

RECENT RESEARCH ON THE GROWTH PLATE

Recent insights into the regulation of the growth plate

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Abstract

For most bones, elongation is driven primarily by chondrogenesis at the growth plates. This process results from chondrocyte proliferation, hypertrophy, and extracellular matrix secretion, and it is carefully orchestrated by complex networks of local paracrine factors and modulated by endocrine factors. We review here recent advances in the understanding of growth plate physiology. These advances include new approaches to study expression patterns of large numbers of genes in the growth plate, using microdissection followed by microarray. This approach has been combined with genome-wide association studies to provide insights into the regulation of the human growth plate. We also review recent studies elucidating the roles of bone morphogenetic proteins, fibroblast growth factors, C-type natriuretic peptide, and suppressor of cytokine signaling in the local regulation of growth plate chondrogenesis and longitudinal bone growth.

Key Words

- growth hormone
- signal transduction
- IGF
- microarray
- skeletal

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Introduction

In the postnatal mammal, elongation of tubular bones occurs at the growth plate. This cartilaginous structure comprises three zones that contain chondrocytes at different stages of differentiation (Kronenberg 2003). The zone closest to the epiphysis is termed the resting zone. The resting zone is thought to contain chondrocytes that serve as progenitor cells, which can generate new clones of rapidly proliferating chondrocytes (Abad *et al.* 2002). Each derivative clone forms a cell column aligned parallel to the long axis of the bone. As these cells replicate, the two daughters line up parallel to the long axis to maintain the columnar organization. The chondrocytes farther from the epiphysis undergo termination differentiation, in which they cease proliferating and enlarge to form the hypertrophic zone. Throughout the growth plate,

chondrocytes secrete proteins and proteoglycans that form the cartilage extracellular matrix. In the resting and proliferative zones, collagen II represents a major component of this matrix, whereas in the hypertrophic zone, there is a shift to production of collagen X (Kronenberg 2003). The hypertrophic chondrocytes farthest from the epiphysis undergo cell death. This cell death has been attributed to apoptosis, but more recent evidence challenges this conclusion (Emons *et al.* 2009). This region is then invaded from the metaphyseal bone by blood vessels and differentiating osteoblasts and osteoclasts, which remodel the cartilage into bone tissue. The net result of this chondrogenesis and ossification is the formation of new bone underneath the growth plate and therefore bone elongation.

The integrated processes of chondrocyte differentiation, proliferation, cartilage matrix secretion, cell death, and vascular and bone cell invasion are regulated and coordinated by a complex array of paracrine signaling molecules, which includes insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), Indian hedgehog (IHH) and parathyroid hormone-related protein (PTHrP), bone morphogenetic proteins (BMPs), WNTs, and vascular endothelial growth factors (VEGFs). In addition, the rate of endochondral bone formation at the growth plate is regulated by an array of endocrine signals, including growth hormone (GH), IGF1, thyroid hormone, glucocorticoids, androgens, and estrogens. One of the principal apparent functions of this endocrine system is to allow rapid growth only when the organism is able to consume abundant nutrients.

Because the growth plate requires so many paracrine and endocrine signaling pathways to function normally, mutations in many genes involved in these signaling pathways lead to bones that are short, which in humans presents as short stature, and often malformed, which presents as a skeletal dysplasia. Thus, mutations in more than 200 genes cause distinct skeletal dysplasias (Warman *et al.* 2011).

Although there has been remarkable progress recently in our understanding of these signaling pathways that regulate the postnatal growth plate, much remains to be learned. In this review, we present some recent studies giving new insights into these control systems. The number of studies to be reviewed had to be limited, and therefore not all important areas of progress could be included.

Delineating gene expression patterns in the mammalian postnatal growth plate

In the past, gene expression within the growth plate has typically been studied by *in situ* hybridization, which provides much useful information but necessarily involves studying one candidate gene at a time. However, recently, methods have been developed to study expression patterns of large numbers of genes in the growth plate, using microdissection, followed by microarray analysis (Nilsson *et al.* 2007). Frozen sections of the growth plate are first microdissected into their constituent zones after which RNA is isolated and mRNA patterns are assessed by microarray analysis. Presumably, the method could readily be modified to use RNA sequencing in place of microarray analysis.

