Current Understanding on the Molecular Basis of Chondrogenesis

Toshimi Michigami

Abstract. Endochondral bone formation involves multiple steps, consisting of the condensation of undifferentiated mesenchymal cells, proliferation and hypertrophic differentiation of chondrocytes, and then mineralization. To date, various factors including transcription factors, soluble mediators, extracellular matrices (ECMs), and cell-cell and cell-matrix interactions have been identified to regulate this sequential, complex process. Moreover, recent studies have revealed that epigenetic and microRNA-mediated mechanisms also play roles in chondrogenesis. Defects in the regulators for the development of growth plate cartilage often cause skeletal dysplasias and growth failure. In this review article, I will describe the current understanding concerning the regulatory mechanisms underlying chondrogenesis.

Key words: chondrocyte, transcription factors, growth factors, extracellular matrix, differentiation

Introduction

In mammals, most of the skeleton including the long bones of the limbs and the vertebral columns is formed through endochondral bone formation, which consists of the mesenchymal condensation of undifferentiated cells, proliferation of chondrocytes and differentiation into hypertrophic chondrocytes, followed by mineralization (1–3). Proliferating chondrocytes form orderly parallel columns in the growth plates, and are characterized by the expression of type II, IX, and XI collagen (Col II, IX and XI) and proteoglycans such as aggrecan. When chondrocytes differentiate, they become hypertrophic and begin to produce a high level of alkaline phosphatase and type X collagen (Col X). Eventually, the terminally differentiated chondrocytes undergo apoptosis, and the cartilaginous matrix is mineralized and replaced by bone (1–3). These mature chondrocytes express vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) (4, 5). VEGF induces blood vessel invasion, and MMPs aid in the degradation of the cartilaginous matrix (4, 5). Accumulating evidence provided by human diseases, mouse models and cell studies has identified a number of factors to be involved in the regulation of proliferation and differentiation of chondrocytes (Fig. 1). Among them are various transcription factors, soluble growth factors, ECMs, and epigenetic factors. In this review...
article, I will provide an updated overview of the molecular mechanisms regulating the development of growth plate cartilage.

**Transcription Factors Involved in Chondrogenesis**

Among the transcription factors involved in chondrogenesis, SOX9 has been the most extensively studied. SOX9 is a member of the Sox family of transcription factors characterized by a high-mobility-group-box DNA binding motif related to that of the sex-determining factor SRY, and is a responsible molecule for campomelic dysplasia characterized by severe skeletal malformation (6, 7). Evidence provided by mouse models has also revealed that Sox9 is indispensable for chondrogenesis. Sox9 begins to be expressed at the mesenchymal osteochondroprogenitor stage, and transactivates several genes specific to proliferating chondrocytes such as Col2a1 encoding Col II (8, 9). Sox5 (L-Sox5) and Sox6 were shown to cooperate with Sox9 to activate the chondrocyte-specific enhancers in these genes (10, 11). The activating transcription factor (ATF)/cyclic AMP response element binding protein (CREB) family and the AP1 family member c-Fos are required to maintain the proliferative capacity of early chondrocytes (12, 13).

Hypertrophic maturation of chondrocytes requires the Runx domain family transcription factors Runx2 and Runx3 as well as a decrease in the expression and/or activity of the Sox proteins. Runx2/Runx3-double knockout mice lack hypertrophic chondrocytes (14, 15). It has been reported that Runx2 directly transactivates the genes Ihh (Indian hedgehog), Col10a1 encoding Col X, and MMP13 (15–17). Recently, it has also been shown that Sox9 suppresses the expression of Runx2 and β-catenin signaling, which inhibits the hypertrophic change of chondrocytes (18). A basic helix-loop-helix type transcription factor, Twist-1 functions as another repressor of Runx2 in the perichondrium (19). Other transcription factors, such as MADS-box transcription factors Mef2c and Mef2d (myocyte enhancer factor 2c and 2d), Msx2, the AP1 family member Fra2, and FoxA family transcription factors, also facilitate chondrocyte hypertrophy (20–25). The transcription factor hypoxia-inducible factor-1α (HIF-1α) is one of the major regulators of the hypoxic response in mammals and plays a role in chondrocyte survival and gene regulation for VEGF, which induces blood vessel invasion into cartilage (26, 27). Thus, a complex transcriptional network governs the process of chondrogenesis from chondrocytic commitment to terminal differentiation.

