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EMBRYONIC DEVELOPMENT AND GROWTH PLATE STRUCTURE

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This article presents modern data on embryonic development and zonal structure of the meta-epiphyseal growth plate in which dysfunction has an important role in the formation of skeletal deformities in children.

Keywords: epiphyseal plate, growth plate, chondrocytes, cartilage.

The question of how bones grow in length is one of the most interesting aspects in the field of biology. Experimental research on the subject began more than two centuries ago, when Hales (1727) noted that the holes drilled in the shaft of the growing bone always remain at the same distance from each other. This led to the conclusion that the growth of long bones occurs at the ends of bones. Subsequent studies performed with fixed markers facilitated the identification of metaepiphyseal cartilage as the exact place of lengthwise growth of tubular bones (du Hamel 1745, Hunter 1772) [1].

The epiphyseal growth plate, also called metaepiphyseal cartilage or physis [2, 3], is a thin layer of hyaline cartilage located between the epiphysis and metaphysis of long bones [4]. The bone grows in length around the growth plate because of the processes of endochondral ossification, which is the gradual replacement of cartilage by bone tissue.

The development of endochondral ossification in humans begins immediately after the formation of the middle germ layer (mesoderm) [5]. The cells of this embryonic layer are undifferentiated, proliferating cells with a round or oval nucleus, with abundant, loosely arranged chromatin. Later, the cells of one of the mesodermal subpopulations manifest a tendency for condensation; they form dense clusters or aggregates of cells. Then, the intercellular matrix, surrounding cell aggregates, acquires tinctorial properties inherent in cartilage. These cells begin to produce cartilage-specific type II collagen and sulfated proteoglycans, chondrogenic differentiation begins, and cartilage blastema forms [2].

Further differentiation of cartilage blastema and development of future long bones is associated with the processes of cell proliferation, active secretion of intercellular matrix, development of perichondrium, and separation into segments.

The blastema contains cells that are in a stage of autosynthetic interphase, as they prepare for mitosis and cell proliferation. The combination of cell proliferation and secretion increases the mass of the cartilage. As a result, the cells of a blastema, which were located close to each other at the early stages of differentiation, are now separated after the matrix surrounded the cells and moved them away from each other. Thus, the cartilage tissue becomes structured and acquires features characteristic of hyaline cartilage. The cells of the perichondrial mesenchyme, which surround the centers of chondrogenesis, form perichondrium [2]. This describes the cartilage model of bone formation (Figure 1).

Afterwards, the chondrocytes in the center of the cartilage no longer actively proliferate, and they become hypertrophied.

The effect of vascular endothelial growth factor (VEGF), which is produced by hypertrophic cartilage cells, promotes the invasion of blood vessels, osteoblasts, and other mesenchymal cells

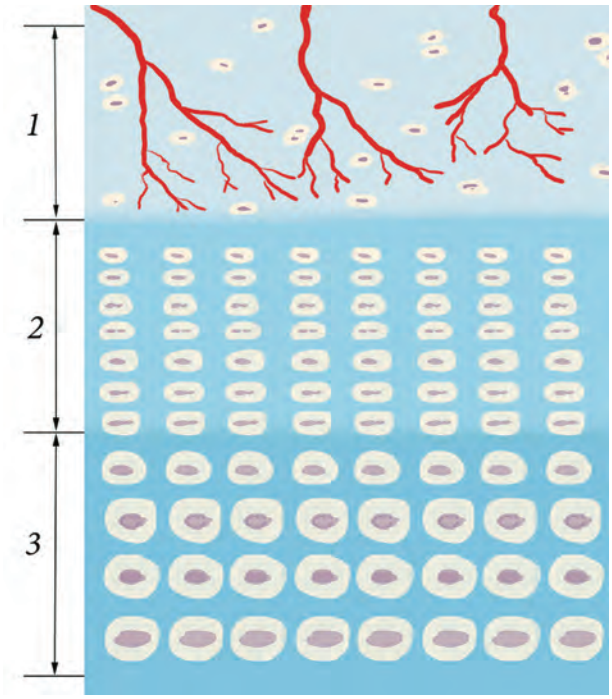

Figure 1

into the cartilage, leading to the appearance of the primary core of ossification and bone formation [6]. As the bone grows, the primary core of the bone ossification moves toward the epiphyseal plates [5].

Endochondral ossification originates from the following two centers of ossification: the diaphyseal (primary) and epiphyseal (secondary) foci. As a result of the spread of endochondral ossification foci toward each other, a boundary forms that delineates the new structure of the bone, called the epiphyseal growth plate.

