3T3-E1 osteoblast-like cells (132).

Several lines of evidence indicate that FGF signaling also regulates apoptosis of cranial sutures. Like its influence on osteoblast differentiation, the effect of FGF signaling on osteoblast apoptosis varies on different developmental stages. Debiais *et al.* found that FGF2 first protects cultured human calvarial osteoblasts from apoptosis induced by serum starvation before stimulating apoptosis (133). *In vivo*, FGF signaling induces apoptosis in more differentiated osteoblasts. FGF2 induces apoptosis in the developing coronal suture (98). In transgenic mice overexpressing FGF2, apoptosis is increased and the apoptotic cells are concentrated at the osteogenic front representing differentiating calvarial osteoblasts (134). Marie *et al.* proposed that acute FGF signaling might reduce apoptosis of immature osteoblasts, whereas continuous signaling may promote apoptosis in more mature osteoblasts (107). Apoptosis may be a mechanism, through which the number of osteoblasts of different stages is maintained at appropriate levels.

Alteration of a number of molecules, including overexpression of IL-1 and FAS, activation of PKC and caspase-8, and increased levels of BAX/BCL-2, was found to be involved in the mechanism by which FGF/FGFR signaling promotes osteoblast apoptosis (117,135,136). By mediating the increased differentiation and premature apoptosis, activation of PKC appears to play a key role in the pathogenesis of craniosynostosis caused by FGFR2 associated mutations (107,136).

6. PERSPECTIVES

Significant advances have been made toward our understanding of skeletal development and related genetic diseases. Over the years, the results of intense studies have helped elucidate more clearly the roles of FGF signaling in skeletogenesis and related skeletal diseases. Nevertheless, many unresolved issues remain in this area. For example, it is not clear why different mutations in the same receptor result in activation of the receptor at varying levels and yield different distinct syndromes. It is unclear whether differences in downstream signaling pathways account for different mutations. It is therefore important to identify upstream and downstream molecules participating in morphogenesis in wild type and mutated FGFRs that ultimately lead to normal skeletal development and skeletal dysplasias respectively. The feedback loop regulating FGFs/FGFRs signaling also needs elucidation. Functions of various FGFs and FGFRs in skeletal development and skeletal dysplasias are yet to be completely understood.

There are an increasing number of signaling molecules found to regulate endochondral and intramembraneous ossification through their distinct action on proliferation, differentiation and apoptosis of chondrocytes and osteoblasts. How these molecules interact with FGFs/FGFRs signaling pathway to coordinate the skeletal development is one of the major unresolved issues in this field. Further understanding of the interaction between FGFs/FGFRs signaling and other important molecules and signaling pathways is also very important. With the advent of an increasing number of transgenic and knock out mice being created, crossing between mouse strains harboring FGFs/FGFRs mutations with other mouse models carrying genetic modifications of genes regulating FGF signaling are expected to shed more light on the interactions between FGFs/FGFRs and their interacting molecules as well as the pathways involved.

Skeleton elements are primarily composed of three major cell types: chondrocytes, osteoblast and osteoclasts. Although our current works on the mechanism of chondrodysplasia and craniosynostosis resulting from mutations of FGFRs focus mainly on the role of chondrocytes and osteoblasts, the role of osteoclasts in the overall scheme of normal skeletal development and maintenance cannot be underestimated. Normal bone structure depends on a balance between chondrocytes and osteoblasts on the one hand, and osteoclasts on the other hand. It is therefore important to determine the precise roles of osteoclasts in the development of long bones and the cranial sutures. In this regard, FGF signaling has been shown to affect osteoclastgenesis and function. FGF2 can induce formation of osteoclast-like cells in murine bone marrow cultures (137) and activate mature osteoclasts (138). FGF18 also promotes osteoclast formation. With respect to FGFRs, mice with activated mutation in FGFR3 showed increased TRAP-staining, suggesting enhanced osteoclast activity.

Our understanding of the role of FGF signaling in bone development is also of some usefulness in the context of the more common skeletal conditions such as bone fractures and metabolic diseases. The role of FGF signaling in fracture healing and metabolic skeletal diseases has been highlighted in the results of a number of studies (139,140). The process of fracture healing also involves the two major bone formation processes, i.e., endochondral ossification

and intramembraneous ossification. Indeed, fracture healing, to a considerable degree, recapitulates the skeletal developmental process. They share some similar morphogenetic processes and signaling networks. Application of our knowledge of the role of FGF signaling in skeletal development to fracture healing and osteoporosis will facilitate our understanding and search for therapeutic measures for these diseases.

Recent advances in genomics and proteomics continue to play significant roles in the biomedical studies of skeletal diseases. For example, the prospect of developing gene chips directed toward screening patients for potential FGFR mutation related skeletal diseases is now quite bright. Our better understanding of the mechanisms of these diseases, and the availability of mouse models orthologous to human diseases will make it possible to test various therapeutic approaches, such as siRNA, antisense, small inhibitory chemical molecule, neutralizing antibodies, and modulators of signaling molecules mediating FGFRs signaling.

