Chondrogenic potential of intramembranous skeletal bones

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Abstract
The analysis of scientific sources regarding the presence of chondrogenic potential in intramembranous bones was carried out. Detailed information is provided on the elements of the general conservative program of enchondral and intramembranous ossification, on the conditions for the formation of cartilage tissue in the cranial sutures and in the area of reparative regeneration of flat bones of the skull, as well as on the possibility of cartilage matrices to have an optimizing effect on reparative osteogenesis in the intramembranous bones of the skeleton.

Keywords: cartilage tissue – intramembranous ossification – reparative osteogenesis

Introduction

It is known that the formation, growth and regeneration of bones of the skeleton occurs by two types of ossification – enchondral and intramembranous [1]. In the embryonic period, as a result of chondroblastic differentiation of cells of mesenchymal condensates of paraxial mesoderm and lateral plate mesoderm, hyaline cartilage models of future bones of the trunk and limbs are formed, which are later replaced by vascular mesh and bone fabric. This type of ossification, which occurs in the middle of the temporary cartilage, is called enchondral [2]. Intramembranous ossification is characteristic of the jaws (viscerocranium), flat bones of the skull that originate from the neural crest (for example, the frontal bone) and partially from the paraxial mesoderm (for example, the parietal bone). However, in some parts of the skeleton and individual bones, both types of ossification occur. For example, the base of the neurocranium, the medial half of the clavicle, the glenoid cavity, the acromion, the coracoid process, the inferior angle, and the medial margin of the scapula are formed due to enchodral ossification, while other parts of the scapula, the vault of the skull and the lateral half of the clavicle exclusively due to intramembranous ossification [3,4]. The essence of intramembranous ossification is that initially the mesenchymal condensates, which are located on the cities of the future flat bones of the skull, mostly take the form of two-layer membranes subcutaneously. Then the cells of mesenchymal condensates enter the path of osteoblastic differentiation and the membranous models (blanks) turn into bones [5]. The formation of the latter occurs in the middle of the membranes and subcutaneously, which explains the name of the process of intramembranous or dermal ossification. Thus, intramembranous ossification differs morphologically from enchondral ossification in that a bone or part of a bone is formed by direct transformation of embryonic mesenchyme condensates into bone tissue, bypassing the stage of chondrogenesis [5,6].

But is everything so clear? The fact is that despite all the expressiveness of the morphological differences between enchondral and intramembranous ossification, both of these processes are controlled by a general conservative program [7].

Elements of a general conservative program of skeletal development

The elements of this program include the absence of angiogenesis in all skeletogenic (prechondrogenic and preosteogenic) condensates at an early stage of their development. Although bone vascularization occurs in the future, and the majority of permanent cartilages remain without vessels [2]. The expression of the main transcription factors of osteoblastic (Runt related transcription

factor 2, Runx2, and Osterix, Osx) and chondroblastic (Sex-determining Region Y related high mobility group box 9, Sox9) differentiation is combined in the embryonic condensates from which the intramembranous bones of the skull vault are formed [2,8]. In addition, in the embryonic condensates from which the intramembranous bones of the skull are formed in the process of osteogenesis, there is a simultaneous expression of not only markers of osteoblasts (type I collagen) but also markers of chondroblasts (types IIA, IX, XI collagens and aggrecan) and chondrocytes (types IIB and X collagen), and cultured mouse calvarial mesenchyme could develop into cartilage [9,10]. Thus, the authors established that normal intramembranous ossification includes a previously unrecognized temporary chondrogenic phase, and cells in this phase retain chondrogenic potential and can undergo overt chondrogenesis in a certain microenvironment.