**Soluble Regulators of Chondrogenesis**

Fibroblast growth factors (FGFs) play critical roles in chondrogenesis by activating signaling through FGF receptors (FGFRs), as indicated by a spectrum of human chondrodysplasias and dwarfism caused by gain-of-function mutations in the FGFR3 gene (28–31). Among the FGFRs, Fgfr3 is expressed in cells undergoing mesenchymal condensation and proliferating chondrocytes. On the other hand, Fgfr1 is expressed in prehypertrophic and hypertrophic chondrocytes (32–34). It has been suggested that FGFR3-mediated signaling negatively regulates chondrocyte proliferation and differentiation (35–
Molecular basis of chondrogenesis

In FGFR3-related chondrodysplasias such as achondroplasia, constitutive activation of FGFR3 results in the activation of the downstream ERK and STAT pathways (28). Although various FGFs are expressed in cartilage, FGF18 is suggested to be a physiological ligand for FGFR3 in chondrocytes, because of similar histology in the growth plates between Fgf18-knockout mice and Fgfr3-knockout mice (38, 39).

Ihh is a secreted signaling molecule, expressed by prehypertrophic chondrocytes. A line of evidence provided by genetically altered mouse models has revealed that Ihh increases the expression of parathyroid hormone-related protein (PTHrP) in perichondrial cells and chondrocytes at the ends of long bones, which delays chondrocyte hypertrophy through the PTH/PTHrP receptor expressed in proliferating chondrocytes. Thus, Ihh and PTHrP function in a local negative feedback loop to regulate the onset of hypertrophic differentiation (40). In addition, it is also reported that Ihh stimulates the proliferation and maturation of chondrocytes independently of PTHrP, in which activation of Wnt and bone morphogenetic protein (BMP) signaling is suggested to be involved (41–43).

The importance of C-type natriuretic peptide (CNP) signaling in chondrogenesis was shown by the severe dwarfism of CNP-knockout mice (44, 45). CNP exerts its signal mainly through the receptor NPR2, which is also called guanylyl cyclase B (GC-B), and Npr2-null mice display a similar phenotype to CNP-knockout mice (46). Based on the mouse studies, it has been suggested that CNP promotes endochondral bone growth through several mechanisms, including the stimulation of chondrocyte proliferation and hypertrophy and an increase in ECM production (44–46). In humans, loss-of-function mutations in the NPR2 gene cause acromesomelic dysplasia, type Maroteaux, characterized by severe dwarfism (47, 48), while a gain-of-function type mutation in the gene has been identified in a family with skeletal overgrowth (49), which indicates that the CNP/NPR2 signaling pathway plays a role in the development of growth plate cartilage both in humans and mice. The similarity in the skeletal phenotype between CNP-deficient mice and human achondroplasia has suggested that CNP/NPR2 signaling is promising as a new therapeutic target for the dwarfism associated with skeletal dysplasia (46). It has been shown that the signaling evoked by CNP inhibits the FGF-induced activation of the ERK pathway (46). The p38MAPK pathway and PI3K/Akt pathway are also suggested to be involved in the regulation of chondrocyte development by CNP (50).

Other soluble factors such as Wnts, bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-β), insulin-like growth factors (IGFs) and thyroid hormone are also involved in chondrogenesis (51–53) but are not further discussed here.