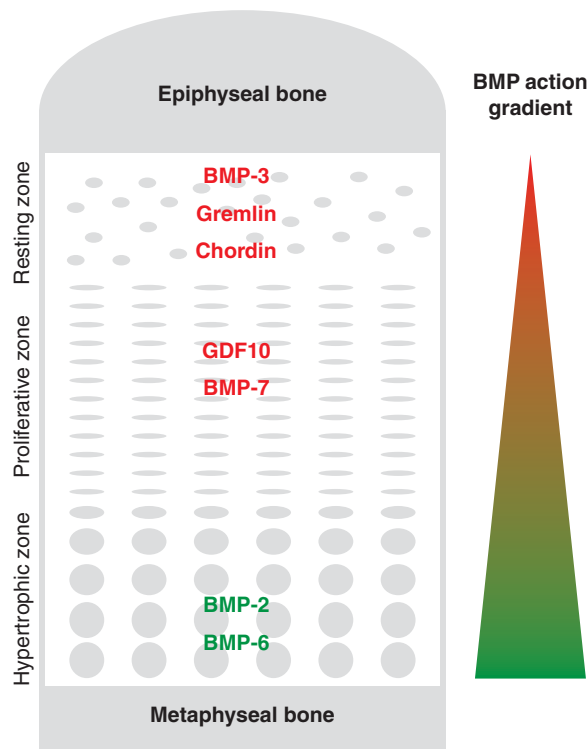
This approach was applied to the proximal tibiae of 1-week-old rats and the resulting expression data were

analyzed using bioinformatic algorithms (Lui *et al.* 2010). Expression in the resting and the proliferative zones were compared to identify pathways involved in the differentiation of resting zone to proliferative zone chondrocytes. This analysis implicated vitamin D receptor/retinoid X receptor (VDR/RXR) activation, platelet-derived growth factor (PDGF) signaling, BMP signaling, and notch signaling. Similar analyses of the proliferative to hypertrophic differentiation step implicated p53 signaling, ephrin receptor signaling, oncostatin M signaling, and BMP signaling (Lui *et al.* 2010).

Evidence for a BMP signaling gradient across the growth plate

As noted above, microarray analysis implicated BMP signaling in both the differentiation of resting zone chondrocytes to proliferative zone chondrocytes and of proliferative zone chondrocytes to hypertrophic zone chondrocytes. More extensive analysis of the BMP signaling pathway using microdissection followed by real-time PCR has shown evidence for a BMP signaling gradient across the growth plate with the greatest BMP signaling occurring in the hypertrophic zone and the least in the resting zone (Nilsson *et al.* 2007). Consistent with this concept, immunolocalization of phosphorylated SMAD1, SMAD5, and SMAD8 in the growth plate increases with increasing distance from the epiphysis (Yoon *et al.* 2006).

These patterns indicate that a BMP signaling gradient across the growth plate may contribute to the progressive differentiation of resting to proliferative to hypertrophic chondrocytes (Fig. 1). Low levels of BMP signaling in the resting zone may help maintain the progenitor cell state. Farther from the epiphysis, greater BMP signaling may induce differentiation to proliferative chondrocytes and, even farther from the epiphysis, yet greater BMP signaling may induce terminal differentiation to hypertrophic chondrocytes. Functional studies support this model. BMP2 stimulates resting zone chondrocytes to proliferate and stimulates proliferative zone chondrocytes to hypertrophy in an organ culture model (De Luca *et al.* 2001). *In vivo* overexpression of constitutively active *Bmpr1a* in mice has no effect on proliferation but accelerates hypertrophic differentiation (Kobayashi *et al.* 2005). Recent evidence specifically implicates *Bmp2* in this process. In mice, conditional targeted ablation of *Bmp2* causes severe defects in chondrocyte proliferation and differentiation through a mechanism involving RUNX2 protein levels (Shu *et al.* 2011). The effects of BMPs on the growth plate appear to involve the canonical BMP

