DDisorders of the Growth Plate

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Abstract

Purpose of the review—To summarize the recent advances in our understanding of the majors genes involved in chondrogenesis and their molecular mechanisms.

Recent findings—Disorders of the growth plate and the resulting skeletal dysplasias are a consequence of defects in genes involved in various stages of the chondrocyte proliferation and differentiation process. Recent identification of disease genes has provided insights into the pathophysiology of many skeletal dysplasias.

Summary—This knowledge enhances our understanding of the physiology and pathophysiology of the growth plate. Many skeletal dysplasias can now be characterized at the molecular level, allowing clinicians to provide accurate molecular diagnoses and counseling. Further research in this area will likely provide insights into possible therapeutic options for disorders of the growth plate.

Keywords

chondrocyte; growth plate; skeletal dysplasia; chondrocyte-specific transcription; extracellular matrix

Introduction

Linear growth results from endochondral bone elongation, which occurs mainly at the cartilaginous growth plate. Events at the growth plate required for bone growth occur in a predictable pattern that reflects the complex molecular events regulating chondrocyte proliferation, hypertrophy and accumulation of extracellular matrix (ECM). This complex process is a result of a balance between the expression of many genes encoding transcription factors, matrix proteins and mediators of cell-matrix interactions [1-3].

The processes of chondrocyte growth and differentiation occur in a spatially-defined relationship within the growth plate. As round, proliferative chondrocytes form a columnar layer; they stop proliferating and become prehypertrophic chondrocytes, which subsequently differentiate into post-mitotic, hypertrophic cells. These stages are defined by cellular phenotype and also by the ECM proteins the cells produce at each stage (Figure 1). Markers of proliferating chondrocytes are collagen II and Aggrecan. As cells progress through the developmental stages, expression of specific transcription factors and ECM proteins occurs in proliferating columnar cells, prehypertrophic and hypertrophic cells [4-6]. Collagen X is highly expressed in hypertrophic chondrocytes as they fully mature. As hypertrophic chondrocytes mature, osteoblast and vascular invasion take place and bone formation occurs. ECM deposited by proliferating and hypertrophic chondrocytes serves as a template for subsequent bone

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formation. Hypertrophic cells also secrete soluble proteins, such as vascular endothelial growth factor (VEGF) [7]. Hypertrophic chondrocytes ultimately undergo apoptosis, resulting in closure of the growth plate and cessation of long bone growth. Interactions between the three critical processes in the growth plate, chondrocyte proliferation, chondrocyte differentiation and hypertrophy, and ECM formation, are critical for normal long bone growth [8].

Defects in genes regulating these critical processes are associated with skeletal dysplasia and short stature. To date, there are over three hundred well defined disorders of skeletal growth and development [3;9]. Achondroplasia and osteogenesis imperfecta are among the most common skeletal dysplasias, while others are less common. Skeletal dysplasia has traditionally been a clinical and radiographic diagnosis.

Over the past decade, geneticists have utilized several strategies to identify the causative genes in many of the skeletal dysplasias.

Most skeletal dysplasias with known genetic etiology are monogenic heritable diseases with variable expressivity. The inheritance patterns are most often autosomal dominant and autosomal recessive. Genetic defects resulting in skeletal dysplasia can be divided into 2 major categories; defects in transcriptional control of skeletogesis and defects in genes involved in ECM formation. Transcriptional dysregulation often results in not only skeletal abnormalities, but in multiple organ system abnormalities [10]. At the cellular level, loss or gain of transcription factor function results in a pathological spectrum ranging from defects in cell proliferation, to dysregulated cell differentiation and cell survival [11]. The defects in ECM formation also result in wide range of skeletal dysplasias, perhaps owing to the fact that ECM proteins are a major component of the growth plate [12;13].

The most abundant ECM constituents in the growth plate are collagen types II, IX and XI. Type II collagens are covalently bound to type IX and XI fibrils. The non-collagenous ECM includes Matrilin (MATN) family members and cartilage oligomeric matrix protein (COMP).

Over the past several years, many of the molecular mechanisms of transcription factor action, chondrocyte proliferation and differentiation and regulation of ECM gene expression have been elucidated, thus explaining the pathophysiology underlying various skeletal dysplasias. This review will provide an update on genetic disorders regulating chondrocyte development within the growth plate.