Regulation of Chondrogenesis by the ECM

The ECM provides a cell type-specific microenvironment. As chondrocytes mature, they produce abundant ECM proteins such as collagens and proteoglycans, and the cell-matrix interactions come to have more important roles than in the earlier stages of chondrogenesis, when the cell-cell interaction via adhesion molecules such as N-cadherin and N-CAM is involved in cellular condensation and subsequent chondrogenesis (54, 55). The ECM is recognized and bound by integrins and cell surface transmembrane receptors. Integrins occur as dimers of an α subunit and a β subunit, and the binding of ligands to integrins leads to transduction of signaling from the ECM to intracellular effectors (56). Chondrocytes express several integrin subunits, and it has been reported that chondrocyte-specific β1 integrin-knockout mice exhibited a chondrodysplasia-like phenotype (57, 58). In these mice, growth plates exhibited unorganized proliferative columns and an abnormal cell shape due to the loss of adhesion to Col II, and the isolated chondrocytes displayed
reduced proliferation (58). Integrin-linked kinase is one of the components of the complex whose formation is triggered by the activation of integrin-mediated signaling, and knockout of its gene resulted in a chondrodysplasia-like phenotype similar to that of chondrocyte-specific β1 integrin-knockout mice (59). These results suggest the importance of integrin-mediated signaling from the ECM in chondrogenesis.

The SLC26A2 gene encodes a sulfate transporter responsible for sulfate uptake by chondrocytes. Mutations in this gene have been identified in a form of chondrodysplasia called diastrophic dysplasia, which is characterized by undersulfation of cartilaginous proteoglycans. Similar to patients with diastrophic dysplasia, dtd mice harboring a knock-in Slc26a2 mutation were reported to exhibit undersulfation of glycosaminoglycans such as chondroitin (60). It has been also reported that mice lacking the gene encoding chondroitin sulfate N-acetylgalactosaminyltransferase 1 (CSGalNAcT-1), an enzyme involved in the initiation of the biosynthesis of chondroitin sulfate, have shorter, disorganized chondrocyte columns in the growth plates with a rapid catabolism of aggrecan (61).

Another function of the cartilaginous ECM is regulation of chondrogenesis through binding, storage, and release of soluble factors. For example, most of FGFs bind to heparan sulfate proteoglycans and bind to FGFRs in the context of heparan sulfate proteoglycans to trigger signal transduction. Analysis of mice lacking sulfate-modifying factor 1 (Sumf1) has suggested that desulfation of proteoglycans regulates chondrocyte proliferation and differentiation by limiting FGF signaling (62).

Vinculin is a major component of the focal adhesion complex and functions in adhesion and/or signaling between the extracellular microenvironment and the cell via integrins and cadherins. Although little is known about its tissue-specific functions, we have recently identified vinculin as having profound roles in chondrogenesis (63). Knockdown of vinculin in primary chondrocytes and organ cultures of metatarsal explants resulted in reduced expression of Col2a1, aggrecan, Col10a1, and Runx2. Moreover, knockdown of vinculin abrogated IGF-I-induced growth of metatarsal explants. The upregulation of Col2a1 and aggrecan expression by IGF-I was also cancelled by the knockdown. These results suggest that vinculin regulates the expression of chondrocyte-specific genes by orchestrating the signaling from the ECM and soluble factors such as IGF-I (63).

**Epigenetic Control of Chondrogenesis**

Recent studies have uncovered the roles of epigenetic mechanism in chondrogenesis. Among the histone deacetylases (HDACs), HDAC4 has been shown to prevent the premature chondrocyte hypertrophy by inhibiting the activity of Runx2/3 and Mef2c/d transcription factors (20, 21, 64). HDAC1 and HDAC2 mediate the repression of some cartilage-specific genes including Col2a1 (65). These findings suggest that histone modification influences the process of endochondral bone formation.

DNA methylation is also involved in the regulation of cartilage-specific genes. For example, it was reported that the demethylation of 2 CpG sites in the COL10A1 promoter was correlated with induction of the COL10A1 gene during the chondrogenic differentiation of human mesenchymal stem cells (66).