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8. REFERENCES

1. F. Coulier, P. Pontarotti, R. Roubin, H. Hartung, M. Goldfarb & D. Birnbaum: Of worms and men: an evolutionary perspective on the fibroblast growth factor (FGF) and FGF receptor families. *J Mol Evol* 44, 43-56 (1997)
2. D. M. Ornitz & N. Itoh: Fibroblast growth factors. *Genome Biol* 2, REVIEWS3005 (2001)
3. C. J. Powers, S. W. McLeskey & A. Wellstein: Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer* 7, 165-197 (2000)
4. D. M. Ornitz, A. Yayon, J. G. Flanagan, C. M. Svahn, E. Levi & P. Leder: Heparin is required for cell-free binding of basic fibroblast growth factor to a soluble receptor and for mitogenesis in whole cells. *Mol Cell Biol* 12, 240-247 (1992)
5. D. E. Johnson & L. T. Williams: Structural and functional diversity in the FGF receptor multigene family. *Adv Cancer Res* 60, 1-41 (1993)
6. J. Z. Hou, M. K. Kan, K. McKeehan, G. McBride, P. Adams & W. L. McKeehan: Fibroblast growth factor receptors from liver vary in three structural domains. *Science* 251, 665-668 (1991)
7. S. Werner, D. S. Duan, V. C. de, K. G. Peters, D. E. Johnson & L. T. Williams: Differential splicing in the extracellular region of fibroblast growth factor receptor 1 generates receptor variants with different ligand-binding specificities. *Molecular & Cellular Biology* 12, 82-88 (1992)
8. A. Orr-Urtreger, M. T. Bedford, T. Burakova, E. Arman, Y. Zimmer, A. Yayon, D. Givol & P. Lonai: Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2). *Dev Biol* 158, 475-486 (1993)
9. X. Xu, M. Weinstein, C. Li, M. Naski, R. I. Cohen, D. M. Ornitz, P. Leder & C. Deng: Fibroblast growth factor receptor 2 (FGFR2)-mediated reciprocal regulation loop between FGF8 and FGF10 is essential for limb induction. *Development* 125, 753-765 (1998)
10. K. Yu, A. B. Herr, G. Waksman & D. M. Ornitz: Loss of fibroblast growth factor receptor 2 ligand-binding specificity in apert syndrome. *Proc Natl Acad Sci U S A* 97, 14536-14541 (2000)
11. J. Schlessinger, A. N. Plotnikov, O. A. Ibrahimi, A. V. Eliseenkova, B. K. Yeh, A. Yayon, R. J. Linhardt & M. Mohammadi: Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization. *Mol Cell* 6, 743-750. (2000)
12. A. Ullrich & J. Schlessinger: Signal transduction by receptors with tyrosine kinase activity. *Cell* 61, 203-212 (1990)
13. T. Pawson: Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. *Cell* 116, 191-203 (2004)
14. J. K. Wang, H. Xu, H. C. Li & M. Goldfarb: Broadly expressed SNT-like proteins link FGF receptor stimulation to activators of Ras. *Oncogene* 13, 721-729 (1996)
15. H. Kouhara, Y. R. Hadari, T. Spivak-Kroizman, J. Schilling, D. Bar-Sagi, I. Lax & J. Schlessinger: A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* 89, 693-702 (1997)
16. S. H. Ong, Y. R. Hadari, N. Gotoh, G. R. Guy, J. Schlessinger & I. Lax: Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc Natl Acad Sci U S A* 98, 6074-6079 (2001)
17. S. Raffioni, D. Thomas, E. D. Foehr, L. M. Thompson & R. A. Bradshaw: Comparison of the intracellular signaling responses by three chimeric fibroblast growth factor receptors in PC12 cells. *Proc Natl Acad Sci U S A* 96, 7178-7183 (1999)
18. Q. Wang, R. P. Green, G. Zhao & D. M. Ornitz: Differential regulation of endochondral bone growth and joint development by FGFR1 and FGFR3 tyrosine kinase domains. *Development* 128, 3867-3876 (2001)
19. N. Hacohen, S. Kramer, D. Sutherland, Y. Hiromi & M. A. Krasnow: sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the Drosophila airways. *Cell* 92, 253-263 (1998)
20. H. Hanafusa, S. Torii, T. Yasunaga & E. Nishida: Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat Cell Biol* 4, 850-858 (2002)
21. A. Sasaki *et al.*: Mammalian Sprouty4 suppresses Ras-independent ERK activation by binding to Raf1. *Cell Cycle* 2, 281-282 (2003)
22. M. Tsang & I. B. Dawid: Promotion and attenuation of FGF signaling through the Ras-MAPK pathway. *Sci STKE* 2004, pe17 (2004)
23. R. T. Bottcher, N. Pollet, H. Delius & C. Niehrs: The transmembrane protein XFLRT3 forms a complex with FGF receptors and promotes FGF signalling. *Nat Cell Biol* 6, 38-44 (2004)
24. F. V. Mariani & G. R. Martin: Deciphering skeletal patterning: clues from the limb. *Nature* 423, 319-325 (2003)