Defects in the Early Stages of Chondrogenesis

Defects in regulatory markers

As mesenchymal cells migrate from the cranial neural crest and mesoderm and form skeletal elements, they start to express Col2a1, a marker for chondrocytes. The transcription factor Sox9 has an important role in specifying the commitment of mesenchymal cells to become chondrocytes [14]. Sox9 belongs to the SRY family, a characteristic of which is the presence of high mobility group box (HMG-box) DNA binding domains. Expression of Sox9 overlaps with that of Col2a1. *In vitro* and *in vivo* studies demonstrated the presence of chondrocyte-specific regulatory sequences in the first intron of the Col2a1 gene [15]. In addition to its ability to interact with this Col2a1 regulatory element, Sox9 binds as a homodimer to the consensus sequences of Col9a1, Col27a1 and MATN-1 [16-18]. Over expression of Sox9 in mice induces the expression of type II collagen [11;19]. The expression of Sox9 is regulated by fibroblast growth factor (FGF), bone morphogenic protein (BMP), members of the Wnt family, insulin-like growth factor-I (IGF-I) and transforming growth factor-β(TGF-β). Sox9 functions as a negative regulator of chondrocyte hypertrophy [11]. Heterozygous mutations in and around the Sox9 gene have been associated with campomelic dysplasia [20].

Defects in ECM

Collagen type II, along with collagens type IX and XI, is one of the major collagens expressed early in chondrogenesis. The collagen family is characterized by a coiled-coiled triple helix. Collagen type II is considered a fibril-forming collagen, a core element of the growth plate. Mutations in collagen type II results in either qualitative changes or quantitative changes depending on the location of the mutation. Therefore, clinical manifestations of collagen II defects vary from perinatal lethality to mild skeletal dysplasia. One consistent phenotype is an abnormality in the epiphysis. Disorders that are associated with mutations in collagen type II include spondyloepiphyseal dysplasia (SED), achondrogenesis type II or hypochondrogenesis, Kniest dysplasia and Stickler syndrome [21-24].

Defects in the Later Stages of Chondrogenesis

Defects in early regulatory markers in late stages of chondrogenesis

Once chondrocytes enter the prehypertrophic stage, they express Indian Hedgehog (Ihh). Ihh is a member of the hedgehog family of signaling molecules, which play a crucial role in regulating a variety of developmental processes. Ihh is one of the key signaling proteins controlling chondrocyte proliferation and hypertrophy in the developing skeletal system [25; 26]. Ihh regulates the onset of hypertrophic differentiation by forming a negative feedback loop with PTHrP [27]. Ihh activates the expression of PTHrP, which then signals through its receptor to inhibit chondrocyte hypertrophy and suppress further Ihh expression by keeping chondrocytes in a proliferating state [28;29]. Ihh has also been found to promote chondrocyte proliferation and the transition from slowly proliferating periarticular chondrocytes to fast proliferating columnar chondrocytes independently of PTHrP signaling [6;28]. More recently, a PTHrP-independent role of Ihh signaling in regulating chondrocyte hypertrophy was demonstrated to be mediated through the activation of Wnt and BMP signaling [30]. Our own data indicate that Ihh is involved in the nutritional regulation of chondrogenesis in the growth plate [31]. Defects in the Ihh gene have been implicated in human skeletal dysplasias; brachydactyly type A1 [32] and acrocapital femoral dysplasia [33]. Recently, a study on genome-wide associations with adult height revealed that Ihh is one of the twenty genes for which variants are associated with adult height [34;35].

Ihh/PTHrP/Wnt/BMP signaling and interactions have been reviewed extensively by others [36-38]. Several defects within these pathways result in skeletal dysplasia. One of the downstream genes of Wnt is Wnt1-inducible signaling protein 3 (WISP3). Reduced WISP3 expression in a human chondrocyte cell line increased the amount of cellular reactive oxygen species (ROS) [39]. Over expression of WIPS3 inhibits BMP and Wnt signaling [40]. Mutations in WISP3 have been found in patients with progressive pseudorheumatoid chondrodysplasia (PPRC) [41]. In contrast, constitutively active mutations of the PTH/PTHrP receptor, which would presumably result in constitutive activation of the pathway's downstream targets, have been associated with metaphyseal chondrodysplasia, Jansen type.

Defects in ECM

COMP is a non-collagenous ECM protein highly expressed in cartilage. Collagen types I, II and IX bind to COMP with high affinity. Defects in the COMP gene have been identified in patients with pseudoachondroplasia and in patients with multiple epiphyseal dysplasia (MED) [42]. Overexpression of mutant COMP leads to decreased chondrocyte viability *in vitro*. The growth failure of patients with COMP defects is presumed to be due to increased cell death of growth-plate chondrocytes [43].

