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Does the epiphyseal cartilage of the long bones have one or two ossification fronts?

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Abstract
Epiphyseal cartilage is hyaline cartilage tissue with a gelatinous texture, and it is responsible for the longitudinal growth of the long bones in birds and mammals. It is located between the epiphysis and the diaphysis. Epiphyseal cartilage also is called a growth plate or physis. It is protected by three bone components: the epiphysis, the bone bar of the perichondrial ring and the metaphysis. The epiphysis, which lies over the epiphyseal cartilage in the form a cupola, contains a juxtaposed bone plate that is near the epiphyseal cartilage and is in direct contact with the epiphyseal side of the epiphyseal cartilage. The germinal zone corresponds to a group of cells called chondrocytes. These chondrocytes belong to a group of chondral cells, which are distributed in rows and columns; this architecture is commonly known as a growth plate. The growth plate is responsible for endochondral bone growth. The aim of this study was to elucidate the causal relationship between the juxtaposed bone plate and epiphyseal cartilage in mammals. Our hypothesis is that cells from the germinal zone of the epiphyseal side of the epiphyseal cartilage are involved in forming a second ossification front that is responsible for the origin of the juxtaposed bone plate. We report the following: (a) The juxtaposed bone plate has a morphology and function that differs from that of the epiphyseal trabeculae; (b) on the epiphyseal edge of the epiphyseal cartilage, a new ossification front starts on the chondrocytes of the germinal area, which forms the juxtaposed bone plate. This ossification front is formed by chondrocytes from the germinal zone through a process of mineralisation and ossification, and (c) the process of mineralisation and ossification has a certain morphological analogy to the process of ossification in the metaphyseal cartilage of amphibians and differs from the endochondral ossification process in the metaphyseal side of the growth plate. The close relationship between the juxtaposed bone plate and the epiphyseal cartilage, in which the chondrocytes that migrate from the germinal area play an important role in the mineralisation and ossification process of the juxtaposed bone plate, supports the hypothesis of a new ossification front in the epiphyseal layer of the epiphyseal plate. This hypothesis has several implications: (a) epiphyseal cartilage is a morphological entity with two different ossification fronts and two different functions, (b) epiphyseal cartilage may be a morphological structure with three parts: perichondrial ring, metaphyseal ossification front or growth plate, and epiphyseal ossification front, (c) all disease (traumatic or dysplastic) that affects some of these parts can have an impact on the morphology of the epiphyseal region of the bone, (d) there is a certain analogy between metaphyseal cartilage in amphibians and mammalian epiphyseal cartilage, although the former is not responsible for bone growth, (e) comparative histological and anatomy studies are also warranted, to shed light on the phylogenetic study of epiphyseal cartilage throughout the changes that occur in the animal species.

Introduction

Epiphyseal cartilage is composed of gelatinous hyaline cartilage tissue. It is located between the epiphysis and the diaphysis. Epiphyseal cartilage also is called a growth plate or physis. The function of a growth plate is longitudinal growth of the long bones. This function is performed through an endochondral ossification front in the hypertrophic area on the metaphyseal side of the epiphyseal cartilage. It is nourished by three vascular systems: epiphyseal, metaphyseal and perichondrial vessels, which do not reach the interior of the growth plate. However, the nutrients reach the chondrocytes through a canalicular system, distributed by a mesh inside the plate that reaches all chondrocytes, allowing oxygen and nutrients to reach the cells [1], [2] and [3].

The growth plate chondrocytes are distributed in columns (isogenic), which in turn interact with each other and with the extracellular matrix. These columns give rise, to three zones: germinal, proliferative

and hypertrophic zones. The function of the germinal zone is an object of discussion in terms of whether it generates growth plate cells [4] or whether the cells above the proliferative area are responsible for generating these new cells [5]. If the latter is the case, then the actual function of the germinal area remains undefined. In a structure with this type of well-defined architecture, which also occurs in other species, the presence of hypertrophic chondrocytes is not necessarily associated with endochondral ossification [6].

