Pubertal growth and epiphyseal fusion

Kye Shik Shim, MD, PhD

Department of Pediatrics, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of Medicine, Seoul, Korea

The complex networks of nutritional, cellular, paracrine, and endocrine factors are closely related with pubertal growth and epiphyseal fusion. Important influencing factors include chondrocyte differentiation capacity, multiple molecular pathways active in the growth plate, and growth hormone-insulin-like growth factor-I axis activation and epiphyseal fusion through estrogen and its receptors. However, the exact mechanisms of these phenomena are still unclear. A better understanding of the detailed processes involved in the pubertal growth spurt and growth plate closure in longitudinal bone growth will help us develop methods to efficiently promote pubertal growth and delay epiphyseal fusion with fewer adverse effects.

Keywords: Growth, Puberty, Growth plate

Introduction

Growth in height is driven by elongation of long bones due to chondrogenesis at the epiphyseal plates, also known as the growth plate. This process results from chondrocyte proliferation, hypertrophy, and extracellular matrix secretion. It is organized by complex networks of nutritional, cellular, paracrine, and endocrine factors[1].

Puberty can be defined as the transitional period from childhood through the development of secondary sexual characteristics to the achievement of final height in adulthood[2].

Growth velocity is increased in early or midpuberty and is known as the pubertal growth spurt. The growth velocity decreases and may even be zero after epiphyseal fusion, that is, after growth plate closure in late puberty. Therefore, the pubertal growth spurt and growth plate closure have opposite effects on bone elongation. However, the exact mechanisms behind these contrary phenomena during puberty are still unknown[2].

Precocious puberty (PP) is a known cause of short stature after earlier puberty due to premature closure of growth plates in long bones. PP is divided into two categories, central and peripheral types, according to pathological lesions. The etiology of most cases of central PP is idiopathic. In industrialized countries, the number of patients with idiopathic central PP is increasing[3].

Gonadotropin-releasing hormone agonist is used as a major therapeutic modality in idiopathic central PP. Several aromatase inhibitors have also been studied as new treatment options. These drugs decrease the secretion of estrogen and delay growth plate closure[3].

The elucidation of the detailed mechanisms of pubertal growth and epiphyseal fusion may help in developing new strategies for the treatment of short stature or PP.

In this article, the processes of bone formation, histology and physiology of the growth plate, and cellular, paracrine, and endocrine factors for bone growth will be reviewed.

Bone formation

The mechanisms of bone formation are divided into two processes, intramembranous and endochondral ossification. The former is involved in the growth of the craniofacial skeleton and the latter is critical for the growth of the axial and appendicular skeleton. Therefore,

endochondral ossification is more important for the increase in human height. It requires several processes, including condensation of mesenchymal cells and differentiation into chondrocytes, osteoblasts, and osteocytes[4].

Histology and physiology of the growth plate

The growth plate is located between the epiphysis and metaphysis and composed of three zones (resting, proliferative, and hypertrophic zone). Each zone contains various chondrocytes in different stages of differentiation. The resting zone contains small chondrocytes that act as stem-like cells with a slow replication rate. The proliferative zone is composed of flat chondrocytes that line up along the long axis of the bone and have high replication rates. The hypertrophic zone is the layer of chondrocytes undergoing terminal differentiation and has an increased thickness, surrounding calcified matrix, and attracted factors for bone and vessel formation[5].

During the pubertal growth spurt, proliferation and differentiation of chondrocytes, secretion of extracellular matrix, calcification of the hypertrophic zone, invasion and differentiation of osteoblast, and formation of blood vessel repeat continuously in the growth plate[5,6].

At the end of the pubertal stage, these processes stop and longitudinal growth completes due to still unknown mechanisms[5,6].

Cellular factors

In early puberty, chondrocytes have the potential to proliferate and differentiate continuously. However, in late puberty, this potential decreases with age. When proliferation completely stops, longitudinal growth also stops and the final adult height is reached. This process is known as senescence of the growth plate. The cellular mechanism of epiphyseal senescence is still unknown. There are four theories on the cellular mechanism of epiphyseal fusion after the pubertal growth spurt. Apoptosis, autophagy, hypoxia, and transdifferentiation have been considered the causes of epiphyseal fusion[7].