Collagen type IX is a heterotrimer mainly expressed in cartilage. Collagen type IX functions as a bridging collagen to other components in the cartilage matrix. MED type II is caused by mutations in the three genes encoding collagen type IX [44].

The MATN family of pericellular matrix proteins is comprised of four structurally related members, MATN-1, -2, -3 and -4). All contain one or two von Willebrand factor A (vWA) domains, at least one EGF-like motif and a C-terminal coiled-coiled domain, which allows the formation of homo and hetero-oligomers. All four MATNs are expressed in cartilage. Expression of MATN family members occurs early in chondrogenesis and persists throughout the process. The regulatory control of MATN family members is not well characterized, although the MATN-1 promoter has been shown to harbor Sox-9 binding sites [45]. The function of MATN proteins remains elusive. MATNs have been shown to bridge collagenous and non-collagenous matrix components. MATN-1, -3 and -4 are associated with collagen VI microfibrils [46] as well as other collagens [47;48]. Other non-collagenous molecules, such as COMP and decorin, also bind to MATNs with high affinity. Mutations in the human gene encoding MATN-3 are associated with MED. The effect of MATN-3 mutations is likely the result of an intracellular retention of the mutant protein in chondrocytes, leading eventually to chondrodysgenesis [49].

Defects in regulatory markers

Runx2 is a member of family of transcription factors that share the DNA-binding domain of the Drosophila "pair rule gene runt." Runx2 is expressed in the late condensation stage of chondrogenesis. Its expression decreases in proliferating chondrocytes and increases again in hypertrophic chondrocytes [50]. Runx2 knock out mice lack hypertrophic chondrocytes in some but not all skeletal elements. Constitutive expression of Runx2 leads to ectopic chondrocyte hypertrophy in transgenic mice and allows chondrocyte hypertrophy to occur in $Runx2^{-/-}$ mice. Runx3 is also important for chondrocyte hypertrophy. Mice lacking both Runx2 and Runx3 do not have recognizable hypertrophic chondrocytes or collagen type X-expressing cells anywhere in the skeleton. Therefore, it seems clear that Runx2 and Runx3 are positive regulators of chondrocyte hypertrophy [51]. Heterozygous mutations in Runx2 are responsible for cleidocranial dysplasia (CCD).

Another transcription factor, Twist-1 is also important to chondrocyte hypertrophy. Its function requires Runx2. Twist-1 is a nuclear protein containing a basic helix-loop-helix domain and a so-called Twist-box at its C-terminus. Twist-1 is expressed in the cells that form the perichondrium that surround the growth plate and inhibit chondrocyte hypertrophy [52]. Defect in Twist-1 has been found in Saethre-Chotzen syndrome [53]. It has been demonstrated that Runx2 positively regulates fibroblast growth factor 18 (FGF18) in the perichondrium. FGF18 activates FGFR3 signaling and inhibits chondrocyte hypertrophy [52;54]. Activating mutations of FGFR3 cause achondroplasia, hypochondroplasia and thanatophoric dysplasia. The pathophysiological connection between Runx2, FGF18 and FGFR3 attests to the intricate interactions of transcription factor and growth factors within the growth plate [55;56].

As chondrocytes continue to differentiate, they become hypertrophic chondrocytes, which are characterized by the synthesis of collagen type X, which exists as a homotrimer under physiological circumstances. The triple helical domain is approximately half the size of that in collagen type II molecules. Mutations in the COL10A1 gene are responsible for Schmid metaphyseal chondrodysplasia. The mutant collagens present in this condition have been demonstrated to remain associated with the endoplasmic reticulum until degraded intracellularly by the proteasome and lysosome pathways [57;58].

C-natriuretic peptide (CNP) and its cognate receptor, the natriuretic peptide receptor B (NPRB), were originally implicated in the regulation of skeletal growth of transgenic and

knockout mice [59]. More recently, mutations in the NPRB have been described in the autosomal recessive skeletal dysplasia known as acromesomelic dysplasia, type Maroteaux (AMDM). In chick chondrocytes, CNP increases the expression of collagen X and N-cadherin, the synthesis of glycosaminoglycan and chondrogenesis [60;61]. Expression of collagen II is unaffected, suggesting an important role of CNP in late chondrocyte differentiation. Pharmacologic inhibition of the p38 mitogen-activated protein (MAP) kinase blocks expansion of the hypertrophic zone of the growth plate, presumably by blocking the anabolic effects of CNP.