**MicroRNAs as Novel Regulators of Chondrogenesis**

MicroRNAs (miRNAs) are a class of ~22 nucleotide noncoding RNAs that regulate the expression of other genes at the posttranscriptional level. The critical roles of miRNAs in chondrogenesis were first indicated by the severe skeletal growth defects in mice lacking Dicer, an enzyme required for miRNA synthesis (67). In the growth plates of Dicer-
knockout mice, proliferation of chondrocytes was decreased, while differentiation was accelerated (67). Since then, an increasing number of specific miRNAs has been identified to have roles in chondrocyte differentiation. Mice lacking miR-140 showed a mild short stature and age-related osteoarthritis-like changes, suggesting that miR-140 regulates both the development and homeostasis of cartilage (68). miR-145 was reported to directly target Sox9 and regulate chondrogenic differentiation of mesenchymal stem cells (69), while miR-199a was shown to be responsive to BMP and to regulate chondrogenesis by targeting Smad1 (70). These findings indicate the importance of miRNA-mediated regulation of chondrogenesis.

**Conclusion**

In this review, I have overviewed the current knowledge concerning the molecular mechanisms underlying the development of growth cartilage. The proliferation and differentiation of chondrocytes is elaborately controlled by various factors, and their defects are often associated with growth failure and skeletal dysplasias. Further clarification of the molecular basis of cartilage development may lead to the discovery of new therapeutic targets for these conditions.

**References**

1. Wagner EF, Karsenty G. Genetic control of skeletal development. Curr Opin Genet Dev 2001;11: 527–32. [Medline] [CrossRef]
2. Kronenberg HM. Developmental regulation of the growth plate. Nature 2003;423: 332–6. [Medline] [CrossRef]
3. Michigami T. Regulatory mechanisms for the development of growth plate cartilage. Cell Mol Life Sci 2013: [CrossRef]. [Medline]
4. Zelzer E, Mamluk R, Ferrara N, Johnson RS, Schipani E, Olsen BR. VEGFA is necessary for chondrocyte survival during bone development. Development 2004;131: 2161–71. [Medline]
5. Stickens D, Behonick DJ, Ortega N, Heyer B, Hartenstein B, Yu Y, et al. Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. Development 2004;131: 5883–95. [Medline] [CrossRef]
6. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanovic M, et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994;372: 525–30. [Medline] [CrossRef]
7. Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, et al. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell 1994;79: 1111–20. [Medline] [CrossRef]
8. Akiyama H, Lefebvre V. Unraveling the transcriptional regulatory machinery in chondrogenesis. J Bone Miner Metab 2011;29: 390–5. [Medline] [CrossRef]
9. Lefebvre V, Huang W, Harley VR, Goodfellow PN, de Crombrugghe B. SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. Mol Cell Biol 1997;17: 2336–46. [Medline]
10. Lefebvre V, Li P, de Crombrugghe B. A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J 1998;17: 5718–33. [Medline] [CrossRef]
11. Akiyama H, Chaboissier MC, Martin JF, Schedl A, de Crombrugghe B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev 2002;16: 2813–28. [Medline] [CrossRef]
12. Vale-Cruz DS, Ma Q, Syme J, LuValle PA. Activating transcription factor-2 affects skeletal growth by modulating pRb gene expression. Mech Dev 2008;125: 843–56. [Medline] [CrossRef]
13. Wang ZQ, Ovitt C, Grigoriadis AE, Mohle-Steinlein U, Ruther U, Wagner EF. Bone and haematopoietic defects in mice lacking c-fos. Nature 1992;360: 741–5. [Medline] [CrossRef]
14. Yoshida CA, Komori T. Role of Runx proteins in chondrogenesis. Crit Rev Eukaryot Gene Expr 2005;15: 243–54. [Medline] [CrossRef]
15. Yoshida CA, Yamamoto H, Fujita T, Furuichi T, Ito K, Inoue K, et al. Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes Dev 2004;18: 952–63. [Medline] [CrossRef]

16. Selvamurugan N, Kwok S, Partridge NC. Smad3 interacts with JunB and Cbfa1/Runx2 for transforming growth factor-beta1-stimulated collagenase-3 expression in human breast cancer cells. J Biol Chem 2004;279: 27764–73. [Medline] [CrossRef]

17. Zheng Q, Zhou G, Morello R, Chen Y, Garcia-Rojas X, Lee B. Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression in vivo. J Cell Biol 2003;162: 833–42. [Medline] [CrossRef]