1. Apoptosis

This theory is the most widely held hypothesis. Hypertrophic chondrocytes of the growth plate undergo death by apoptosis, leaving behind a frame of cartilage matrix for osteoblasts that invade and lay down bone. Some supporting and opposing results for this hypothesis have been reported in several studies[8]. Apoptosis-regulating proteins (such as caspases) were expressed in the growth plate and typical histologic changes in cell during apoptosis were shown in several studies in rat[9]. However, another study found no signs of classical apoptosis in fusing human growth plates[10].

2. Autophagy

Autophagy is another method of programmed cell death that involves a catabolic process in which the cell degrades its own components through autophagosomes. Signs of autophagy (autophagosomes, double-membrane structures, and condensed chromatin) were observed in avian hypertrophic chondrocytes and chondrocytes of newborn mice[11,12]. However, there were no autophagosomes or signs of autophagy in the growth plate in a study in humans[7].

3. Hypoxia

Emons et al.[10] reported a dense border of thick bone surrounding growth plate remnants in a human growth plate tissue specimen undergoing epiphyseal fusion. They postulated that the dense border might act as a physical barrier preventing oxygen and nutrients from reaching the fusing growth plate, resulting in hypoxia and eventually cell death in a nonclassical apoptotic manner.

Stewart et al.[13] also observed increased expression of hypoxia-inducible factor 2α mRNA during chick and murine chondrocyte differentiation in vitro.

4. Transdifferentiation

This is the oldest hypothesis, in which terminal hypertrophic chondrocytes transdifferentiate into osteoblasts at the chondro-osseous junction of the growth plate[14]. This theory is mostly based on organ and cell culture models and there is a lack of direct evidence in human studies[7].

Paracrine factors

1. Proliferation and differentiation of chondrocytes

The conversion of progenitor cells in the mesenchymal condensation into chondrocyte lineage is controlled by the expression of the transcription factors Sox9, 5, and 6[8]. Parathyroid hormone-related peptide (PTHrP) signaling, Indian hedgehog (IHH) pathway, and runt-related transcription factor 2 (Runx2) are required for further differentiation and hypertrophy of chondrocytes. In addition to those factors, fibroblast growth factor (FGF) pathway and bone morphogenic proteins (BMP) are necessary for the development of the perichondrium, periosteum, chondrocytes, and osteoblasts[7,15].

Of these paracrine factors, the best studied one is PTHrP that is known as secreted by periarticular chondrocytes of long bones[16-18]. Hirai et al.[19] reported that PTHrP diffuses across the growth plate cartilage maintaining chondrocytes in the proliferative state.

IHH is secreted by prehypertrophic and hypertrophic chondrocytes and positively regulates PTHrP production. And it also

has independent effects on chondrocyte differentiation[18]. BMP signaling across the growth plate is considered to contribute to the progressive differentiation of resting to proliferative to hypertrophic chondrocytes[20-22]. FGF and its receptor (FGFR) system are also important for growth plate development. Results from various in vivo studies indicate that FGFR1 and FGFR3 are growth-inhibiting, while FGFR-2 is growth-promoting[23,24].

2. Blood vessel formation

Vascular invasion is a prerequisite for the replacement of avascular cartilage by vascular bone and marrow. The most important stimulating factor for vessel invasion is hypoxia. Vessel formation is mediated by transcription factors, including hypoxia-inducible factor-1 and vascular endothelial growth factor (VEGF)[25,26].

Runx2, FGFs, BMPs, transforming growth factor (TGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) are also required for VEGF expression and vessel formation[27].

3. Osteoblast differentiation and ossification

There are five steps in differentiation of the osteoblastic lineage. These steps are the preosteoblast, mature osteoblast, osteoid osteocyte, early osteocyte, and mature osteocyte[28]. Wingless-type mouse mammary tumor virus integration site (Wnts)/β-catenin signaling, Runx2, Osterix, Ihh, BMPs, and IGFs are required for these processes. During these steps, bone can deposit minerals from the extracellular matrix rich in type I collagen, completing ossification[4].