Three bone structures protect the epiphyseal cartilage from the vicissitudes of bone biomechanics [7] and regulate the forces that reach the growth plate [8]. These structures are the epiphyseal bone structure, the bone bar of the perichondrial ring and the metaphyseal bone. The perichondrial ring is a structure that is considered part of the epiphyseal cartilage. However, the origins and function of cells in the upper area of the perichondrial ring are still being investigated [9]. In our laboratory, we have studied the morphology and the effect of various types of experimental lesions on the perichondrial ring, and we have also examined perichondrial ring injuries in the pathology of human clinical dysplasias [10], [11], [12], [13] and [14]. From these studies we can conclude that there is an intimate relationship between the epiphyseal cartilage and perichondral ring, although this relationship is not well defined. With regard to the region of the epiphysis, alongside the trabecular bone, a bone layer located on the upper edge of the germinal area of the epiphyseal cartilage has been described [15] and [16]. The mechanism of formation of this layer of bone is unknown, but it has been suggested that it forms by partial ossification of the germinal area [15].

The close relationship between this bone layer and some cells from the germinal zone leads to the hypothesis that a new ossification front is generated in the epiphyseal cartilage, which is responsible for the formation of this bone layer in the epiphysis, a bone layer we call the juxtaposed bone plate. If this hypothesis regarding the physiology of the epiphyseal cartilage is correct, we should find a new ossification front in the epiphyseal edge of the epiphyseal cartilage that is produced in a different manner from the endochondral ossification front described in the metaphyseal side of the growth plate.

One might ask why the information obtained from further study will be important. We have observed that the small zone above the epiphyseal cartilage is a “dark zone”, meaning it is a poorly studied zone in the literature. In the literature, epiphyseal cartilage is also called growth plate or physis, which implies that it has a single function. Due to the predominance of trabecular bone in the epiphysis, we believe that this small zone of the epiphyseal cartilage has been overlooked. We find it compelling to define whether the epiphyseal cartilage has one or two functions, because the latter would imply that what is normally known as growth plate is actually a region of the epiphyseal cartilage plate located on its metaphyseal side. We propose is to study what happens on the epiphyseal side of the epiphyseal cartilage.

Hypothesis

Our hypothesis is: There is an ossification front on epiphyseal side of the epiphyseal cartilage. This ossification front explains the formation of the juxtaposed bone plate.

From the hypothesis two research questions are generated: Which is the function of the juxtaposed bone plate? Are there any differences between the epiphyseal trabecular bone and juxtaposed bone plate?

Verification of the hypothesis

Methodological plan for testing the critical elements of the hypothesis:
Given there is a juxtaposed bone plate over the epiphyseal cartilage, the following questions arise:

Does epiphyseal cartilage participate in juxtaposed bone plate formation?
Are there differences between the juxtaposed bone plate and the trabecular bone of the epiphysis?
What is the function of the juxtaposed bone plate?
Are the growth plate and epiphyseal cartilage are the same thing or different things?

To answer these questions we revisited four doctoral theses in our laboratory [17], [18], [19] and [20]. Their objective was to study the growth plate injury. However, we had not yet elucidated the difference between epiphyseal cartilage and the growth plate, because the ultimate goals of the four theses were different.

This paper focuses on studying epiphyseal cartilage under two conditions: normal epiphyseal cartilage and epiphyseal cartilage under stress conditions. The stress situation helps us observe physiological structural adaptations in response to the stress.

Experiment design

The experiments performed in this study were approved by the Institutional Animal Care Committee of the School of Medicine at the Universidad Autónoma de Madrid. European Union and domestic guidelines for the care of animals were followed during the experiment and for other scientific purposes. (Guideline 86/609/CEE and Spanish Royal Decree 1201/2005, Official State Journal (B.O.E.)).

93 female rats aged 21 days under general anesthesia were divided into three types of trials, with the aim answering each research question. Anaesthesia was induced and sustained with ketamine (10 mg/kg, i.m.), diazepam (4 mg/kg, i.m.) and atropine (0.05 mg/kg, i.m.). Surgery was performed with microsurgery material (microsurgery case, Allgaier Instrumente Schreiber 70–0950, Germany) and a stereoscopic loupe with epillumination and transillumination. The skin was prepared with a chlorhexidine solution.