25. X. Sun, F. V. Mariani & G. R. Martin: Functions of FGF signalling from the apical ectodermal ridge in limb development. *Nature* 418, 501-508 (2002)
26. X. Xu, M. Weinstein, C. Li & C. X. Deng: Fibroblast growth factor receptors (FGFRs) and their roles in limb development. *Cell & Tissue Research* 296, 33-43 (1999)
27. X. Coumoul & C. X. Deng: Roles of FGF receptors in mammalian development and congenital diseases. *Birth Defects Res Part C Embryo Today* 69, 286-304 (2003)
28. E. Laufer, C. E. Nelson, R. L. Johnson, B. A. Morgan & C. Tabin: Sonic hedgehog and Fgf-4 act through a signaling cascade and feedback loop to integrate growth and patterning of the developing limb bud. *Cell* 79, 993-1003 (1994)
29. L. Niswander, S. Jeffrey, G. R. Martin & C. Tickle: A positive feedback loop coordinates growth and patterning in the vertebrate limb. *Nature* 371, 609-612 (1994)
30. D. Summerbell, J. H. Lewis & L. Wolpert: Positional information in chick limb morphogenesis. *Nature* 244, 492-496 (1973)
31. P. H. Crossley & G. R. Martin: The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* 121, 439-451 (1995)
32. M. P. Savage, C. E. Hart, B. B. Riley, J. Sasse, B. B. Olwin & J. F. Fallon: Distribution of FGF-2 suggests it has a role in chick limb bud growth. *Dev Dyn* 198, 159-170 (1993)
33. G. R. Martin: The roles of FGFs in the early development of vertebrate limbs. *Genes Dev* 12, 1571-1586 (1998)
34. C. Tickle & A. Munsterberg: Vertebrate limb development--the early stages in chick and mouse. *Curr Opin Genet Dev* 11, 476-481 (2001)
35. A. M. Boulet, A. M. Moon, B. R. Arenkiel & M. R. Capecchi: The roles of Fgf4 and Fgf8 in limb bud initiation and outgrowth. *Dev Biol* 273, 361-372 (2004)
36. A. T. Dudley, M. A. Ros & C. J. Tabin: A re-examination of proximodistal patterning during vertebrate limb development. *Nature* 418, 539-544 (2002)
37. J. M. Revest, B. Spencer-Dene, K. Kerr, L. De Moerlooze, I. Rosewell & C. Dickson: Fibroblast Growth Factor Receptor 2-IIIb Acts Upstream of Shh and Fgf4 and Is Required for Limb Bud Maintenance but Not for the Induction of Fgf8, Fgf10, Msx1, or Bmp4. *Dev Biol* 231, 47-62. (2001)
38. V. P. Eswarakumar, E. Monsonego-Ornan, M. Pines, I. Antonopoulou, G. M. Morriss-Kay & P. Lonai: The IIIc alternative of Fgfr2 is a positive regulator of bone formation. *Development* 129, 3783-3793. (2002)
39. C. X. Deng, A. Wynshaw-Boris, M. M. Shen, C. Daugherty, D. M. Ornitz & P. Leder: Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev* 8, 3045-3057 (1994)
40. T. P. Yamaguchi, K. Harpal, M. Henkemeyer & J. Rossant: fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev* 8, 3032-3044 (1994)
41. C. Deng, M. Bedford, C. Li, X. Xu, X. Yang, J. Dunmore & P. Leder: Fibroblast growth factor receptor-1 (FGFR-1) is essential for normal neural tube and limb development. *Dev Biol* 185, 42-54 (1997)
42. X. Xu, C. Li, K. Takahashi, H. C. Slavkin, L. Shum & C. X. Deng: Murine fibroblast growth factor receptor 1alpha isoforms mediate node regression and are essential for posterior mesoderm development. *Dev Biol* 208, 293-306 (1999)
43. A. Montero, Y. Okada, M. Tomita, M. Ito, H. Tsurukami, T. Nakamura, T. Doetschman, J. D. Coffin & M. M. Hurley: Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. *J Clin Invest* 105, 1085-1093 (2000)
44. J. S. Colvin, A. C. White, S. J. Pratt & D. M. Ornitz: Lung hypoplasia and neonatal death in Fgf9-null mice identify this gene as an essential regulator of lung mesenchyme. *Development* 128, 2095-2106 (2001)
45. J. D. Coffin *et al.*: Abnormal bone growth and selective translational regulation in basic fibroblast growth factor (FGF-2) transgenic mice. *Mol Biol Cell* 6, 1861-1873 (1995)
46. S. Garofalo, M. Kliger-Spatz, J. L. Cooke, O. Wolstin, G. P. Lunstrum, S. M. Moshkovitz, W. A. Horton & A. Yayon: Skeletal dysplasia and defective chondrocyte differentiation by targeted overexpression of fibroblast growth factor 9 in transgenic mice. *J Bone Miner Res* 14, 1909-1915 (1999)
47. Z. Liu, J. Xu, J. S. Colvin & D. M. Ornitz: Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. *Genes Dev* 16, 859-869 (2002)
48. N. Ohbayashi, M. Shibayama, Y. Kurotaki, M. Imanishi, T. Fujimori, N. Itoh & S. Takada: FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. *Genes Dev* 16, 870-879 (2002)
49. L. Guo, L. Degenstein & E. Fuchs: Keratinocyte growth factor is required for hair development but not for wound healing. *Genes Dev* 10, 165-175 (1996)
50. E. N. Meyers, M. Lewandoski & G. R. Martin: An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. *Nature Genetics* 18, 136-142 (1998)
51. J. Xu, M. Nakahara, J. W. Crabb, E. Shi, Y. Matuo, M. Fraser, M. Kan, J. Hou & W. L. McKeehan: Expression and immunochemical analysis of rat and human fibroblast growth factor receptor (flg) isoforms. *Journal of Biological Chemistry* 267, 17792-17803 (1992)
52. G. Szebenyi, M. P. Savage, B. B. Olwin & J. F. Fallon: Changes in the expression of fibroblast growth factor receptors mark distinct stages of chondrogenesis *in vitro* and during chick limb skeletal patterning. *Dev Dyn* 204, 446-456 (1995)
53. K. Peters, D. Ornitz, S. Werner & L. Williams: Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis. *Developmental Biology* 155, 423-430 (1993)
54. C. Deng, A. Wynshaw-Boris, F. Zhou, A. Kuo & P. Leder: Fibroblast growth factor receptor 3 is a negative regulator of bone growth. *Cell* 84, 911-921 (1996)
55. J. S. Colvin, B. A. Bohne, G. W. Harding, D. G. McEwen & D. M. Ornitz: Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. *Nat Genet* 12, 390-397 (1996)