Endocrine factors

1. Growth hormone–insulin–like growth factor–I axis

Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are the main stimulators of longitudinal bone growth. They are also important for the acquisition of bone mass during the prepubertal period and maintenance of bone homeostasis throughout life. GH stimulates the synthesis and secretion of IGF-I in the liver and growth plate. Longitudinal bone growth is mediated by GH, circulating IGF-I, and more importantly, local IGF-I in the growth plate. For differentiation, proliferation, and hypertrophy of chondrocytes; the production of extracellular matrix; and ossification in the growth plate, IGF-I produced from chondrocytes of the epiphyseal plate is important[29]. There is a close interplay between estrogen and GH in the regulation of growth and development in puberty. During puberty, there can be a 1.5- to 3-fold increase in the pulsatile secretion of GH and a more than 3-fold increase in the concentration of serum IGF-I. However, the interactions between estrogen and GH at the growth plate remain unclear because there is a lack of evidence of the independent roles of these two hormones at the

cellular level in the growth plate[30,31].

2. Estrogen

During puberty, estrogen induces the stimulation of the GH-IGF-I axis and a pubertal growth spurt. In the classical pathway, estrogen acts after binding with its receptor (estrogen receptor, ER). There are two subtypes of ER, ERα, and ERβ. Both subtypes seem to be involved in the augmentation of GH secretion and are expressed in the resting, proliferative, and hypertrophic zones of the growth plate. Indirect evidence suggests that epiphyseal fusion occurs when the proliferative capacity of growth plate chondrocytes is exhausted and estrogen acts by advancing growth plate senescence. Therefore, the binding of estrogen with each subtype of ER is thought to be related to the pubertal growth spurt and epiphyseal fusion[32-35].

In mice studies, ERα looks like the dominant mediator of estrogen actions in bone. ERβ has some repressive functions in bone of females. Evidence for a role of ERβ in males is lacking. A definite role for ERβ in humans remains unclear[36].

Borjesson et al.[37] studied the mechanisms of the pubertal growth spurt and epiphyseal fusion with cartilage-specific ERα knockout mice and reported that the effects of estrogen and ERα on growth plate thickness were unremarkable in early puberty, but obvious in late puberty. They suspected that activation of the GH-IGF-I axis with low doses of estrogen is important for the pubertal growth spurt in early puberty, while high doses of estrogen binding to its receptors in growth plate cartilage are essential for epiphyseal fusion in late puberty.

The effects of estrogen on the growth plate have been studied in many rodent models. However, there are some differences in growth plate physiology between rodents and humans. In rats or mice, the pubertal growth spurt is unremarkable and longitudinal bone growth continues even after sexual maturation, as epiphyseal fusion does not occur at the time of sexual maturation. Therefore, there are difficulties in the direct application of growth plate studies in rodents to humans[5].

Further molecular studies are needed to elucidate the consequences of estrogen and the ER in growth plates. Estrogen is known to promote bone formation by stimulating osteoblastogenesis and inhibiting apoptosis of mature osteoblasts. It is also known to decrease the osteoclast production by inhibiting the reaction with receptor activator of nuclear factor-κB ligand or production of interleukin-1, -6, -7, and tumor necrosis factor-α. Estrogen may also increase osteoclast apoptosis by stimulating the Fas/FasL pathway[4].

3. Androgen

Androgen itself also contributes to bone formation and the pubertal growth spurt, perhaps through a direct interaction with growth plate chondrocytes[38]. Dihydrotestosterone can stimulate proliferation and proteoglycan synthesis in growth plate chondrocytes *in vitro* and testosterone stimulates chondrocyte

proliferation in an organ culture model study with increased local IGF-I production[5].

In epiphyseal fusion, the activity of androgen is due to the aromatization of androgens to estrogens in various peripheral tissues, including growth plate cartilage[36].