In addition, signaling molecules, transcription factors Indian hedgehog (Ihh) and Parathyroid hormone-related peptide (PTHrP) are expressed in the forming bones of the skull vault, which determine the course of enchondral ossification, longitudinal growth of the bones of the axial and appendicular skeleton. However, Ihh and PTHrP in the condensates from which the bones of the skull vault are formed regulate not the proliferation of chondroblasts, as in enchondral bones, but the differentiation of preosteoblasts into osteoblasts [2,8]. The joint action of BMP-2, BMP-4 and BMP-7 (bone morphogenetic protein) in the intramembranous bones of the skull leads to the fact that during the differentiation of mesenchymal progenitor cells into osteoblasts, part of the cells undergo a short-term state, which was named chondrocyte-like osteoblast (GLO, similar to a chondrocyte osteoblast). However, without the involvement of BMP-7, intramembranous bone progenitor cells differentiate directly into osteoblasts. GLO is able to simultaneously express a marker of osteoblasts (osteopontin) and cartilage cells (collagen II, IX types, PTHrP and Ihh). At the same time, GLO does not express such important markers of chondrogenesis as Sox9 and aggrecan. In the next phase, the cell stops expressing cartilaginous collagens and begins to express not only osteopontin, but also the marker of mature osteoblast – bone sialoprotein II (BspII) [8].

It should also be noted that after birth, the bones of the skull vault are separated and at the same time connected to each other with the help of sutures. The latter originate from mesoderm or neural crest cells and are niches for stem cells that support bone growth and regeneration after birth [3,11]. However, in bone seams there are also islands of cartilage tissue or islands of secondary cartilage, which was named so because its formation occurs not before, but after the formation of bone tissue [12]. In addition, Sahar DE and co-authors established the presence of cartilaginous tissue in the posterior frontal suture in mice. The authors noted that the posterior frontal suture (analogous to metopic suture in humans) is the only suture that closes in mice in the first month after birth, and all other sutures (coronal, sagittal, lambdoid sutures) remain open throughout life. The frontal suture is the only suture in the skull, which originates from the cells of the neural crest (neural crest cells) and is surrounded on both sides by the plates of the frontal bones, which are also formed from neural crest cells. At the same time, the gene that expresses the transcription factor Sox9, a regulator of chondrogenesis, is a determinant for neural crest cells and contributes to the unique fate of the posterior frontal suture through the implementation of a separate morphogenetic program. The latter manifests itself in the closure of the posterior frontal suture due to endochondral ossification, which is evidenced by a number of consecutive changes. Thus, initially the authors established increased expression of the chondrogenesis regulator Sox9, specific cartilage markers of type II and X collagens, the formation of cartilage tissue, and then the replacement of cartilage by bone tissue with the expression of such bone markers as type I collagen and osteocalcin. At the same time, the deficiency of the Sox9 gene disrupts the formation of cartilage and leads to a delay in the closure of the posterior frontal suture [13]. In turn, He F and co-authors established the presence of cartilage rudiments in the coronal suture in mice. The latter originate from the cells of the neural crest and paraxial mesoderm, express collagen IIa1, Sox9, collagen Xa1 and subsequently, under the partial regulation of the platelet-derived growth factor receptor alpha (PDGFRα), can transdifferentiate into osteoblasts of the bones of the skull vault and coronary suture [3]. However, increased signaling activity of PDGFRα causes abnormal expansion and premature differentiation of the cartilage bud in the coronal suture and its pathological closure (craniosynostosis) by enchondral ossification [14]. Other authors established that the mutation of the fibroblast growth factor receptor 2 (Fgfr2) [15] and zinc finger protein Gli3 with ectopic expression of Patched1 (Hedgehog receptor) and with reduced expression of the transcription factor Twist1 [16] leads in the first case to the formation of cartilage in the sagittal suture, and in the second – in the lambdoid suture, as well as to their pathological closure (craniosynostosis).

And finally, in our opinion, the most impressive example of the chondrogenic potential of the bones of the skull is the determining by Govindarajan V and Overbeek PA of the conditions under which the parietal bone cell differ-

entiation program switches from intramembranous to enchondral ossification. This is observed in transgenic mice, with the expression of fibroblast growth factor 9 (Fibroblast growth factor – FGF-9) acting through the Fgfr2 receptor by cells of the cranial mesenchyme. The effect of only these factor (FGF-9) is sufficient to induce enchondral ossification in intramembranous bones. These changes are accompanied by the activation of the expression of Sox9, Ihh, collagen 2a1, collagen Xa1 types, as well as the suppression of the expression of Core-binding factor alpha 1(Cbfal) and osteocalcin [5].