**Figure 1**

Hypothesized BMP action gradient in the growth plate. Based on microarray and real-time PCR data, BMP agonists (green) and antagonists (red) are expressed primarily in the hypertrophic zone and resting zone respectively. These findings are indicative of a BMP signaling gradient across the growth plate that may be important for spatial control of chondrocyte differentiation within the growth plate.

signaling pathway in that combined loss of regulatory *Smad1* and *Smad5* in mice causes a severe skeletal dysplasia with impaired proliferation and hypertrophic differentiation (Retting *et al.* 2009). Although this review focuses on the function of the postnatal growth plate, it is important to recognize that BMP signaling affects embryonic development of the cartilaginous skeleton and thus genetic manipulations in mice may have combined embryonic and postnatal effects.

In addition to BMPs, other paracrine systems also appear to form gradients across the growth plate. Of these, the best studied one involves PTHrP. In the embryonic skeleton, PTHrP is secreted by periarticular chondrocytes of long bones (Kronenberg 2003). PTHrP diffuses across the growth cartilage maintaining chondrocytes in the proliferative state (Hirai *et al.* 2011). Cells more distant from the source of PTHrP undergo hypertrophic differentiation. The prehypertrophic and hypertrophic chondrocytes then secrete IHH, which positively regulates PTHrP production and also has independent effects on chondrocyte

differentiation. More recent evidence has indicated that the IHH-PTHrP system is maintained in the postnatal growth plate, but that the PTHrP source shifts to the resting zone (Koziel *et al.* 2005, Chau *et al.* 2011, Hirai *et al.* 2011).

Genome-wide association studies provide insights into the regulation of the human growth plate

A recent large meta-analysis of genome-wide association (GWA) studies identified at least 180 loci that influence adult height (Lango *et al.* 2010). Some of the genes within these loci probably affect height through endocrine mechanisms, such as *GH1*, which encodes GH, and *GHSR*, which encodes the GH secretagogue receptor. However, other genes probably affect height through a direct, local effect on the growth plate, such as *ACAN*, which encodes aggrecan, a critical proteoglycan component of the cartilage matrix. Thus, GWA studies of height have the potential to provide important insights into the molecular pathways regulating the human growth plate.

However, one challenge in the analysis of GWA data is identification the causative gene(s) at each position. At most positions, there are multiple genes that are sufficiently close to account for the linkage to adult stature, and thus additional information is needed to determine which of these genes modulates height and which are merely located close to the causative genes. We therefore used a mouse knockout phenotype database and human disease databases to identify genes within the GWA loci that are probably required for normal growth plate function. We also used expression microarray studies of mouse and rat growth plate to identify genes that have higher expression in growth plate cartilage than in other tissues, genes that are spatially regulated across different zones in the growth plate and/or genes that are temporally regulated in the growth plate during postnatal life, as growth plate function declines.

The combined phenotype-expression-GWA analysis implicated 78 genes in human growth plate function (Lui *et al.* 2012). Of these, some were already known to function in the human growth plate because human mutations affect the growth plate. In addition, many of the implicated genes participate in molecular pathways that have previously been implicated in the regulation of growth plate chondrocyte proliferation and differentiation in the mouse, such as the IHH-PTHrP system (*GLI2*, *IHH*, *HHIP*, *PTCH1*, and *PTH1LH* lie within GWA loci), BMP/TGF superfamily signaling (*TGFB2*, *BMP6*, *LTBP3*, *NOG*, *BMP2*, and *GDF5*), C-type natriuretic peptide signaling (*NPPC*, *PRKG2*, and *NPR3*), GH-IGF1 signaling (*IGF2BP2*, *IGF2BP3*, and *IGF1R*),

and FGF signaling (*FGF18*). This analysis indicates that these pathways are important not only in the mouse but also in the human growth plate.

In addition, the method implicates many genes not previously known to regulate either the mouse or human growth plate (Lui *et al.* 2012). For example, the analysis implicates *IGF2BP2* and *IGF2BP3* based on presence in the GWA loci and expression patterns in the growth plate. These mRNA-binding proteins have previously been implicated in mRNA localization, turnover, and translational control (Christiansen *et al.* 2009), and mRNA targets include *IGF2*, *H19*, *c-Myc*, β -actin (*ACTB*) and *GDF1*. Although neither *IGF2BP2* nor *IGF2BP3* has a recognized mouse or human phenotype, targeted ablation of the third member of the gene family, *IGF2BP1*, impairs bone growth and advances mineralization (Hansen *et al.* 2004). Thus, the data indicate that this family of proteins regulates growth plate chondrogenesis in both mice and humans.