Conclusions

Many factors are related with the stimulation of bone formation and growth, the pubertal growth spurt, epiphyseal senescence, and fusion, including nutritional, cellular, paracrine, and endocrine factors.

An important cellular factor in these processes is the differentiation and aging of chondrocytes in the growth plate. Important paracrine factors include the many molecular pathways involved in chondrocyte differentiation, vascularization, and ossification. Estrogen and the GH-IGF-I axis are important endocrine factors.

But, hitherto, the exact processes of interactions between paracrine and endocrine systems in growth plate, and the cellular mechanisms of the decreasing capacity of proliferation of chondrocytes and growth of organ with age are unknown.

Elucidation of the detailed mechanisms of the pubertal growth spurt and growth plate fusion will allow a better understanding of the molecular mechanisms responsible for short stature, PP, and skeletal disorders. It will also contribute to the development of new therapeutic modalities with fewer side effects and better efficacy.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

References

1. Lui JC, Nilsson O, Baron J. Recent research on the growth plate: Recent insights into the regulation of the growth plate. J Mol Endocrinol 2014;53:T1-9.
2. Murray PG, Clayton PE. Endocrine control of growth. Am J Med Genet C Semin Med Genet 2013;163C:76-85.
3. Styne DM, Grumbach MM. Puberty: ontogeny, neuroendocrinology, physiology, and disorders. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, editors. Williams textbook of endocrinology. 12th ed. Philadelphia: Saunders, 2011:1054-202.
4. Maes C, Kronenberg HM. Postnatal bone growth: growth plate biology, bone formation, and remodeling. In: Glorieux FH, Pettifor JM, Juppner H, editors. Pediatric bone. 2nd ed. San Diego: Elsevier Co., 2012:55-82.
5. Nilsson O, Marino R, De Luca F, Phillip M, Baron J. Endocrine regulation of the growth plate. Horm Res 2005;64:157-65.
6. van der Eerden BC, Karperien M, Wit JM. Systemic and local regulation of the growth plate. Endocr Rev 2003;24:782-801.
7. Emons J, Chagin AS, Sävendahl L, Karperien M, Wit JM. Mechanisms of growth plate maturation and epiphyseal fusion. Horm Res Paediatr 2011;75:383-91.
8. 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.
9. Chrysis D, Nilsson O, Ritzen EM, Savendahl L. Apoptosis is developmentally regulated in rat growth plate. Endocrine 2002;18:271-8.
10. Emons J, Chagin AS, Hultenby K, Zhivotovsky B, Wit JM, Karperien M, et al. Epiphyseal fusion in the human growth plate does not involve classical apoptosis. Pediatr Res 2009;66:654-9.
11. 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.
12. Shapiro IM, Adams CS, Freeman T, Srinivas V. Fate of the hypertrophic chondrocyte: microenvironmental perspectives on apoptosis and survival in the epiphyseal growth plate. Birth Defects Res C Embryo Today 2005;75:330-9.
13. Stewart AJ, Houston B, Farquharson C. Elevated expression of hypoxia inducible factor-2alpha in terminally differentiating growth plate chondrocytes. J Cell Physiol 2006;206:435-40.
14. Moskalewski S, Malejczyk J. Bone formation following intrarenal transplantation of isolated murine chondrocytes: chondrocyte-bone cell transdifferentiation? Development 1989;107:473-80.
15. Karimian E, Chagin AS, Savendahl L. Genetic regulation of the growth plate. Front Endocrinol (Lausanne) 2012;2:1-10.
16. Karp SJ, Schipani E, St-Jacques B, Hunzelman J, Kronenberg H, McMahon AP. Indian hedgehog coordinates endochondral bone growth and morphogenesis via parathyroid hormone related-protein-dependent and -independent pathways. Development 2000;127:543-8.
17. van der Eerden BC, Karperien M, Gevers EF, Lowik CW, Wit JM. Expression of Indian hedgehog, parathyroid hormone-related protein, and their receptors in the postnatal growth plate of the rat: evidence for a locally acting growth restraining feedback loop after birth. J Bone Miner Res 2000;15:1045-55.
18. Kindblom JM, Nilsson O, Hurme T, Ohlsson C, Savendahl L. Expression and localization of Indian hedgehog (Ihh) and parathyroid hormone related protein (PTHrP) in the human growth plate during pubertal development. J Endocrinol 2002;174:R1-6.
19. Hirai T, Chagin AS, Kobayashi T, Mackem S, Kronenberg HM. Parathyroid hormone/parathyroid hormone-related