56. F. Rousseau, V. el Ghouzzi, A. L. Delezoide, L. Legeai-Mallet, M. Le Merrer, A. Munnich & J. Bonaventure: Missense FGFR3 mutations create cysteine residues in thanatophoric dwarfism type I (TD1). *Hum Mol Genet* 5, 509-512. (1996)
57. W. A. Horton & G. P. Lunstrum: Fibroblast growth factor receptor 3 mutations in achondroplasia and related forms of dwarfism. *Rev Endocr Metab Disord* 3, 381-385 (2002)
58. F. Rousseau, J. Bonaventure, L. Legeai-Mallet, A. Pelet, J. M. Rozet, P. Maroteaux, M. Le Merrer & A. Munnich: Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. *Nature* 371, 252-254 (1994)
59. M. M. Cohen, Jr.: Achondroplasia, hypochondroplasia and thanatophoric dysplasia: clinically related skeletal dysplasias that are also related at the molecular level. *Int J Oral Maxillofac Surg* 27, 451-455 (1998)
60. D. M. Ornitz & P. J. Marie: FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes Dev* 16, 1446-1465 (2002)
61. V. A. McKusick, T. E. Kelly & J. P. Dorst: Observations suggesting allelism of the achondroplasia and hypochondroplasia genes. *J Med Genet* 10, 11-16 (1973)
62. R. Stanescu, V. Stanescu & P. Maroteaux: Homozygous achondroplasia: morphologic and biochemical study of cartilage. *Am J Med Genet* 37, 412-421 (1990)
63. R. Shiang, L. M. Thompson, Y. Z. Zhu, D. M. Church, T. J. Fielder, M. Bocian, S. T. Winokur & J. J. Wasmuth: Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell* 78, 335-342 (1994)
64. A. Superti-Furga, G. Eich, H. U. Bucher, J. Wisser, A. Giedion, R. Gitzelmann & B. Steinmann: A glycine 375-to-cysteine substitution in the transmembrane domain of the fibroblast growth factor receptor-3 in a newborn with achondroplasia. *Eur J Pediatr* 154, 215-219 (1995)
65. J. Briner, A. Giedion & M. A. Spycher: Variation of quantitative and qualitative changes of enchondral ossification in heterozygous achondroplasia. *Pathol Res Pract* 187, 271-278 (1991)
66. M. C. Naski, Q. Wang, J. Xu & D. M. Ornitz: Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. *Nat Genet* 13, 233-237 (1996)
67. L. Chen, R. Adar, X. Yang, E. O. Monsonego, C. Li, P. V. Hauschka, A. Yayon & C. X. Deng: Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. *J Clin Invest* 104, 1517-1525 (1999)
68. J. Y. Cho, C. Guo, M. Torello, G. P. Lunstrum, T. Iwata, C. Deng & W. A. Horton: Defective lysosomal targeting of activated fibroblast growth factor receptor 3 in achondroplasia. *Proc Natl Acad Sci U S A* 101, 609-614 (2004)
69. S. G. Brodie & C. X. Deng: Mouse models orthologous to FGFR3-related skeletal dysplasias. *Pediatr Pathol Mol Med* 22, 87-103 (2003)
70. C. Li, L. Chen, T. Iwata, M. Kitagawa, X. Y. Fu & C. X. Deng: A Lys644Glu substitution in fibroblast growth factor receptor 3 (FGFR3) causes dwarfism in mice by activation of STATs and ink4 cell cycle inhibitors. *Hum Mol Genet* 8, 35-44 (1999)
71. T. Iwata, L. Chen, C. Li, D. A. Ovchinnikov, R. R. Behringer, C. A. Francomano & C. X. Deng: A neonatal lethal mutation in FGFR3 uncouples proliferation and differentiation of growth plate chondrocytes in embryos. *Hum Mol Genet* 9, 1603-1613 (2000)
72. L. Chen, C. Li, W. Qiao, X. Xu & C. Deng: A Ser(365)-->Cys mutation of fibroblast growth factor receptor 3 in mouse downregulates Ihh/PTHrP signals and causes severe achondroplasia. *Hum Mol Genet* 10, 457-465. (2001)
73. W. C. Su, M. Kitagawa, N. Xue, B. Xie, S. Garofalo, J. Cho, C. Deng, W. A. Horton & X. Y. Fu: Activation of Stat1 by mutant fibroblast growth-factor receptor in thanatophoric dysplasia type II dwarfism. *Nature* 386, 288-292 (1997)
74. M. Sahni, D. C. Ambrosetti, A. Mansukhani, R. Gertner, D. Levy & C. Basilico: FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. *Genes Dev* 13, 1361-1366 (1999)
75. M. Sahni, R. Raz, J. D. Coffin, D. Levy & C. Basilico: STAT1 mediates the increased apoptosis and reduced chondrocyte proliferation in mice overexpressing FGF2. *Development* 128, 2119-2129 (2001)
76. S. Murakami, G. Balmes, S. McKinney, Z. Zhang, D. Givol & B. de Crombrugghe: Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. *Genes Dev* 18, 290-305 (2004)
77. J. M. Wang, X. L. Du, C. L. Li, L. J. Yin, C. X. Deng & L. Chen: Gly374Arg mutation in FGFR3 causes achondroplasia in mice. *Chinese Journal of Medical Genetics* 21, 537-541 (2004)
78. E. Laplantine, F. Rossi, M. Sahni, C. Basilico & D. Cobrinik: FGF signaling targets the pRb-related p107 and p130 proteins to induce chondrocyte growth arrest. *J Cell Biol* 158, 741-750 (2002)
79. L. Dailey, E. Laplantine, R. Priore & C. Basilico: A network of transcriptional and signaling events is activated by FGF to induce chondrocyte growth arrest and differentiation. *J Cell Biol* 161, 1053-1066 (2003)
80. N. Amizuka, H. Warshawsky, J. E. Henderson, D. Goltzman & A. C. Karaplis: Parathyroid hormone-related peptide-depleted mice show abnormal epiphyseal cartilage development and altered endochondral bone formation. *J Cell Biol* 126, 1611-1623 (1994)
81. A. C. Karaplis, A. Luz, J. Glowacki, R. T. Bronson, V. L. Tybulewicz, H. M. Kronenberg & R. C. Mulligan: Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. *Genes Dev* 8, 277-289 (1994)
82. B. Lanske *et al.*: PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science* 273, 663-666 (1996)
83. B. St-Jacques, M. Hammerschmidt & A. P. McMahon: Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev* 13, 2072-2086 (1999)
84. A. Vortkamp, K. Lee, B. Lanske, G. V. Segre, H. M. Kronenberg & C. J. Tabin: Regulation of rate of cartilage