Trial A. Methodology to analyze the questions: Are there a new ossification front on the epiphyseal side of the epiphyseal cartilage? Does epiphyseal cartilage participate in juxtaposed bone plate formation? For trial A, a growth plate allograft was performed [17]. 29 rats were divided into three groups: Group-A-1, in 14 rats the distal growth plate of the left radius was exposed and resected by epiphyseal osteotomy and a metaphyseal osteotomy. In its bed the allograft growth plates were placed. Group A-2, in 15 rats with the same operation the growth plate allograft free on all edges was placed in the bed. Group A-3, the 29 radii on the right side of the animals was used as controls.

The operated limb was placed in a plastic tubular splint with limb traction, which was attached to the skin in the proximal area with silk sutures and in the distal area using transmetacarpal silk sutures. The splint was withdrawn on the tenth day of monitoring. The results were assessed at 12 weeks post-surgery. In the normal epiphyseal cartilage group (Group A-3) and epiphyseal cartilage under stress groups (Groups A-1 and A-2) the following parameters were analysed: the phenotype and distribution of chondrocytes on the upper edge of the epiphyseal cartilage, the juxtaposed bone plate and its two zones, the onset of mineralisation fronts in the stress groups.

Trial B. Methodology to analyze the second question: Are there any differences between the juxtaposed bone plate and the trabecular bone of the epiphysis? For trial B, we studied the epiphyseal trabeculae, the juxtaposed bone plate and cortical bone cells in a rat model of experimental osteoporosis by ovariectomy [18]. A total of 32 ovariectomised female rats were divided into two groups: Group B-1, 20 rats were treated with mesenchymal stem cells and followed for 6 weeks ($n = 10$) and 6 months ($n = 10$). Group B-2, the control group, 12 ovx-rats not treated with cell therapy and were followed for 6 weeks ($n = 6$) and 6 months ($n = 6$). The following parameters were stereologically analyzed: the number of cells per mm^3, volume cell (μ^3) and cell volume fraction (%).

Trial C. Methodology for analyzing the third question: What is the function of the juxtaposed bone plate? For trial C, a growth plate fracture and an epiphysiolisis Salter II were performed [19] and [20]. 32 rats were divided into three groups. Group C-1, in 22 rats in the upper third of the left tibia an epiphysiolysis Salter II was manually performed. In Group C-2, in 10 rats a growth plate upper left tibia injury was inflicted horizontally at the central region of the growth plate using a scalpel (No. 11). In Group C-3, the contralateral right tibia of both groups was used as a control. Follow-up was at 12 weeks. The following parameters were evaluated: the course of the fracture line in the growth plate, the presence or absence of fractures in the germinal zone, the presence or absence of cell-free areas in the germinal zone, the presence or absence of a mineralisation front, the presence or absence of the bone juxtaposed plate.

Postoperative care and treatments: In all cases, a local antibiotic was administered after surgery (Terramycin® in powder; Pfizer, S.A., Madrid, Spain) to the surgical wound. All animals received Meloxicam, 5 mg/kg, i.m. (Metacam® Beoehtinger Ingelheim, España) and Ibuprofen, oral suspension in water *ad libitum* (Dalsy, Abbott, Laboratories S.A., Madrid, España) as an analgesic therapy. Tacrolimus (Prograft®, Astellas Pharma S.A. España) suspension was administered to group A-2, doses of 0.1 mg every other day during the test period. Euthanasia was performed with CO_2. After being excised, all specimens were fixed in 4% formalin, decalcified, embedded in paraffin, serial frontal sections (7 μ) were performed, and stained with hematoxylin-eosin.

Evaluation of the hypothesis

Control groups outcomes show an apparent “migration” of the germinal zone chondrocytes to the edge of the epiphyseal cartilage. There are also a close relationship between these chondrocytes and juxtaposed bone plates. Even some chondrocyte-like cell morphology was observed, including in the juxtaposed bone plate (Fig. 1).