Cartilage tissue in the healing area of intramembranous bone defect

The above types of ossification, due to which bones are formed in the embryonic period, are also repeated during their reparative regeneration [17–19]. However, is it possible to form cartilage tissue in the area of healing of the intramembranous bone defect?

For example, Hermann CD and co-authors in the area of trepanation of the posterior frontal suture in mice (C57Bl/6J, Jackson Labs, Bar Harbor, Maine, USA) aged 21 days established increased expression of genes associated not only with osteoblastic (transforming growth factor, beta-2, dentin matrix acidic phosphoprotein 1, TGFb2, Dmp1) but also chondroblastic (Sox9, Col2, ColX, cartilage oligomeric matrix protein, Comp) differentiation. At the same time, in the area of the defect, cartilage tissue was detected on the 5th day, and the peak expression of the specified genes – on the 4th and 5th day after the injury. In addition, the authors established the acceleration of the formation of bone tissue in the area of the posterior frontal suture after the impact of a traumatic factor on it with the formation of a 1.5/2.5 mm defect. This was evidenced by the filling of the entire defect cavity within 14 days with mature, mineralized, trabeculated bone tissue in animals whose age at that time was 35 days (animal age 21 days + 14 days after injury). However, in uninjured mice, a similar pattern is observed in the developed suture only on the 50th day after birth. In this study, the authors also found that a frontal bone defect of identical size (1.5/2.5 mm) that was placed 1 mm lateral to the posterior frontal suture, centered between the interfrontal ridge and the coronal suture at 14th day after the injury in mice of both age groups (21 and 50 days) does not heal and cartilage tissue does not form in it. A delay in the formation of regenerated bone tissue was also observed in the defect of the posterior frontal suture in mice aged 50 days after birth. Thus, the formation of cartilage tissue occurred only in the defect of the posterior frontal suture, which was completely healed in 14 days in 21-day-old mice, and the closure of the defect of the frontal bone with bone tissue and the formation of cartilage tissue in it did not occur, despite the fact that the frontal bone and the posterior frontal suture have the same embryonic origin [20].

In turn, Inoue S and co-authors established the absence of increased mRNA expression of markers of chondrogenesis (Sox 9, type 2 collagen) and the formation of cartilage tissue in an artificially created defect of the scapula and parietal bone with a diameter of 0.8 mm in 8-week-old mice. At the same time, the authors reported the restoration of the thickness of the cortical layer of the scapula in 21 days and the lack of complete healing of the parietal bone defect on the 49th day after injury [21]. Lim J et al, comparing the healing of an experimental defect of 3 mm in the tibial and parietal bones of 6-week-old rats, also reported the absence of cartilage tissue in the defect of the skull vault and its presence in the defect of the long bone of the skeleton. In addition, the authors found that the healing of the tibial bone defect is approximately twice as fast (in 80 % in 3 weeks) as in the parietal bone (in 60 % in 6 weeks) [22].

Thus, the above-mentioned researchers, whose works were published in 2013 and 2020, reported the absence of cartilage tissue in the healing area of the parietal bone defect. However, in the scientific work, which was published at the beginning of the second half of the 20th century, facts are given about the possibility of cartilage tissue formation in the area of the parietal bone injury in conditions of significant hypoxia. The authors of this article, Girgis FG and Pritchard JJ, proposed several types of defects of the parietal bone in rats aged from 3 to 34 days, which led to varying degrees of impaired blood supply to the trauma site. The most serious limitations of blood supply occurred in those animals that were first scraping the periosteum, and then making bone incisions to the dura mater: two longitudinal incisions in the left parietal bone, one longitudinal incision in the right parietal bone and a transverse incision that connected three longitudinal. With such an injury, cartilage tissue was found 7–12 days after the operation in the area of the second (central) longitudinal incision, where the greatest disruption of the blood supply was observed. At the same time, the authors noted that cartilage tissue was not present in every histological section and in most cases the cartilage was in the form of isolated nodules between which bone tissue was found. Accordingly, the hypothesis that ischemia in the area of the bone defect induces the formation of cartilage tissue was experimentally confirmed on parietal bones as early as 1958 [23]. In turn, Schmitz JP and co-authors in a work published in 1990 also de-

scribed the presence of cartilage cells in the central area of a round defect with a diameter of 8 mm in the parietal bone of adult rats [24].