In humans, loss-of-function mutations of the short stature homoebox (SHOX) gene are associated with Leri-Weill dyschondrosteosis and Langer mesomelic dysplasia [62]. Most importantly, SHOX accounts for the skeletal dysplasia associated with Turner syndrome. SHOX mutations have also been identified as accounting for rare cases of "idiopathic short stature," the phenotype of which tends to closely resemble that of Turner syndrome. At the molecular level, SHOX is upregulated in hypertrophic chondrocytes. In the murine model, deficiency of SHOX2, closely related to human SHOX, results in mesomelia. Histologically, $SHOX2^{-/-}$ mutants have a decreased number of hypertrophic chondrocytes, supporting the purported role of SHOX in chondrocyte differentiation [63;64].

Conclusion

Our current knowledge of the molecular mechanisms of skeletal dysplasia has provided insights into the pathophysiology of specific skeletal defects as well as the normal process required for chondrocyte development, as broadly summarized in Figure 2. Defects in individual genes result in a spectrum of disease ranging from mild to severe and to various classes of skeletal dysplasia. It is intriguing that despite the critical role of each gene within the growth plate, mutations in a specific gene may not produce universal effects in every growth plate. This observation suggests that within each growth plate there is a threshold requirement for the particular gene. The precise pathophysiology accounting for this is yet to be characterized. As knowledge in this field advances, it is likely that we will identify more factors that regulate ciritical genes within growth plate. Defects in these genes will likely reduce the number of skeletal dysplasias with molecular defects that are presently unidentified.

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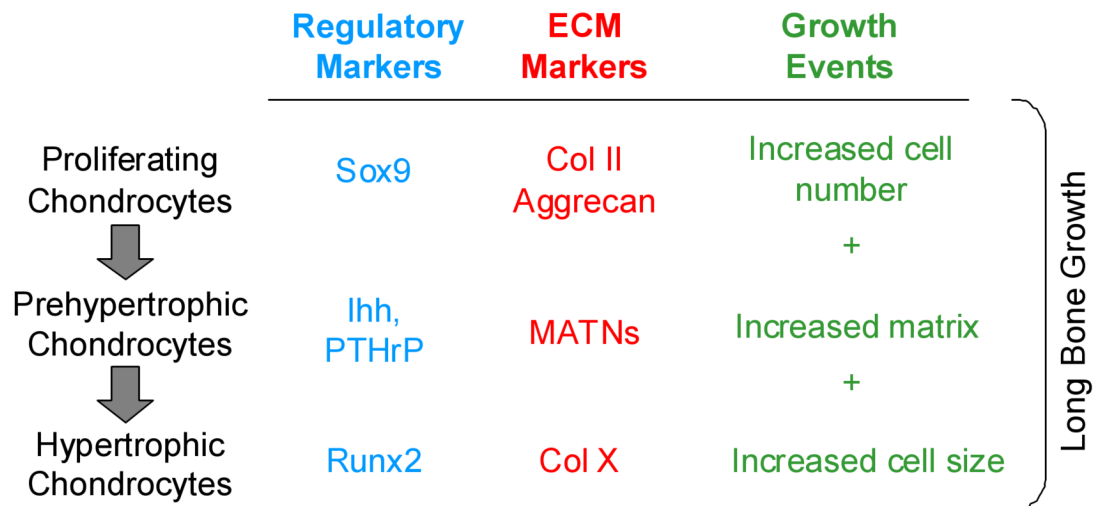
Figure 1.
The organization of the growth plate, showing the stages of chondrogenesis, the matrix constituents and the transcription factors that serve as markers for the stages of chondrocyte differentiation.

	Regulatory Markers	ECM Markers	Skeletal Dysplasia
Proliferating Chondrocytes	Sox9		Campomelic dysplaia
↓		Col II	SED
Prehypertrophic Chondrocytes	Ihh PTHrP		Brachydactyl Type A1 Metaphyseal chondrodysplasia, Jansen type
↓		MATNs	MED
Hypertrophic Chondrocytes	Runx2		CCD
		Col X	Schmid metaphyseal chondrodysplasia

Figure 2.
Examples of genetic defects in key chondrocyte regulators or ECMs during various stages of chondrogenesis resulting in specific skeletal dysplasia.