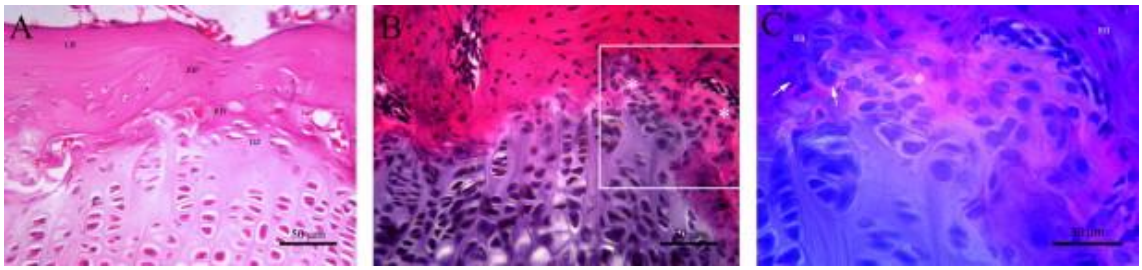

Fig. 1. Study of normal epiphyseal cartilage. (A) On the germinal area (GA), we observe the juxtaposed bone plate (JBP) and its two areas: the laminar bone area (LB), consisting of multiple bone lamellae and the rough bone area (RB) in close contact with the germinal area. The juxtaposed bone plate presents a number of continuity solutions through which the epiphyseal vessels that nourish the epiphyseal cartilage penetrate. By these means, a space is generated between the ABP and the germinal area occupied by vessels and cells (200×). (B) Interface between the germinal area and the juxtaposed bone plate. On the germinal area, we observe the presence of cells with chondrocyte phenotypes (asterisk), cellular lacunae and large nuclei, very close to the rough bone area, which suggest a closer contact between the germinal area and the RB (200×). (C) Inset of B, where we observe cells (asterisk) of the germinal area in a "balloon release" in close contact with the rough bone (RB) immersed in an extracellular eosinophilic matrix. In the upper left part, we observe cells with nuclear polymorphism (arrows) (400×) (H&E).

In the test groups, trial A, the mineralisation and neo-formation process of the juxtaposed bone plate were produced by a number of tissue and cell mechanisms that were very different from the endochondral ossification process that occurs in the metaphyseal side of the epiphyseal cartilage. The most notable differences in the histological analysis were that columns of cells were not produced, cartilage septa did not appear, and the osteocytic cells did not have the typical polyhedral morphology described in the metaphyseal area. The mineralisation (manifested in the appearance of a tide-line) and ossification occured on an area of chondrocytes that have separated from the germinal area, changed their cell phenotype by acquiring a hypertrophic phenotype, and then died by a process of karyorrhexis followed by karyolysis (in contrast to the process of apoptosis that is described in the hypertrophic chondrocyte on the metaphyseal side) (Fig. 2). A new ossification front formed on these cells, which later formed the juxtaposed bone plate. However, when the germinal zone was affected by trauma or by ovariectomy (ovx) the juxtaposed bone plate did not appear or was delayed. Thus, we found an ossification front in the epiphyseal cartilage on the epiphyseal side and an endochondral ossification front on the metaphyseal side.