Loss-of-function mutations of *CNP* impair and gain-of-function mutations stimulate bone growth

One interesting pathway implicated by the combined microarray–GWA analysis and by previous studies is C-type natriuretic peptide (*CNP* or *NPPC*) signaling (Lui *et al.* 2012). *CNP* belongs to a family of three natriuretic peptides, with *ANP* and *BNP* being the other two members (Potter *et al.* 2006). Unlike the other two members, *CNP* does not stimulate ‘natriuresis’ at physiological concentrations. Instead, *CNP* is found at high concentrations in cartilage (Hagiwara *et al.* 1994) and functions primarily as a local cartilage growth factor to stimulate growth plate chondrocytes (Pejchalova *et al.* 2007). Interestingly, homozygous loss-of-function mutations of the *CNP* receptor, natriuretic peptide receptor B (*NPRB* or *NPR2*), which is also highly expressed in the growth plate, cause acromesomelic dysplasia type Maroteaux in humans (Bartels *et al.* 2004), while heterozygous mutations of *NPR2* are associated with short stature (Olney *et al.* 2006, Vasques *et al.* 2013). Conversely, activating mutations of *NPR2* (Miura *et al.* 2012, Hannema *et al.* 2013) and overexpression of *NPPC* (Agoston *et al.* 2007) in humans both cause overgrowth disorders. These growth phenotypes have been replicated in knockout and transgenic mice, with *Nppc* or *Npr2* knockout causing severely shortened bones (Chusho *et al.* 2001, Tsuji & Kunieda 2005) and transgenic expression of activated *Npr2* causing increased bone length (Miura *et al.* 2012). At the cellular level, *CNP* stimulates

chondrocyte proliferation, chondrocyte hypertrophy, and cartilage matrix production (Mericq *et al.* 2000, Agoston *et al.* 2007). At the molecular level, *CNP* inhibits the ERK and p38 MAPK pathways (Ozasa *et al.* 2005), therefore counteracting the growth-inhibitory downstream signaling of FGFs in the growth plate (Yasoda *et al.* 2004), which will be discussed in the next section. Owing to its potent effect on offsetting FGF signaling, the use of *CNP* in treating achondroplasia (ACH) caused by activating mutation of FGF receptor 3 (*FGFR3*) is under active investigation. It is currently unclear whether all the growth-stimulating effects of *CNP* on chondrocytes are dependent on FGF signaling.

In addition to *CNP*, a related peptide, brain natriuretic peptide (*BNP*), also has been implicated in growth plate regulation. There is evidence that *BNP* is transcriptionally regulated by the transcription factor *SHOX* (Marchini *et al.* 2007). As *SHOX* deficiency underlies the growth plate dysfunction in Leri–Weill, Langer mesomelic dysplasia, and Turner’s syndromes, the findings indicate that decreased *NPPB* expression may play a role in the pathogenesis of these disorders.

Elucidating the role of FGFs in growth plate

FGF signaling is important for growth plate development, as mutations in various *FGFR* genes can lead to skeletal disease in humans (Chen & Deng 2005). Results from various *in vivo* studies indicate that *FGFR1* and *FGFR3* signaling are growth-inhibiting, while *FGFR2* signaling is growth-promoting. Cartilage-specific (*Col2a1-Cre*) inactivation of *Fgfr1* in mice showed a transient increase in height of the hypertrophic zone, and delayed terminal differentiation of hypertrophic chondrocytes (Jacob *et al.* 2006). However, an increase in adult body length has not been reported. In contrast, inactivation of *Fgfr2* in the mesenchymal condensations (*Dermo1-cre*), which affects both the osteoblast and chondrocyte lineages, resulted in mice with skeletal dwarfism (Yu *et al.* 2003), indicating a growth-promoting effect of *FGFR2* signaling. Clinically, *FGFR3* signaling is perhaps most relevant to growth plate development, as gain-of-function mutations of *FGFR3* in humans cause ACH, hypochondroplasia, and thanatophoric dysplasia (Rousseau *et al.* 1994, Shiang *et al.* 1994, Foldynova-Trantirkova *et al.* 2012).