By the time of skeletal maturity, epiphysis and metaphysis synostosis results in the growth plate being replaced by the growth line in most mammals. In some mammals, such as rat, the epiphyseal plate is retained in the mature skeleton but in the inactive state [7].

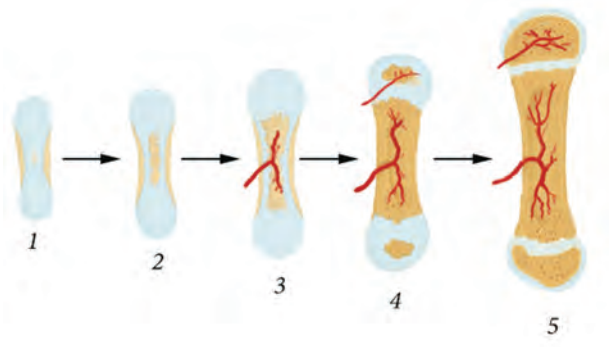

Figure 2

It should also be noted that the epiphyseal growth plate is "open" for resorption and replacement by bone tissue from the diaphyseal side and at the same time is relatively "closed" for the process of ossification from the epiphyseal side. From the epiphyseal side the death of chondrocytes, their calcification, and vascular invasion are less pronounced. In addition, there are boundary plates, blocking the path of the vessels, which lay parallel to the boundary between bone and cartilage [2]. These features provide long-term preservation of the metaphyseal cartilage as growth plate in the long bones.

Metaphyseal cartilage has zoning and polarity properties [2]. The growth plate is divided into zones on the basis of the rate of chondrocyte proliferation, by their degree of differentiation, and the cellular composition [8]. For example, the following earlier three zones were recognized in the epiphyseal cartilage: (in the direction from the epiphysis toward the diaphysis) the surface area or resting cartilage zone, the columnar cartilage zone, and the spongy cartilage zone [2]. Later it was also proposed to distinguish the border, intermediate, and internal zones.

In 1971, E. Gardner proposed to classify the four areas of epiphyseal cartilage as the zone of resting cartilage, the zone of proliferation, the zone of maturation, and the zone of cartilage calcification, where chondocytes die.

In 1980, V.G. Koveshnikov described the five zones of metaphyseal cartilage, which included the region of active osteogenesis as the fifth zone.

At the moment, scientists disagree on the classification of metaepiphyseal cartilage zones.

T. Ballock, I. Villemure et al., described the growth plate on the basis of the morphological and biochemical characteristics during the differentiation of chondrocytes: a rest zone, proliferation zone, and hypertrophy zone [9, 10].

C. Anenisia and O. Nilsson proposed, in addition to the above mentioned areas, a pre-hypertrophy zone, which is located between the proliferation and hypertrophy zones. In this zone, as in the zone of proliferation, chondrocytes have the same distinct arrangement, but the cells increase in size and the concentration of collagen type X is

higher. In the hypertrophy zone, vascular invasion and replacement of newly formed cartilage by bone tissue takes place [8].

As the names suggest, cell composition is strongly considered when classifying the major zones. According to V.M. Pavlova et al., this is correct from the point of zone function [2]. In the following description, the classification of the main zones of metaepiphyseal cartilage takes into account the contradictions between researchers (Figure 2).

As for polarities, the growth plate is considered to be monopolar because the growth of the structure is only due to chondrocytes of the proliferative zone, whereas other cartilages, e.g., apophyses, are bipolar. The growth of bipolar cartilage is because of reserve chondrocytes of the middle zone and chondrocytes of proliferative and hypertrophy zones on both sides. Thus, cartilage growth occurs in two directions [10].

The percentage ratio of areas in the growth plate relative to each other is different for each mammalian species. A growth plate of a newborn piglet is characterized by the following percentages: the resting zone, 70%; proliferative chondrocytes zone, 17%; and hypertrophic chondrocytes zone, 13%. Conversely, in rats aged 21 days, the resting zone accounts for 6%, the proliferative chondrocytes zone 35%, and the hypertrophic chondrocytes zone 59% [10].

The resting zone has direct contact with the epiphysis, and in turn, is a place where the epiphyseal plate is attached to the trabeculae of the epiphyseal bone [2]. Characteristic for this zone is a high ratio of the volume of the extracellular matrix to the volume of cells, the chondrocytes are in a relatively stationary state, the cells have a slightly spherical shape, and arranged either individually or in pairs [8, 9].