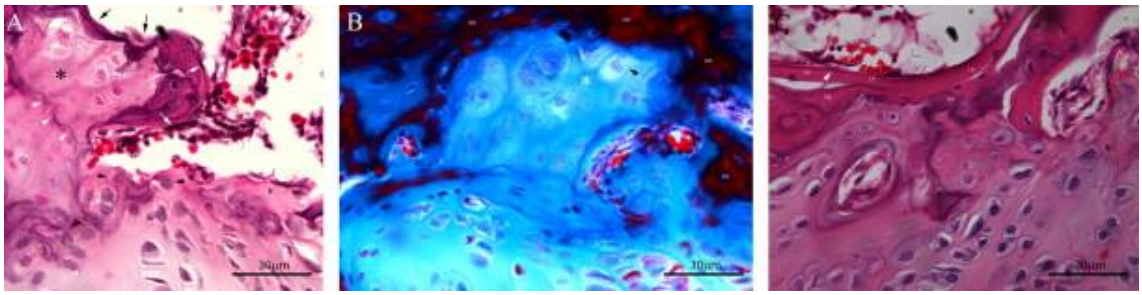

Fig. 2. Stress trial using an epiphyseal cartilage allograft with viable evolution. (A) epiphyseal cartilage: we observe the presence of viable chondrocytes in the germinal area that have migrated to the epiphyseal region. At the start of the migration, the chondrocytes increase in size and have a ball shape, and within their capsules they present hypertrophy. The cytoplasm is enlarged and has a basophilic and round nucleus (black arrowhead). The further these cells are from the germinal area, the more they are immersed in an eosinophilic matrix (asterisk), increasing cell hypertrophy. The nucleus is eosinophilic with an image of karyolysis, with the most peripheral cells presenting an image of karyorrhexis. The cytoplasm is eosinophilic and presents vacuolation with a granular appearance. On the edges of these cells, newly formed bone is deposited by the participation of osteocytic cells (white arrow) from the epiphysis. The formation of rough bone begins (black arrows). These osteocytes do not have the polyhedral configuration of the osteoblasts in the metaphyseal trabeculae. We observe that the chondrocytes are not distributed in cell columns, and there are no longitudinal septa, as occurs in the hypertrophic area of the growth plate. However, we do observe a tide-line image (white arrowheads) representative of a mineralisation process (H&E, ×400). (B) We observe the hypertrophic chondrocytes from the germinal area immersed in a connective tissue (collagen) matrix (asterisk). In the most peripheral areas, we observe cells with nuclei in the karyorrhexis phase and granular cytoplasm (black arrowhead). An area of rough bone (RB) is deposited on this row of cells. We observe the presence of vascular lumina with erythrocytes (Masson, ×400). (C). The first bone lamellae of the laminar component (LB) of the juxtaposed bone plate forms on the rough bone (RB) from the osteocytic cells of the epiphysis (white arrowhead) (H&E, ×400).

There are two aspect of the above paragraph that deserve comment. The first is the process of "separation" or "migration" of the germinal zone chondrocytes. This process is consistent with the hypothesis that chondrocytes exhibit highly controlled and specialized movements during tissue growth and remodelling in vivo [21]. In a previous paper we describe the potential of the germinal zone cells in cultures of postnatal rat epiphyseal cartilage to regenerate the chondro-epifisis [22]. However, the case for in vivo chondrocyte motility remains to be proben [21].

The second aspect arises the instant the ossification front stars. What cells are responsible for this process? The most accepted hypothesis today is the recruitment of osteoblast progenitor cells from the bone marrow. However, there is also the hypothesis that chondrocytes may be responsible for the process of bone formation. The latter would occur in two phases. In the first phase, the phenotypic switch from chondrocytes to bone-forming cells occurs [23]. At this step the asymmetric cell division involves chondrocytes [4] and [23]. In the second phase, the chondrocytes could be induced to differentiate into bone forming cells [24]. However, it has also suggested that only chondrocytes positioned at the "borderland" between cartilage and (non-cartilage) osteogenic tissues undergo further differentiation into bone producing cell [25]. Further work is needed to support these hypotheses.

The initial ossification process we describe in the ossification front of the germinal area is similar to the mineralisation process described in the degenerated hypertrophic cartilage cells in the metaphyseal cartilage of frogs during the second and third hibernation [26]. However, the intrinsic mechanisms of this mineralisation process are not well understood.

The term "metaphyseal cartilage" in frogs may cause confusion. Metaphyseal cartilage is part of epiphyseal cartilage and is stratified into four areas: the reserve area, the proliferative area, the hypertrophic area and the boundary area [6] and [26]. The metaphyseal cartilage located in the epiphyseal cartilage is responsible for the growth of the epiphysis. It does not generate endochondral ossification and does not participate in the growth of the long bone. We should not confuse it with the growth plate of mammals, which does generate endochondral ossification and participates in the growth of the long bones [6] and [26]. In metaphyseal cartilage, the hypertrophic chondrocytes are not distributed in columns and have a swollen cytoplasm and rounded nucleus. The metaphyseal cartilage edge, in the second and third hibernation, appears to be mineralised, and in the fourth hibernation, the bone formation starts [26].