Consistently, transgenic mice with activated *Fgfr3* in the growth plate show reduced chondrocyte proliferation, decreased numbers of hypertrophic chondrocytes, and decreased height of the hypertrophic zone (Chen *et al.* 1999), while *Fgfr3* knockout mice showed increased

chondrocyte proliferation, increased height of the hypertrophic zone, and increased skeletal growth (Eswarakumar & Schlessinger 2007).

Several signaling pathways downstream of FGFR3 activation have been elucidated, including the phosphoinositide 3-kinase–AKT pathway (Priore *et al.* 2006, Ulici *et al.* 2010), the ERK and p38 MAPK pathway (Krejci *et al.* 2008, Matsushita *et al.* 2009), and the STAT pathway (Li *et al.* 1999). These advancements in our understanding of the FGFR3 signaling pathway have contributed to the ongoing development of therapeutics for ACH. For example, growth-plate-specific overexpression of CNP (*Col2a1-Nppc*) or administration of a CNP analog has been shown to counteract FGF-induced MAPK activation and rescue the growth phenotype of ACH mice (Yasoda *et al.* 2004, Lorget *et al.* 2012). Other recently described potential therapeutics for ACH include meclizine, an antihistaminic drug that promotes chondrocyte proliferation (Matsushita *et al.* 2013); and a soluble form of human FGFR3 (sFGFR3) that acts as a decoy receptor to interfere with FGF binding and signaling (Garcia *et al.* 2013).

Expression studies in rodents have provided clues about the physiological ligands for FGFRs in the growth plate. In growth plates of 1-week-old rats, only *Fgf2*, *Fgf7*, *Fgf18*, and *Fgf22* expression were detectable by real-time PCR (Lazarus *et al.* 2007), whereas expression was far higher in the perichondrium adjacent to the growth plate, particularly for *Fgf1*, *Fgf2*, *Fgf6*, *Fgf7*, *Fgf9*, and *Fgf18* (Lazarus *et al.* 2007). In human fetal growth plate, expression of *FGF1*, *FGF2*, *FGF5*, *FGF8–FGF14*, *FGF16–FGF19*, and *FGF21* were detected at the mRNA level and *FGF1*, *FGF2*, *FGF17*, and *FGF19* at the protein level (Krejci *et al.* 2007). Functional studies in mice indicate signaling by *FGF9* and *FGF18* both contribute to growth plate development. Knockout mouse models of *Fgf9* (Hung *et al.* 2007) and *Fgf18* (Liu *et al.* 2002) indicate that both *Fgf9* and *Fgf18* promote chondrocyte proliferation during early development of the growth plate, but then function to inhibit chondrocyte proliferation and promote hypertrophic differentiation at later stages of development.

An interesting crosstalk between FGF signaling and GH–IGF1 signaling in the growth plate has recently been discovered that primarily involves *FGF21* (Inagaki *et al.* 2008). *FGF21* is a part of a subfamily of FGFs (other members include *FGF15*, *FGF19* and *FGF23*) that lack the FGF heparin-binding domain (Kharitonov *et al.* 2005), and therefore can act both locally in a paracrine fashion and diffuse from the tissue of synthesis to act as an endocrine factor. *FGF21* can activate *FGFR1* and *FGFR3* (Suzuki *et al.* 2008), both of which elicit growth-inhibitory

signaling as discussed earlier. Consistently, transgenic mice overexpressing *Fgf21* exhibit reduced bone growth, and interestingly, hepatic GH insensitivity (Inagaki *et al.* 2008).

