Immunohistochemical Examination of Developing Mandibular Angle in Fetal Mice

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Abstract: In fetal period, on the 15th fetal day, mouse mandibular angle development started as a coagulation of mesemchymal cells. On the 16th fetal day, cells of the central portion of the cell coagulation showed metachromasia to toluidine blue, and type 2 collagen positive chondrocytes were immunohistochemically detected. After the 17th fetal day, cartilaginous osteogenesis occurred with invasion of capillaries. At the same stage, membranous (perichondral) ossification occurred in the periphery of the chondrocyte mass. These proliferating chondrocytes showed positive reactions to type 2 collagen, type 1 collagen and osteopontin. These results suggest that the characteristics of mandibular angular cartilage are slightly different from those of normal physiological articular cartilage

Key words: mandible angular cartilage, mouse, immunohistochemistry, type 2 collagen, type 1 collagen, osteopontin

Introduction

There are nemerous published literatures on the development of the mandible [1)], especially on the mandible condylar cartilage [2-5)]. Mandible cartilage is recognized as a secondary cartilage and is slightly different from primary cartilage, which composes the mandible articule. However, there have been almost no published literatures on mandible angular cartilage which developes the same as condylar cartilage. In this examination, we examined developing mandible angular cartilage in fetal mice, using mainly immunohistochemical techniques.

Materials and Methods

The chracteristics of developmental mandible angular cartilage was examined using fetal ddY mice, purchased from Japan SLC Co., Hamamatsu, Japan, aged from the 14th fetal day to just after the birth (equivalent to the 19th fetal day). Immediately after removal from the mice, the materials were fixed in 10 % neutral buffered formalin fixative solution. The materials were decalcified by 10%EDTA and then dehydrated by passage through a series of ethanols and embedded in paraffin.

After being sectioned, the series specimens were examined by histology, histochemistry (toluidine blue: TB) and immunohistochemistry using 3 monoclonal antibodies: anti-type 1 collagen (LB-1102, LSL, 1/800), anti-type 2 collagen (LB-1297, LSL, 1/800) and anti-osteopontin (OPN: MPIIIB10, 1/50). The OPN developed by Solursh and Franzen [6)] was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, USA. DAB was applied for visualization of immunohistochemical activity. We included immunohistochemical staining using PBS in place of the primary antibody as a negative control.

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Results

In histological sepecimens, there were no developmental features of mandible angle, with some osteoblastic cell proliferation and a little bone matrices with mesencymal cell proliferation, in the 14th fetal day. Mouse mandible angle development started as a coagulation of mesenchymal cells at the end of the developmental mandible in the 15th fetal day, although there was no metachromasia reaction to TB. On the 16th fetal day, cells of the central portion of the cell coagulation showed metachromasia to TB (Figure 1). After that, the mandible angular cartilage developed through a similar course of intrachondral ossification with invasion of capillaries. At the 17th fetal day, direct (perichondral) bone formation was observed at the anterior portion (Figure 2).

Immunohistochemically, at the 16th fetal day, type 2 collagen positive chondrocytes were detected (Figure 3), although there was no positive reaction at the 14th and 15th fetal day. Furthermore, these proliferating chondrocytes showed positive reactions to type 1 collagen (Figure 4) and OPN through the examination period.

Discussion

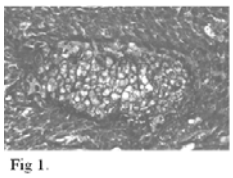
Fig 1.

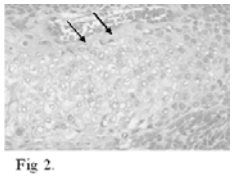
Fig 2.

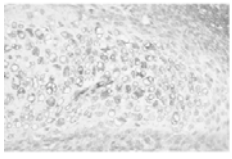
Fig 3.

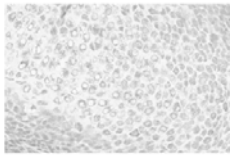
Fig 4.

Regarding the mandible angular cartilage, Tengan [7] reported the examination results of developmental aspects of mandible condylar and angular cartilages. In the report using C57BL/6N mice, the developmental start of the mandible angular cartilage is observed as a coagration of msenchymal cell proliferation in the end of the mandibular bone at the 14.5th fetal day. Our examination results of histological findings and TB reactions of ddY mice mandible angular cartilage suggest that the development starts nearly the same fetal day. Histologically and histochemically (TB), after the 17th fetal day, endochondral ossification occurred with invasion of capillaries, and perichondral ossification occurred in the periphery of the cartilage mass.

In the immunohistochemical examination, the proliferating chondrocytes of the mandible angular cartilage showed positive reactions to type 2 collagen, as well as to type 1 collagen and OPN. In an examination of mandible chondylar cartilage, Mizoguchi [3] reported the same immunohistochemical aspects, and Ishiwari et al. [4] reported the gene expression using in situ hybridization technique. Therefore, present immunohistochemical results of mandible angular cartilage show that the characteristics of proliferating mandible angular cartilage are nearly the same as mandibule condylar cartilage, and slightly different from normal physiological articular cartilage.

References

1. Kimura A. Matrix protein production and gene expression in bone forming cells on mandible bone formation of mouse. Jpn J Tokyo Dent Coll Soc 103:335-345,2003
2. Ishii M. An immunohistochemical study on fetal mouse condylar cartilage. Jpn J Tokyo Med Dent Univ 62:16-28,1995
3. Mizoguchi I (1999) Growth behavior of the mandible and biological aspects of mandibular condylar cartilage. Jpn TohokuUniv Dent J 18:1-21,1999
4. Ishiwari Y, Nagatsuka H, Inoue M, Nagai N. Localized expression of type I, II and X collagen genes in the mandibular condyle of immature mice. Jpn Okayama Dent J 723: 688-698,1999
5. Shimizu T, Tsujigiwa H, Nagatsuka H, Okafuji N, Kurihara S, Nagai N, Kawakami T. Expression of Notch1 and Math1 in Mandibular Condyle Cartilage in Neonatal Mice. Angle Orthodont 75: 787-789,2005
6. Gorski JP, Gorski JP, Griffin D, Dudley G, Stanford C, Thomas R, Huang C, Lai E, Karr B, Solursh M. Bone acidic glycoprotein-75 is a major synthetic product of osteoblastic cells and localized as 75- and/or 50-kDa forms in mineralized phases of bone and growth plate and in serum. J Biol Chem 265: 14956-14963,1990
7. Tengan T. Histogenesis and three-dimensional observation on condylar cartilage in prenatal mice. Jpn J Tokyo Med Dent Univ 57: 32-57,1990