As an initial conclusion, on the epiphyseal side of epiphyseal cartilage a new ossification front can be observed. At the onset of ossification front, chondrocytes coming from germinal zone chondrocytes of the growth plate are involved. Some germinal zone chondrocytes of the growth plate migrate toward the epiphyseal edge of epiphyseal cartilage. These chondrocytes hypertrophy and die. An ossification front occurs on the epiphyseal side of the epiphyseal cartilage on the edge of the hypertrophic chondrocytes, originating from osteocytic cells from the epiphysis, which participate in the new formation of rough bone. This ossification process can be compared with the process described in the metaphyseal cartilage edge of frogs, although the molecular biology mechanisms of this ossification process are unknown. Finally, a rough bone is deposited over the upper edge of the epiphyseal cartilage. Later, a few lamellar bones are deposited, and they both form the juxtaposed bone plate.

These observations suggest to us that, in an unknown manner, the mammalian epiphyseal cartilage preserves a number of phenotypes of cell and tissue behavior that are described in the metaphyseal cartilage of frogs. The functional and phylogenetic correlation of theses cartilages (the metaphyseal cartilage of frogs and the epiphyseal cartilage of mammals) may help us, through the study of cell signaling molecules, to better understand the mammalian epiphyseal cartilage.

Evaluation of the research questions

The comparative histological study in the stress trials of the epiphyseal cartilage showed that the juxtaposed bone plate and the cortex of the long bones were morphologically and functionally similar but diferent from the bone trabeculae of the epiphysis. In a stress situation, such as in rats (300 g weight) that were ovariectomized (ovx) and treated with MSC stem cells (doses 1×10^6 cells/animal), trial B, mesenchimal stem cells express abundant chondro-osseous and estrogen signalling molecules [18], the juxtaposed bone plate and the bone cortex show a very similar response, the number of cells previously reduced post-ovx does not change. This result is in contrast to the response of the trabecular bone in which the number of post-treatment cells increases. This outcome suggests that the juxtaposed bone plate has a morphological and metabolic behavior that is very different from that of the epiphyseal trabeculae.

What functional role can be attributed to the juxtaposed bone plate? We hypothesize that an analogy can be established between the bone epiphyseal region and a cupola, in which stability is achieved through a balance of forces. Initially, we attempted an analogy with a classic cupola, which is observed paradigmatically in the femoral head. Traditionally, the classic cupola is defined by its generatrix and

thickness. The classic cupola is subjected to its own weight and the weight that it must support, and it distributes the load throughout its membrane and surface [27]. Up to this point, the epiphysis meets these criteria. However, the intent behind the construction of cupolas is to open the space that they contain, while the space in the epiphysis is occupied by multidirectional trabeculae that partially occupy the inner volume along with hematopoietic tissue. In addition, the bone epiphyseal region space is closed by the juxtaposed bone plate. The observation of the configuration of this system, which initially works as a unit, eliminates the possibility of comparing the epiphysis with a classic cupola.

One of the trials we are conducting [17] consists of removing the juxtaposed bone plate. The results showed that the architecture and hence the morphology of the epiphysis considered as a cupola changed completely both in its membranes, or walls, and in the architecture of the trabeculae contained within it. This observation was interpreted as an expression of a number of tension and compression forces acting on the juxtaposed bone plate that comprised a stable structural system through the three-dimensional web configured by the bone trabeculae of the epiphysis. The trial, therefore, showed that the juxtaposed bone plate also participates in maintaining the integrity of the structure through the perimetral bracing of the bone membrane.

The epiphysis, as a part of all biological systems, may be interpreted using the paradigm of tensegrity. In biology, the paradigm of tensegrity postulates that tissues, by means of the cell molecules, are sensitive to the mechanobiological forces of compression and tension and convert them into intracellular biochemical changes and gene expression, a process called mechanotransduction [28]. As with all geometric structures, the growth and function of the epiphysis is modulated by uniaxial and multiaxial mechanical forces [29]. As a functional mechanobiological model, we could consider the analogy of the epiphysis as an enveloping cupola, with the participation of a tensegrity structure configured by a wireframe dome. A paradigmatic image in current architecture is China's National Stadium, known as the Birds' Nest, designed by the architects Herzog and De Meuron in 2008. The epiphysis has one of the main properties of tensegrities, which is the ability to dissipate forces and distribute them among all elements of the system, instead of concentrating them in just those elements that receive the forces [30]. Thus the juxtaposed bone plate may participate in a vector component that stabilises the epiphysis and relieves the growth plate from excessive external forces. The external forces that act on the bone [7] and the epiphyseal cartilage [8], in turn, may be part of a more complex tensegrity system, composed of both the anatomical bone structure and the body [31].