FGF21 expression does not seem to be required for normal development of the growth plate, as *Fgf21* knockout mice showed no significant difference in body weight and body length as compared with WT mice (Kubicky *et al.* 2012). However, mounting evidence indicates that *FGF21* plays an important role in fasting-induced growth inhibition (Fig. 2). It is well established that reduced caloric intake in mammals causes reduced skeletal growth and hepatic GH insensitivity, which is partly attributed to decreased GH receptor (GHR) expression in the liver (Bornfeldt *et al.* 1989, Straus & Takemoto 1990). Numerous studies have shown that *FGF21* expression is induced by fasting (Galman *et al.* 2008). Interestingly, when WT and *Fgf21* knockout mice were placed under food restriction, *Fgf21* knockout mice showed significantly greater linear growth and growth plate thickness when compared with WT mice, indicating that the growth suppression induced by fasting is elicited by *Fgf21* (Kubicky *et al.* 2012). Most importantly, many of the molecular changes induced by fasting, including

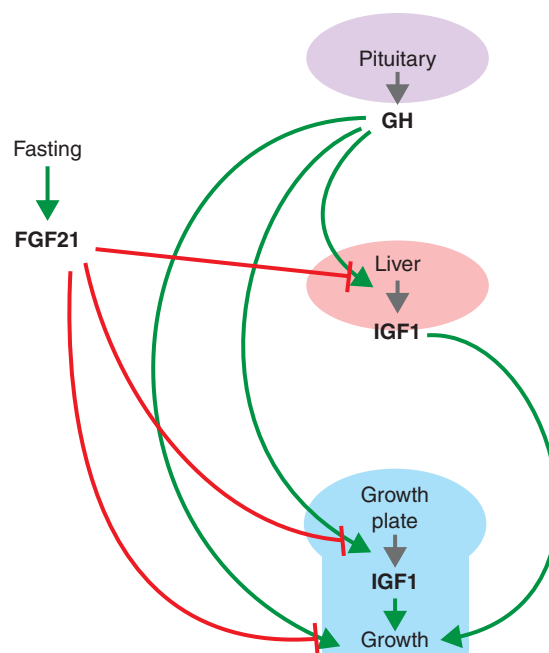


Figure 2

Proposed role of *FGF21* in fasting-induced growth inhibition. Green arrows, stimulation; red blunt-ended arrows, inhibition; and gray arrows, production. Evidence indicates that fasting-induced *FGF21* inhibits GH-induced IGF1 production in the liver, as well as the local effects of GH (IGF1-dependent and IGF1-independent) at the growth plate.

decreased hepatic GH sensitivity and decreased GHR and IGF1 expression in the growth plate, were corrected by *Egf21* deletion (Kubicky *et al.* 2012). More recently, results from *in vitro* studies using cultured growth plate chondrocytes have indicated that FGF21 may inhibit bone growth by directly suppressing chondrogenesis and GH action locally at the growth plate (Wu *et al.* 2012, 2013). Whether FGF21 mediates the effects of malnutrition on childhood growth in humans is less clear. Circulating FGF21 levels in humans appear to be less responsive to fasting than those in rodents and are actually elevated in obese humans (Woo *et al.* 2013).

Modulation of the GH/IGF1 axis by suppressor of cytokine signaling 2

The importance of GH and IGF1 in stimulating longitudinal growth has long been established. GH excess caused by pituitary adenomas in childhood can lead to gigantism. Conversely, GH deficiency or GH insensitivity caused by mutations in the GHR or signaling pathways markedly impairs postnatal growth (Rosenfeld *et al.* 2007). Patients with untreated isolated GH deficiency have an average final height standard deviation scores of -4.7 (range: -6.1 to -3.9) (Wit *et al.* 1996). Interestingly, GH has no apparent role in fetal growth, despite the presence of its receptor (GHR) in embryos (Garcia-Aragon *et al.* 1992). Experimental ablation of the pituitary in animals, or mutations of *GHR* that affect GH actions in both mice and humans have no significant effect on prenatal growth (Laron *et al.* 1993, Lupu *et al.* 2001). In contrast, IGF1 is important for both fetal and postnatal growth, as indicated by the observations that mutations of *IGF1* or *IGF1R*, the gene encoding its receptor, in humans lead to intrauterine (Abuzzahab *et al.* 2003, Fang *et al.* 2012) and postnatal (Baker *et al.* 1993) growth retardation.