differentiation by Indian hedgehog and PTH-related protein. *Science* 273, 613-622 (1996)
85. M. C. Naski, J. S. Colvin, J. D. Coffin & D. M. Ornitz: Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. *Development* 125, 4977-4988 (1998)
86. A. Yasoda *et al.*: Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. *Nat Med* 10, 80-86 (2004)
87. A. Aszodi, E. B. Hunziker, C. Brakebusch & R. Fassler: Beta1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis. *Genes Dev* 17, 2465-2479 (2003)
88. L. Legeai-Mallet, C. Benoist-Lasselin, A. L. Delezoide, A. Munnich & J. Bonaventure: Fibroblast growth factor receptor 3 mutations promote apoptosis but Do not alter chondrocyte proliferation in thanatophoric dysplasia. *J Biol Chem* 273, 13007-13014 (1998)
89. M. K. Hajihosseini & J. K. Heath: Expression patterns of fibroblast growth factors-18 and -20 in mouse embryos is suggestive of novel roles in calvarial and limb development. *Mech Dev* 113, 79-83 (2002)
90. A. M. Gonzalez, D. J. Hill, A. Logan, P. A. Maher & A. Baird: Distribution of fibroblast growth factor (FGF)-2 and FGF receptor-1 messenger RNA expression and protein presence in the mid-trimester human fetus. *Pediatr Res* 39, 375-385 (1996)
91. H. J. Kim, D. P. Rice, P. J. Kettunen & I. Thesleff: FGF-, BMP- and Shh-mediated signalling pathways in the regulation of cranial suture morphogenesis and calvarial bone development. *Development* 125, 1241-1251 (1998)
92. R. K. Globus, J. Plouet & D. Gospodarowicz: Cultured bovine bone cells synthesize basic fibroblast growth factor and store it in their extracellular matrix. *Endocrinology* 124, 1539-1547 (1989)
93. A. L. Delezoide, C. Benoist-Lasselin, L. Legeai-Mallet, M. Le Merrer, A. Munnich, M. Vekemans & J. Bonaventure: Spatio-temporal expression of FGFR 1, 2 and 3 genes during human embryo-fetal ossification. *Mech Dev* 77, 19-30 (1998)
94. A. Molteni, D. Modrowski, M. Hott & P. J. Marie: Differential expression of fibroblast growth factor receptor-1, -2, and -3 and syndecan-1, -2, and -4 in neonatal rat mandibular condyle and calvaria during osteogenic differentiation *in vitro*. *Bone* 24, 337-347 (1999)
95. D. P. Rice, T. Aberg, Y. Chan, Z. Tang, P. J. Kettunen, L. Pakarinen, R. E. Maxson & I. Thesleff: Integration of FGF and TWIST in calvarial bone and suture development. *Development* 127, 1845-1855 (2000)
96. D. P. Rice, R. Rice & I. Thesleff: Fgfr mRNA isoforms in craniofacial bone development. *Bone* 33, 14-27 (2003)
97. S. Iseki, A. O. Wilkie & G. M. Morriss-Kay: Fgfr1 and Fgfr2 have distinct differentiation- and proliferation-related roles in the developing mouse skull vault. *Development* 126, 5611-5620. (1999)
98. I. M. Mathijssen, H. van Leeuwen, C. Vermeij-Keers & J. M. Vaandrager: FGF-4 or FGF-2 administration induces apoptosis, collagen type I expression, and mineralization in the developing coronal suture. *J Craniofac Surg* 12, 399-400 (2001)
99. J. A. Greenwald *et al.*: *In vivo* modulation of FGF biological activity alters cranial suture fate. *Am J Pathol* 158, 441-452 (2001)