The juxtaposed bone plate has two functions from a biomechanical standpoint: (a) it participates in maintaining the tensegrity system of the epiphysis and (b) it protects and relieves the epiphyseal cartilage and particularly the growth plate from external force overload. These aspects require more specific studies.

Conclusion

In conclusion, in the upper area of the epiphyseal cartilage we describe the presence of an ossification front. This ossification front is responsible for the juxtaposed bone plate formation.

These findings suggest the hypothesis that in the epiphyseal cartilage of the long bones, two ossification fronts can be described: (a) an endochondral ossification front located on the metaphyseal edge of the epiphyseal cartilage called chondral growth plate, which is responsible for longitudinal bone growth; (b) and another front located in the epiphyseal edge formed by the chondrocytes that migrate from the germinal area, which is responsible for the formation of juxtaposed bone plate. These two ossification fronts are initially formed from chondrocytes that have different morphological characteristics of the cells. They also have different mechanisms of cell death, and ultimately they generate two different types of bone morphology and function. (c) The juxtaposed bone plate participates in protecting the epiphyseal cartilage, and therefore also the chondral growth plate, from the vicissitudes of biomechanics.

This hypothesis has several implications. (a) That the epiphyseal cartilage is a morphological entity with two different ossification fronts and two different functions. (b) The epiphyseal cartilage is a morphological structure with three parts: perichondrial ring, metaphyseal ossification front or cartilaginous growth plate, and an epiphyseal ossification front. (c) All kinds of disease (traumatic or dysplastic) that affect these parts can have an impact on the morphology of the epiphyseal region of the bone. (d) Comparative histological and anatomy studies are also warranted, along with molecular biology studies, to help unravel the evolutionary origin of the mammalian epiphyseal cartilage.

The present hypothesis must be corroborated through research into the germinal zone of the epiphyseal side of the mammalian epiphyseal cartilage and in the juxtaposed bone plate.

Conflict of interest statement

The authors report that there is no conflict of interest.

References

[1]. C. Farnun, M. Lenox, W. Zipfel, W. Horton, R. Williams. In vivo delivery of fluoresceinated dextrans to the murine growth plate: Imaging of the three vascular routes by multiphoton microscopy. Anat Rec A Discov Mol Cell Evol Biol, 288 (1) (2006), pp. 91–103.

[2]. R. Williams, W.R. Zipfel, M.L. Tinsley, C.E. Farnun. Solute transport in growth plate cartilage: in vitro and in vivo. Biophys J, 93 (2007), pp. 1039–1050.

[3]. Cajal SR. In: de Histología Manual de Histologia. Normal y de Técnica Micrográfica. Madrid: Imprenta y Librería de Nicolás Moya; 1910. p. 279–83.

[4]. V. Abad, J.L. Meyers, M. Weise, *et al*. The role of the resting zone in growth plate chondrogenesis. Endocrinology, 143 (2002), pp. 1851–1857.

[5]. C.T. Brighton. The growth plate. Orthop Clin North Am, 15 (1984), pp. 571–595.

[6]. S.L. Felisbino, H.F. Carvalho. The epiphyseal cartilage and growth of long bones in Rana catesbeiana. Tissue Cell, 31 (3) (1999), pp. 301–307.

[7]. J. Wolff. Ueber die innere Arcuitectur der Knochen und ihre Bedeutung fur die Frage von Knochenwaschtum. Vichows Arch, 50 (1870), pp. 389–450.

[8]. C. Hueter. Anatomische studien an den Extremitätengelenken Neugebboreener und Erwachsener. Virchows Archiv, 25 (1862), pp. 572–599.

[9]. V. Lefebvre, P. Smits. Transcriptional control of chondrocyte fate and differentiation. Birth Defects Research C Embryo Today, 75 (2005), pp. 200–212.

[10]. J.I. Rodríguez-González. Anillo pericondral Aportaciones experimentales y clinico-patologicas (thesis). Autónoma University of Madrid, Spain, Madrid (1982).