GH affects the growth plate through several mechanisms. Some stimulatory effects are mediated through circulating IGF1, as evidenced by the observation that combined deficiency in the acid-labile subunit and liver-specific deficiency of IGF1 modestly decreases longitudinal bone growth in mice (Yakar *et al.* 2002). However, Col2-driven ablation of IGF1 in mice also decreases linear growth indicating a role for local skeletal IGF1 production in regulating growth plate function (Govoni *et al.* 2007), although not necessarily from chondrocytes (Parker *et al.* 2007). Furthermore, mice lacking both GHR and IGF1 have shorter bones than mice lacking only IGF1, indicating that GH, at least at supraphysiological circulating

concentrations, has an IGF1-independent effect on bone growth (Lupu *et al.* 2001).

Much work has been devoted to distinguish between the effects of GH, and excellent reviews on this subject are available elsewhere (Ahmed & Farquharson 2010, Wit & Camacho-Hubner 2011) and therefore will not be discussed further. Instead, herein, we highlight some of the recent work that established suppressor of cytokine signaling 2 (SOCS2) as a key modulator of local GH action in the growth plate.

The SOCS family contains eight members, SOCS1–SOCS7 and cytokine inducible SH2-containing protein (CISH). SOCS proteins are upregulated in response to cytokine stimulation and can subsequently bind through their SH2 domain to phosphorylated tyrosines in the cytokine receptor–JAK complex to inhibit further cytokine receptor activation. As such, SOCS proteins form part of a classical negative feedback circuit (Krebs & Hilton 2001). The role of SOCS2 in postnatal growth was demonstrated by the overgrowth phenotype of *Socs2* knockout mice (Metcalf *et al.* 2000), *Socs2*^{−/−} mice showed increased body length and body weight, and increased GH/IGF1 signaling with wider proliferative and hypertrophic zones in the growth plate (Metcalf *et al.* 2000, MacRae *et al.* 2009). Recent evidence has indicated that SOCS2 acts locally at the growth plate to modulate GH signaling. Chondrocytes isolated from *Socs2*^{−/−} mice showed increased phosphorylation of STATs upon incubation with GH (Pass *et al.* 2012), while cells overexpressing SOCS2 did not. Similarly, GH was able to stimulate growth in fetal metatarsals isolated from *Socs2*^{−/−} mice, but not that from WT mice (Pass *et al.* 2012), indicating that local GH action at the growth plate is negatively regulated by SOCS2. Some evidence indicates such local modulation of GH action is IGF-independent, as the GH-induced *Socs2*^{−/−} metatarsal bone growth is not accompanied by an increase in *Igf1* or *Igfbp3* transcript levels and occurred in the presence of an IGF1 receptor inhibitor (NVP-AEW541) (Dobie *et al.* 2013). A role for SOCS2 in human growth is indicated by the identification of SOCS2 at a locus associated with human height variation in GWA studies (Weedon *et al.* 2008, Lango *et al.* 2010, Lui *et al.* 2012). Interestingly, a missense mutation in *SOCS2* has been reported (in meeting abstract form) to cause gigantism (Suda *et al.* 2011).

Summary and future prospects

The understanding of the paracrine regulation of longitudinal bone growth at the growth plate has advanced

substantially in recent years. In this brief review, we have focused on some of the recent advances that have been possible due to microdissection, microarray analysis, inducible and tissue-specific gene targeting in mice, GWA studies, and genetic studies of rare diseases. These studies have not only described important biological mechanisms and processes, but also identified many new genes and indicated a promising potential treatment for ACH that is currently being evaluated in human studies. However, many important questions remain to be elucidated. For example, information on how the endocrine system interacts with the paracrine signals to regulate growth plate chondrogenesis is mostly lacking, as well as information on molecular mechanisms for the orientation of proliferative chondrocytes into columns and mechanisms, which cause the proliferation rate and growth rate to slow with age and thus limit the overall size of the skeleton and thus the organism. Continued methodological advancements promise to accelerate progress in our understanding of skeletal development, skeletal growth, and the disorders affecting these processes and will probably yield new therapeutic targets and approaches.

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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