Cartilage matrices have an optimizing effect on reparative osteogenesis in intramembranous bones of the skeleton

Analyzing the above data on the possibility of cartilage tissue formation in the intramembranous bones of the skull vault during their reparative regeneration, we also drew attention in the scientific literature to information on the use of tissue engineered constructs with chondroblasts and chondrocytes for the treatment of skull bone defects. Thus, in 1994, Kim WS and co-authors first demonstrated the effectiveness of cartilage cells as part of a biodegradable polymer template in optimizing the healing of large defects of the skull vault that affected the frontal, parietal, and temporal bones [25]. Doan L et al also confirmed the ability of cartilage to optimize reparative osteogenesis in a 2 mm diameter mouse skull defect, which healed in 6 weeks [26]. But Freeman FE and co-authors conducted a comparative analysis of the influence of intramembranous and endochondral primed scaffolds on the healing of a critical 4 mm diameter skull vault defect in one-month-old female mice over the course of 8 weeks. Both scaffolds were constructed from polycaprolactone and stem mesenchymal cells, which were exposed to osteogenic growth factors in the intramembranous construct, and chondrogenic growth factors in the endochondral constructs. 8 weeks after the implantation of these structures into the skull vault defect, the authors established that the cartilage template provided the conditions for the formation of regenerated bone tissue and was subject to rapid integration with it. At the same time, the amount of regenerated bone tissue slightly prevailed in the group of animals with an intramembranous structure, but on the contrary, there were more vessels in the area of cartilage matrix implantation [27]. In turn, the work of Fu R and co-authors explained the very idea of using chondrogenic cells for optimization of reparative osteogenesis. Thus, at the expense of the latter, a solution to the problems associated with poor vascularization of tissue-engineered structures after implantation into a bone defect is proposed, since chondroblasts and chondrocytes are born and live in avascular environment, so they are adapted to hypoxia conditions. Unlike chondroblasts, stem cells and osteoblasts are less resistant to a hypoxic environment [28]. Also, due to the expression of vascular endothelial growth factors (VEGF), bone morphogenetic proteins and the formation of hydroxylapatite, hypertrophied chondrocytes are able to induce neovascularization and ossification [29,30]. In addition, until recently, the scientific literature claimed that chondrocytes are terminally differentiated cells doomed to apoptosis, and osteoblasts, together with vessels from the periosteum or endosteum, penetrate the acellular cartilage matrix and replace it with bone tissue [31,32]. However, despite this statement, some scientists, for example, Girgis FG and Pritchard JJ in 1958 [23], Kahn AJ and Simmons DJ in 1977 [33], and Scammell BE and Roach HI in 1992 and 1996 [34,35] assumed the transformation of chondrocytes into osteoblasts. In 2014, Zhou X and Yang L et al presented evidence that chondrocytes are capable of transdifferentiation and are one of the sources of osteoblasts during endochondral bone formation, growth, and reparative regeneration [19,36]. Scientists also noted that osteoblasts, which are formed from chondrocytes, make up about 60 % of all mature osteoblasts in the enchondral bones of mice [19].

Conclusion

In the process of intramembranous ossification, there is a combination of molecular mechanisms of chondrogenesis and osteogenesis, in the embryonic period, the intramembranous formation of flat bones passes through a hidden temporary chondrogenic phase, the closure of the posterior frontal suture in the postnatal period occurs due to endochondral ossification, cartilage cells in tissue engineering structures are able to exert an optimizing effect on reparative osteogenesis in the flat bones of the skull, and the manifestation of their chondrogenic potential during reparative regeneration depends on the age, shape, size, localization of the defect, the level of its blood supply and the integrity of the periosteum.

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