[11]. Rodríguez Peralto JI. Osteocondroma Experimental Estudio de la Participación de las Cubiertas Pericondrales (thesis). Madrid: Autónoma University of Madrid, Spain; 1985.

[12]. J.I. Rodriguez, E. Delgado, R. Paniagua. Changes in young rat radious following excision of the perichondral ring. Calcif Tissue Int, 37 (1985), pp. 677–683.

[13]. E. Delgado, J. Rodriguez, A. Serrada, M. Tellez, R. Paniagua. Radiation-induced osteochondroma-like lesion in young rat radius. Clin Orthop Relat Res, 201 (1985), pp. 251–258.

[14]. E. Delgado, J. Rodriguez, C. Miralles, R. Paniagua. Osteochondroma induced by reflection of the perichondral ring in young rat radii. Calcif Tissue Int, 40 (1987), pp. 85–90.

[15]. W.R. Harris, R. Martin, M. Tile. Transplantation of epiphyseal plates. An experimental study. J Bone Joint Surg, 47-A (1965), pp. 897–914.

[16]. R.W. Haines. The histology of epiphyseal union in mammals. J Anat, 120 (1) (1975), pp. 1–25.

[17]. A. Touza. Placa de Crecimiento Alotrasplante Simple y Alotrasplante Combinada Con Hueso Modelo Experimental en rata (thesis). Autónoma University of Madrid, Spain, Madrid (2012).

[18]. Delgado-Martos MJ. Terapia celular en un modelo murino de osteoporosis. Implicaciones de dos moduladores de la vía JAK-STATs (thesis). Madrid: Autónoma University of Madrid, Spain; 2010.

[19]. Santos del Riego S. Epifisiolisis. análisis histométrico. estudio experimental (thesis). Madrid: Autónoma University of Madrid, Spain; 1988.

[20]. B. Quintana-Villamandos. Historia natural del cartílago articular tras la lesión de la placa de crecimiento (thesis). Autónoma University of Madrid, Spain, Madrid (2004).

[21]. T.I. Morales. Chondrocytes moves: clever strategies?. Osteoarthritis and Cartilage, 15 (2007), pp. 861–871.

[22]. E. Delgado-Baeza, M. Gimenez-Ribotta, C. Miralles-Flores, A. Nieto-Chaguaceda, I. Santos-Alvarez. Growth of the perichondrium and the chondroepiphysis: experimental approach in the rat proximal tibial epiphysis. Acta Anat, 145 (1992), pp. 195–200.

[23]. Roach HI, Erenpreisa JE. The phenotypic switch from chondrocytes to bone-forming cells involves asymmetric cell division and apoptosis. Connect Tissue Res 1996; 35(1–4):85–91 (139–145).

[24]. H.I. Roach, J.E. Erenpreisa, T. Aigner. Osteogenic differentiation of hyperthrophic chondrocytes involves asymmetric cell división and apoptosis. J Cell Biol, 131 (2) (1995), pp. 483–494.

[25]. P. Bianco, F. Descalzi Cancedda, M. Riminucci, Cancedda. Bone formation via cartilage models: the "bordeline" chondrocyte. Matrix Biol, 17 (1998), pp. 185–192.

[26]. Rozenblut B, Ogielska M. Development and growth of long bones in European water frogs (Amphibia: Anura: Ranidae), with remarks on age determination. J Morphol 2005; 265(3):304–317.

[27]. S. Huertas. Arcos, bóvedas y cúpulas. Geometría y equilibrio en el cálculo tradicional de estructuras de fábrica. Instituto Juán de Herrera, Madrid (2004).

[28]. D.E. Ingber. Tensegrity-based mechanosensing from macro to micro. Prog Biophys Mol Biol, 97 (2008), pp. 163–179.

[29]. S. Piszczatowski. Geometrical aspects of growth plate modelling using Carter's and Stokes's approaches. Acta Bioeng Biomech, 14 (2012), pp. 93–106.

[30]. Gómez-Jauregui V. Tensegridad. Estructuras tensegríticas en ciencias y arte. Publicaciones UC, Universidad de Cantabria, 2007, pp. 57–75.