The absence of proliferating cells has allowed this zone to be designated as a zone of "resting" cartilage. In 1973, it was proposed that the resting zone be called the "reserve zone," as it is related to the underlying areas. However, no signs of chondrocyte proliferation has been discovered as a result of the research. On the basis of these data, it was suggested that the cells of this zone do not serve as a reserve for underlying zones [2]. However, later in 2001, after *in vivo* studies on rabbits, it was discovered that the removal of proliferative and hypertrophic zones while retaining only the reserve zone of the growth plate leads to a complete restoration of the epiphyseal plate structure in one week. This suggests that chondrocytes of the reserve zone act as stem cell cache that continually supplies cells to the proliferative zone [11].

The proliferative zone has the ability to actively proliferate, hence the name [2]. Most cells in this zone have a flattened, wedge-like shape, are in autosynthetic interphase, and mitotic figures are seen in a number of cells These cells are located mostly in the plane of the longitudinal axis of the epiphyseal growth plate, which corresponds to the features of the collagen fibers arrangement of the extracellular matrix in this zone. This cellular arrangement organises dividing cells into a column. Metaepiphyseal cartilage growth is achieved both by increasing the number of the cells in the zone and by increasing the amount of extracellular matrix because of the formation of collagen types II and IX and proteoglycans [2].

Chondrocytes of the hypertrophic zone lose their ability to divide. There is a 10-fold increase in their intracellular volume because of an increase in the number of organelles, such as the endoplasmic reticulum, an increased amount of alkaline phosphatase, and the synthesis and secretion of collagen type X. Although the exact function of collagen type X in the growth plate is still unclear, mutations of its gene cause Schmid metaphyseal chondrodysplasia. In addition, chondrocytes of this zone are involved in the metabolic functions; they prepare extracellular matrix for calcification and form calcified cartilage matrix, which subsequently is subject to resorption and is replaced by cancellous bone [9, 12].

As for the cartilage extracellular matrix, its function in the epiphyseal growth plate is also important. It is actively involved in the calcification of cartilage, which serves as a template for bone formation by osteoblasts [9].

In the growth plate, the extracellular matrix is represented by certain types of collagen and proteoglycans. The main type of collagen in the growth plate is collagen type II, it consists of three identical α1 chains and is encoded by the COL2A1 gene. Mutations of this gene

causes chondrodysplasia of varying severity, including achondrogenesis type II and Stickler syndrome [13].

Other types of collagens, such as XI, IX, and X types, are also present in the growth plate and play an important role in its normal functioning.

Type XI collagen is a heterodimer and contains chains of the collagen α1 (XI), α2 (XI), and α3 (XI). Chains α1 (XI) and α2 (XI) are encoded by the COL11A1 gene, whereas α3 (XI) is encoded by the COL11A2 gene. Collagen of this type is present only in thin fibrils of collagen type II, and it adjusts the diameter of the fibrils. Mutations in COL11A1 and COL11A2 genes result in various skeletal dysplasias, such as Stickler syndrome or Marshall syndrome. It was established that mutations in these genes in homozygous mice leads to abnormalities in the structure of the growth plate, a severe degree of chondrodysplasia, and animals die shortly after birth [13].

Collagen IX also is a heterodimer, composed of the following three distinct collagen chains: α1 (IX), α2 (IX), and α3 (IX), which are encoded by COL9A1, COL9A2, and COL9A3 genes, respectively. Mutations in these genes result in various epiphyseal dysplasia with autosomal dominant type of inheritance.

COL10A1 codes for collagen type X, which consists of thee alpha1 (X) chains. Mutations of this gene causes Schmidt dysplasia.

The main proteoglycan in the epiphyseal plate is aggrecan, followed by perlecan, decorin, fibromodulin, and lumican. In addition, the growth plates contain the non-collagenous proteins matrilin 1, tenascin C, and cartilage oligomeric matrix protein.

Aggrecan is known as a heavy proteoglycan because it has a large molecular weight and contains more than 100 glycosaminoglycan chains. It gives strength to the epiphyseal growth plates. Matrilin 1 is regarded as an adaptor protein for the formation of extracellular matrix. Perlecan is the main heparan sulfate proteoglycan of the basal membrane; it provides a connection between cells and the extracellular matrix components.

In addition to the above mentioned chondrodysplasia, other musculoskeletal abnormalities in the metaepiphyseal cartilage may cause diseases such as valgus or varus deformity of the knee, idiopathic scoliosis, brachymetacarpia [14], and brachymetatarsia.

Recent data on the structure and functional significance of growth zones presented in this paper provide new insights into the mechanisms of the development of skeletal deformities in children.

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