18. Dy P, Wang W, Bhattaram P, Wang Q, Wang L, Ballock RT, et al. Sox9 directs hypertrophic maturation and blocks osteoblast differentiation of growth plate chondrocytes. Dev Cell 2012;22: 597–609. [Medline] [CrossRef]

19. Hinoi E, Bialek P, Chen YT, Rached MT, Groner Y, Behringer RR, et al. Runx2 inhibits chondrocyte proliferation and hypertrophy through its expression in the perichondrium. Genes Dev 2006;20: 2937–42. [Medline] [CrossRef]

20. Arnold MA, Kim Y, Czubryt MP, Phan D, McAnally J, Qi X, et al. MEF2C transcription factor controls chondrocyte hypertrophy and bone development. Dev Cell 2007;12: 377–89. [Medline] [CrossRef]

21. Kozhemyakina E, Cohen T, Yao TP, Lassar AB. Parathyroid hormone-related peptide represses chondrocyte hypertrophy through a protein phosphatase 2A/histone deacetylase 4/MEF2 pathway. Mol Cell Biol 2009;29: 5751–62. [Medline] [CrossRef]

22. Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, et al. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Nat Genet 2000;24: 391–5. [Medline] [CrossRef]

23. Amano K, Ichida F, Sugita A, Hata K, Wada M, Takigawa Y, et al. MSX2 stimulates chondrocyte maturation by controlling Ihh expression. J Biol Chem 2008;283: 29513–21. [Medline] [CrossRef]
34. Peters KG, Werner S, Chen G, Williams LT. Two FGF receptor genes are differentially expressed in epithelial and mesenchymal tissues during limb formation and organogenesis in the mouse. Development 1992;114: 233–43. [Medline]

35. Chen L, Adar R, Yang X, Monsonego EO, Li C, Hauschka PV, et al. Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. J Clin Invest 1999;104: 1517–25. [Medline] [CrossRef]

36. Dailey L, Laplantine E, Priore R, Basilico C. A network of transcriptional and signaling events is activated by FGF to induce chondrocyte growth arrest and differentiation. J Cell Biol 2003;161: 1053–66. [Medline] [CrossRef]

37. Li C, Chen L, Iwata T, Kitagawa M, Fu XY, Deng CX. 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 1999;8: 35–44. [Medline] [CrossRef]

38. Ohbayashi N, Shibayama M, Kurotaki Y, Imanishi M, Fujimori T, Itoh N, et al. FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. Genes Dev 2002;16: 870–9. [Medline] [CrossRef]

39. Liu Z, Xu J, Colvin JS, Ornitz DM. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor receptor 3 (FGFR3) causes dwarfism in mice by activation of STATs and ink4 cell cycle inhibitors. Hum Mol Genet 1999;8: 35–44. [Medline] [CrossRef]

40. Kronenberg HM. PTHrP and skeletal development. Ann NY Acad Sci 2006;1068: 1–13. [Medline] [CrossRef]

41. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes Dev 1999;13: 2072–86. [Medline] [CrossRef]

42. Kobayashi T, Soegiarto DW, Yang Y, Lanske B, Schipani E, McMahon AP, et al. Indian hedgehog stimulates periarticular chondrocyte differentiation to regulate growth plate length independently of PTHrP. J Clin Invest 2005;115: 1734–42. [Medline] [CrossRef]

43. Mak KK, Kronenberg HM, Chuang PT, Mackem S, Yang Y. Indian hedgehog signals independently of PTHrP to promote chondrocyte hypertrophy. Development 2008;135: 1947–56. [Medline] [CrossRef]

44. Chusho H, Tamura N, Ogawa Y, Yasoda A, Suda M, Miyazawa T, et al. Dwarfism and early death in mice lacking C-type natriuretic peptide. Proc Natl Acad Sci USA 2001;98: 4016–21. [Medline] [CrossRef]

45. Yasoda A, Ogawa Y, Suda M, Tamura N, Mori K, Sakuma Y, et al. Natriuretic peptide regulation of endochondral ossification. Evidence for possible roles of the C-type natriuretic peptide/guanylyl cyclase-B pathway. J Biol Chem 1998;273: 11695–700. [Medline] [CrossRef]