100. R. Moore, P. Ferretti, A. Copp & P. Thorogood: Blocking endogenous FGF-2 activity prevents cranial osteogenesis. *Dev Biol* 243, 99-114 (2002)
101. E. P. Newberry, T. Latifi & D. A. Towler: Reciprocal regulation of osteocalcin transcription by the homeodomain proteins Msx2 and Dlx5. *Biochemistry* 37, 16360-16368 (1998)
102. N. Funato, K. Ohtani, K. Ohyama, T. Kuroda & M. Nakamura: Common regulation of growth arrest and differentiation of osteoblasts by helix-loop-helix factors. *Mol Cell Biol* 21, 7416-7428 (2001)
103. L. A. Opperman: Cranial sutures as intramembranous bone growth sites. *Dev Dyn* 219, 472-485 (2000)
104. M. M. Cohen, Jr.: Transforming growth factor beta s and fibroblast growth factors and their receptors: role in sutural biology and craniosynostosis. *J Bone Miner Res* 12, 322-331 (1997)
105. F. Debiais, M. Hott, A. M. Graulet & P. J. Marie: The effects of fibroblast growth factor-2 on human neonatal calvaria osteoblastic cells are differentiation stage specific. *J Bone Miner Res* 13, 645-654 (1998)
106. T. Sobue *et al.*: Regulation of fibroblast growth factor 2 and fibroblast growth factor receptors by transforming growth factor beta in human osteoblastic MG-63 cells. *J Bone Miner Res* 17, 502-512 (2002)
107. P. J. Marie, F. Debiais & E. Hay: Regulation of human cranial osteoblast phenotype by FGF-2, FGFR-2 and BMP-2 signaling. *Histol Histopathol* 17, 877-885 (2002)
108. S. M. Warren, L. J. Brunet, R. M. Harland, A. N. Economides & M. T. Longaker: The BMP antagonist noggin regulates cranial suture fusion. *Nature* 422, 625-629 (2003)
109. X. Zhang, T. Sobue & M. M. Hurley: FGF-2 increases colony formation, PTH receptor, and IGF-1 mRNA in mouse marrow stromal cells. *Biochem Biophys Res Commun* 290, 526-531 (2002)
110. R. A. Power, U. T. Iwaniec & T. J. Wronski: Changes in gene expression associated with the bone anabolic effects of basic fibroblast growth factor in aged ovariectomized rats. *Bone* 31, 143-148 (2002)
111. P. B. Saadeh, B. J. Mehrara, D. S. Steinbrech, J. A. Spector, J. A. Greenwald, G. S. Chin, H. Ueno, G. K. Gittes & M. T. Longaker: Mechanisms of fibroblast growth factor-2 modulation of vascular endothelial growth factor expression by osteoblastic cells. *Endocrinology* 141, 2075-2083 (2000)
112. J. T. Katzen & J. G. McCarthy: Syndromes involving craniosynostosis and midface hypoplasia. *Otolaryngol Clin North Am* 33, 1257-1284, vi. (2000)
113. D. Renier, E. Lajeunie, E. Arnaud & D. Marchac: Management of craniosynostoses. *Childs Nerv Syst* 16, 645-658. (2000)
114. U. Hehr & M. Muenke: Craniosynostosis syndromes: from genes to premature fusion of skull bones. *Mol Genet Metab* 68, 139-151. (1999)
115. S. M. Warren, J. A. Greenwald, J. A. Spector, P. Bouletreau, B. J. Mehrara & M. T. Longaker: New developments in cranial suture research. *Plast Reconstr Surg* 107, 523-540 (2001)
116. Y. X. Zhou, X. Xu, L. Chen, C. Li, S. G. Brodie & C. X. Deng: A Pro250Arg substitution in mouse Fgfr1 causes increased expression of Cbfa1 and premature fusion of calvarial sutures. *Hum Mol Genet* 9, 2001-2008 (2000)