protein receptor signaling is required for maintenance of the growth plate in postnatal life. Proc Natl Acad Sci U S A 2011;108:191-6.

20. Nilsson O, Parker EA, Hegde A, Chau M, Barnes KM, Baron J. Gradients in bone morphogenetic protein-related gene expression across the growth plate. J Endocrinol 2007;193:75-84.

21. Pizette S, Niswander L. BMPs are required at two steps of limb chondrogenesis: formation of prechondrogenic condensations and their differentiation into chondrocytes. Dev Biol 2000;219:237-49.

22. Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP, et al. BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. Development 2001;128:4523-34.

23. Minina E, Kreschel C, Naski MC, Ornitz DM, Vortkamp A. Interaction of FGF, Ihh/Pthlh, and BMP signaling integrates chondrocyte proliferation and hypertrophic differentiation. Dev Cell 2002;3:439-49.

24. Lazarus JE, Hegde A, Andrade AC, Nilsson O, Baron J. Fibroblast growth factor expression in the postnatal growth plate. Bone 2007;40:577-86.

25. Cramer T, Schipani E, Johnson RS, Swoboda B, Pfander D. Expression of VEGF isoforms by epiphyseal chondrocytes during low-oxygen tension is HIF-1 alpha dependent. Osteoarthritis Cartilage 2004;12:433-9.

26. Lin C, McGough R, Aswad B, Block JA, Terek R. Hypoxia induces HIF-1alpha and VEGF expression in chondrosarcoma cells and chondrocytes. J Orthop Res 2004;22:1175-81.

27. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004;25:581-611.

28. Noble BS. The osteocyte lineage. Arch Biochem Biophys 2008;473:106-11.

29. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 2008;29:535-59.

30. Albin AK, Niklasson A, Westgren U, Norjavaara E. Estradiol and pubertal growth in girls. Horm Res Paediatr 2012;78:218-25.

31. Leung KC, Johannsson G, Leong GM, Ho KK. Estrogen regulation of growth hormone action. Endocr Rev 2004;25:693-721.

32. Chagin AS, Sävendahl L. Oestrogen receptors and linear bone growth. Acta Paediatr 2007;96:1275-9.

33. Borjesson AE, Lagerquist MK, Windahl SH, Ohlsson C. The role of estrogen receptor α in the regulation of bone and growth plate cartilage. Cell Mol Life Sci 2013;70:4023-37.

34. Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of estrogen on growth plate senescence and epiphyseal fusion. Proc Natl Acad Sci U S A 2001;98:6871-6.

35. Borjesson AE, Windahl SH, Karimian E, Eriksson EE, Lagerquist MK, Engdahl C, et al. The role of estrogen receptor-α and its activation function-1 for growth plate closure in female mice. Am J Physiol Endocrinol Metab 2012;302:E1381-9.

36. Vanderschueren D, Laurent MR, Claessens F, Gielen E, Lagerquist MK, Vandenput L, et al. Sex steroid actions in male bone. Endocr Rev 2014;35:906-60.

37. Borjesson AE, Lagerquist MK, Liu C, Shao R, Windahl SH, Karlsson C, et al. The role of estrogen receptor α in growth plate cartilage for longitudinal bone growth. J Bone Miner Res 2010;25:2690-700.

38. Nilsson O, Chrysis D, Pajulo O, Boman A, Holst M, Rubinstein J, et al. Localization of estrogen receptors-alpha and -beta and androgen receptor in the human growth plate at different pubertal stages. J Endocrinol 2003;177:319-26.