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. 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. 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.

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.

**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.

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.

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

**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 stage. Apoptosis, autophagy, hypoxia, and transdifferentiation have been considered the causes of epiphyseal fusion.

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. 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. However, another study found no signs of classical apoptosis in fusing human growth plates.

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. However, there were no autophagosomes or signs of autophagy in the growth plate in a study in humans.

3. **Hypoxia**

Emons et al. 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. 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. This theory is mostly based on organ and cell culture models and there is a lack of direct evidence in human studies.

**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 transcription factors Sox9, 5, and 6. 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.

Of these paracrine factors, the best studied one is PTHrP that is known as secreted by periarticular chondrocytes of long bones. Hirai et al. 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\textsuperscript{18}. BMP signaling across the growth plate is considered to contribute to the progressive differentiation of resting to proliferative to hypertrophic chondrocytes\textsuperscript{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\textsuperscript{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)\textsuperscript{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\textsuperscript{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\textsuperscript{28}. Wingless-type mouse mammary tumor virus integration site (Wnts)/\(\beta\)-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\textsuperscript{29}.

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\textsuperscript{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\textsuperscript{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, ER\(\alpha\), and ER\(\beta\). 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\textsuperscript{2,33}.

In mice studies, ER\(\alpha\) looks like the dominant mediator of estrogen actions in bone. ER\(\beta\) has some repressive functions in bone of females. Evidence for a role of ER\(\beta\) in males is lacking. A definite role for ER\(\beta\) in humans remains unclear\textsuperscript{26}.

Borjesson et al.\textsuperscript{37} studied the mechanisms of the pubertal growth spurt and epiphyseal fusion with cartilage-specific ER\(\alpha\) knockout mice and reported that the effects of estrogen and ER\(\alpha\) 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\textsuperscript{38}.

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-\(\kappa\)B ligand or production of interleukin-1, -6, -7, and tumor necrosis factor-\(\alpha\). Estrogen may also increase osteoclast apoptosis by stimulating the Fas/Fasl. pathway\textsuperscript{39}.

3. Androgen

Androgen itself also contributes to bone formation and the pubertal growth spurt, perhaps through a direct interaction with growth plate chondrocytes\textsuperscript{40}. 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\(^3\).

In epiphyseal fusion, the activity of androgen is due to the aromatization of androgens to estrogens in various peripheral tissues, including growth plate cartilage\(^2\).

**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, PI, 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.

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