117. L. Chen, D. Li, C. Li, A. Engel & C. Deng: A Ser250Trp substitution in mouse fibroblast growth factor receptor 2 (Fgfr2) results in craniosynostosis. *Bone* 130, 6143-6153 (2003)
118. V. P. Eswarakumar, M. C. Horowitz, R. Locklin, G. M. Morriss-Kay & P. Lonai: A gain-of-function mutation of Fgfr2c demonstrates the roles of this receptor variant in osteogenesis. *Proc Natl Acad Sci U S A* 101, 12555-12560. Epub 12004 Aug 12517. (2004)
119. S. C. Robertson, A. N. Meyer, K. C. Hart, B. D. Galvin, M. K. Webster & D. J. Donoghue: Activating mutations in the extracellular domain of the fibroblast growth factor receptor 2 function by disruption of the disulfide bond in the third immunoglobulin-like domain. *Proc Natl Acad Sci U S A* 95, 4567-4572 (1998)
120. A. O. Wilkie & G. M. Morriss-Kay: Genetics of craniofacial development and malformation. *Nat Rev Genet* 2, 458-468 (2001)
121. K. Yu & D. M. Ornitz: Uncoupling fibroblast growth factor receptor 2 ligand binding specificity leads to Apert syndrome-like phenotypes. *Proc Natl Acad Sci U S A* 98, 3641-3643 (2001)
122. K. Mangasarian, Y. Li, A. Mansukhani & C. Basilico: Mutation associated with Crouzon syndrome causes ligand-independent dimerization and activation of FGF receptor-2. *J Cell Physiol* 172, 117-125 (1997)
123. Y. H. Liu, Z. Tang, R. K. Kundu, L. Wu, W. Luo, D. Zhu, F. Sangiorgi, M. L. Snead & R. E. Maxson: Msx2 gene dosage influences the number of proliferative osteogenic cells in growth centers of the developing murine skull: a possible mechanism for MSX2-mediated craniosynostosis in humans. *Dev Biol* 205, 260-274 (1999)
124. S. Mundlos *et al.*: Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. *Cell* 89, 773-779. (1997)
125. P. Ducy, R. Zhang, V. Geoffroy, A. L. Ridall & G. Karsenty: Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* 89, 747-754. (1997)
126. P. Ducy *et al.*: Increased bone formation in osteocalcin-deficient mice. *Nature* 382, 448-452 (1996)
127. J. M. Boudreaux & D. A. Towler: Synergistic induction of osteocalcin gene expression: identification of a bipartite element conferring fibroblast growth factor 2 and cyclic AMP responsiveness in the rat osteocalcin promoter. *J Biol Chem* 271, 7508-7515 (1996)
128. E. P. Newberry, J. M. Boudreaux & D. A. Towler: The rat osteocalcin fibroblast growth factor (FGF)-responsive element: an okadaic acid-sensitive, FGF-selective transcriptional response motif. *Mol Endocrinol* 10, 1029-1040 (1996)
129. H. J. Kim, J. H. Kim, S. C. Bae, J. Y. Choi & H. M. Ryoo: The protein kinase C pathway plays a central role in the fibroblast growth factor-stimulated expression and transactivation activity of Runx2. *J Biol Chem* 278, 319-326 (2003)
130. G. Xiao, D. Jiang, R. Gopalakrishnan & R. T. Franceschi: Fibroblast growth factor 2 induction of the osteocalcin gene requires MAPK activity and phosphorylation of the osteoblast transcription factor, Cbfa1/Runx2. *J Biol Chem* 277, 36181-36187 (2002)
131. F. Debiais, J. Lemonnier, E. Hay, P. Delannoy, J. Caverzasio & P. J. Marie: Fibroblast growth factor-2 (FGF-2) increases N-cadherin expression through protein kinase C and Src-kinase pathways in human calvaria osteoblasts. *J Cell Biochem* 81, 68-81 (2001)
132. A. Suzuki, G. Palmer, J. P. Bonjour & J. Caverzasio: Stimulation of sodium-dependent phosphate transport and signaling mechanisms induced by basic fibroblast growth factor in MC3T3-E1 osteoblast-like cells. *J Bone Miner Res* 15, 95-102 (2000)