46. Yasoda A, Komatsu Y, Chusho H, Miyazawa T, Ozasa A, Miura M, et al. Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. Nat Med 2004;10: 80–6. [Medline] [CrossRef]

47. Bartels CF, Bukulmez H, Padayatti P, Rhee DK, van Ravenswaaij-Arts C, Pauli RM, et al. Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. Am J Hum Genet 2004;75: 27–34. [Medline] [CrossRef]

48. Hachiya R, Ohashi Y, Kamei Y, Suganami T, Mochizuki H, Mitsui N, et al. Intact kinase homology domain of natriuretic peptide receptor-B is essential for skeletal development. J Clin Endocrinol Metab 2007;92: 4009–14. [Medline] [CrossRef]

49. Miura K, Namba N, Fujiwara M, Ohata Y, Ishida H, Kitaoka T, et al. An overgrowth disorder associated with excessive production of cGMP due to a gain-of-function mutation of the natriuretic peptide receptor 2 gene. PLoS One 2012;7: e42180. [Medline] [CrossRef]

50. Ulici V, Hoenselaar KD, Gillespie JR, Beier F. The PI3K pathway regulates endochondral bone growth through control of hypertrophic chondrocyte differentiation. BMC Dev Biol 2008;8: 40. [Medline] [CrossRef]

51. Pogue R, Lyons K. BMP signaling in the cartilage growth plate. Curr Top Dev Biol 2006;76: 1–48. [Medline] [CrossRef]

52. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS, Mirams M. Endochondral ossification: how
cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol 2008;40: 46–62. [Medline] [CrossRef]

53. Olney RC, Mougey EB. Expression of the components of the insulin-like growth factor axis across the growth-plate. Mol Cell Endocrinol 1999;156: 63–71. [Medline] [CrossRef]

54. Tavella S, Raffo P, Tacchetti C, Cancedda R, Castagnola P. N-CAM and N-cadherin expression during in vitro chondrogenesis. Exp Cell Res 1994;215: 354–62. [Medline] [CrossRef]

55. Oberlender SA, Tuan RS. Expression and functional involvement of N-cadherin in embryonic limb chondrogenesis. Development 1994;120: 177–87. [Medline]

56. ffrench-Constant C, Colognato H. Integrins: versatile integrators of extracellular signals. Trends Cell Biol 2004;14: 678–86. [Medline] [CrossRef]

57. Loeser RF. Chondrocyte integrin expression and function. Biorheology 2000;37: 109–16. [Medline]

58. Aszodi A, Hunziker EB, Brakebusch C, Fassler R. Beta1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis. Genes Dev 2003;17: 2465–79. [Medline] [CrossRef]

59. Terpstra L, Prud'homme J, Arabian A, Takeda S, Karsenty G, Dedhar S, et al. Reduced chondrocyte proliferation and chondrodysplasia in mice lacking the integrin-linked kinase in chondrocytes. J Cell Biol 2003;162: 139–48. [Medline] [CrossRef]

60. Mertz EL, Facchinini M, Pham AT, Gualeni B, De Leonardis F, Rossi A, et al. Matrix disruptions, growth, and degradation of cartilage with impaired sulfation. J Biol Chem 2012;287: 22030–42. [Medline] [CrossRef]

61. Sato T, Kudo T, Ikehara Y, Ogawa H, Hirano T, Kiyohara K, et al. Chondroitin sulfate N-acetylgalactosaminyltransferase 1 is necessary for normal endochondral ossification and aggrecan metabolism. J Biol Chem 2011;286: 5803–12. [Medline] [CrossRef]

62. Settembre C, Arteaga-Solis E, McKee MD, de Pablo R, Al Awqati Q, Ballabio A, et al. Proteoglycan desulfation determines the efficiency of chondrocyte autophagy and the extent of FGF signaling during endochondral ossification. Genes Dev 2008;22: 2645–50. [Medline] [CrossRef]