133. F. Debiais, G. Lefevre, J. Lemonnier, S. Le Mee, F. Lasmoles, F. Mascarelli & P. J. Marie: Fibroblast growth factor-2 induces osteoblast survival through a phosphatidylinositol 3-kinase-dependent, -beta-catenin-independent signaling pathway. *Exp Cell Res* 297, 235-246 (2004)
134. A. Mansukhani, P. Bellosta, M. Sahni & C. Basilico: Signaling by fibroblast growth factors (FGF) and fibroblast growth factor receptor 2 (FGFR2)-activating mutations blocks mineralization and induces apoptosis in osteoblasts. *J Cell Biol* 149, 1297-1308. (2000)
135. J. Lemonnier, E. Hay, P. Delannoy, A. Lomri, D. Modrowski, J. Caverzasio & P. J. Marie: Role of N-cadherin and protein kinase C in osteoblast gene activation induced by the S252W fibroblast growth factor receptor 2 mutation in Apert craniosynostosis. *J Bone Miner Res* 16, 832-845. (2001)
136. J. Lemonnier, E. Hay, P. Delannoy, O. Fromigue, A. Lomri, D. Modrowski & P. J. Marie: Increased osteoblast apoptosis in apert craniosynostosis: role of protein kinase C and interleukin-1. *Am J Pathol* 158, 1833-1842. (2001)
137. M. M. Hurley, S. K. Lee, L. G. Raisz, P. Bernecker & J. Lorenzo: Basic fibroblast growth factor induces osteoclast formation in murine bone marrow cultures. *Bone* 22, 309-316 (1998)
138. D. Chikazu, Y. Hakeda, N. Ogata, K. Nemoto, A. Itabashi, T. Takato, M. Kumegawa, K. Nakamura & H. Kawaguchi: Fibroblast growth factor (FGF)-2 directly stimulates mature osteoclast function through activation of FGF receptor 1 and p42/p44 MAP kinase. *J Biol Chem* 275, 31444-31450 (2000)
139. W. J. Chen, S. Jingushi, I. Aoyama, J. Anzai, G. Hirata, M. Tamura & Y. Iwamoto: Effects of FGF-2 on metaphyseal fracture repair in rabbit tibiae. 303-309.
140. T. Larsson, R. Marsell, E. Schipani, C. Ohlsson, O. Ljunggren, H. S. Tenenhouse, H. Juppner & K. B. Jonsson: Transgenic mice expressing fibroblast growth factor 23 under the control of the alpha1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. *Endocrinology* 145, 3087-3094. Epub 2004 Feb 3026. (2004)
141. M. K. Hajihosseini, M. D. Lalioti, S. Arthaud, H. R. Burgar, J. M. Brown, S. R. Twigg, A. O. Wilkie & J. K. Heath: Skeletal development is regulated by fibroblast growth factor receptor 1 signalling dynamics. *Development* 131, 325-335 (2004)
142. M. K. Hajihosseini, S. Wilson, L. De Moerlooze & C. Dickson: A splicing switch and gain-of-function mutation in FgfR2-IIIc hemizygotes causes Apert/Pfeiffer-syndrome-like phenotypes. *Proc Natl Acad Sci U S A* 98, 3855-3860. (2001)
143. Y. Wang *et al.*: A mouse model for achondroplasia produced by targeting fibroblast growth factor receptor 3. *Proc Natl Acad Sci U S A* 96, 4455-4460. (1999)

144. E. Monsonego-Ornan, R. Adar, T. Feferman, O. Segev & A. Yayon: The transmembrane mutation G380R in fibroblast growth factor receptor 3 uncouples ligand-mediated receptor activation from down-regulation. *Mol Cell Biol* 20, 516-522 (2000)
145. T. Iwata, C. L. Li, C. X. Deng & C. A. Francomano: Highly activated Fgfr3 with the K644M mutation causes prolonged survival in severe dwarf mice. *Hum Mol Genet* 10, 1255-1264. (2001)
146. M. B. Carlton, W. H. Colledge & M. J. Evans: Crouzon-like craniofacial dysmorphology in the mouse is caused by an insertional mutation at the Fgf3/Fgf4 locus. *Dev Dyn* 212, 242-249 (1998)

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