

Short Communication

IMMUNOHISTOCHEMICAL CHARACTERISTICS OF DEVELOPING MANDIBULAR ANGLE IN FETAL MICE*

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Abstract: Mouse mandibular angle development started as a coagulation of mesenchymal cells on the 15th fetal day. 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: mandibular angular cartilage; mouse; immunohistochemistry; type 2 collagen; type 1 collagen; osteopontin

INTRODUCTION

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

MATERIALS AND METHODS

The nature of developmental mandibular angular cartilage was investigated 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: MPIIB10, 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.

RESULTS AND DISCUSSION

Histologically, there were no developmental features of mandibular angle, with some osteoblastic cell proliferation and a little bone matrices with mesenchymal cell proliferation, in the 14th fetal day. Mouse mandibular 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 (Fig. 1). After that, the mandibular 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 (Fig. 2).

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

Regarding the mandibular angular cartilage, Tengan [7] reported the examination results of developmental aspects of mandibular condylar and angular cartilages. In the report using C57BL/6N mice, the developmental start of the mandibular angular cartilage is observed as a coagulation of mesenchymal 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 mandibular angular

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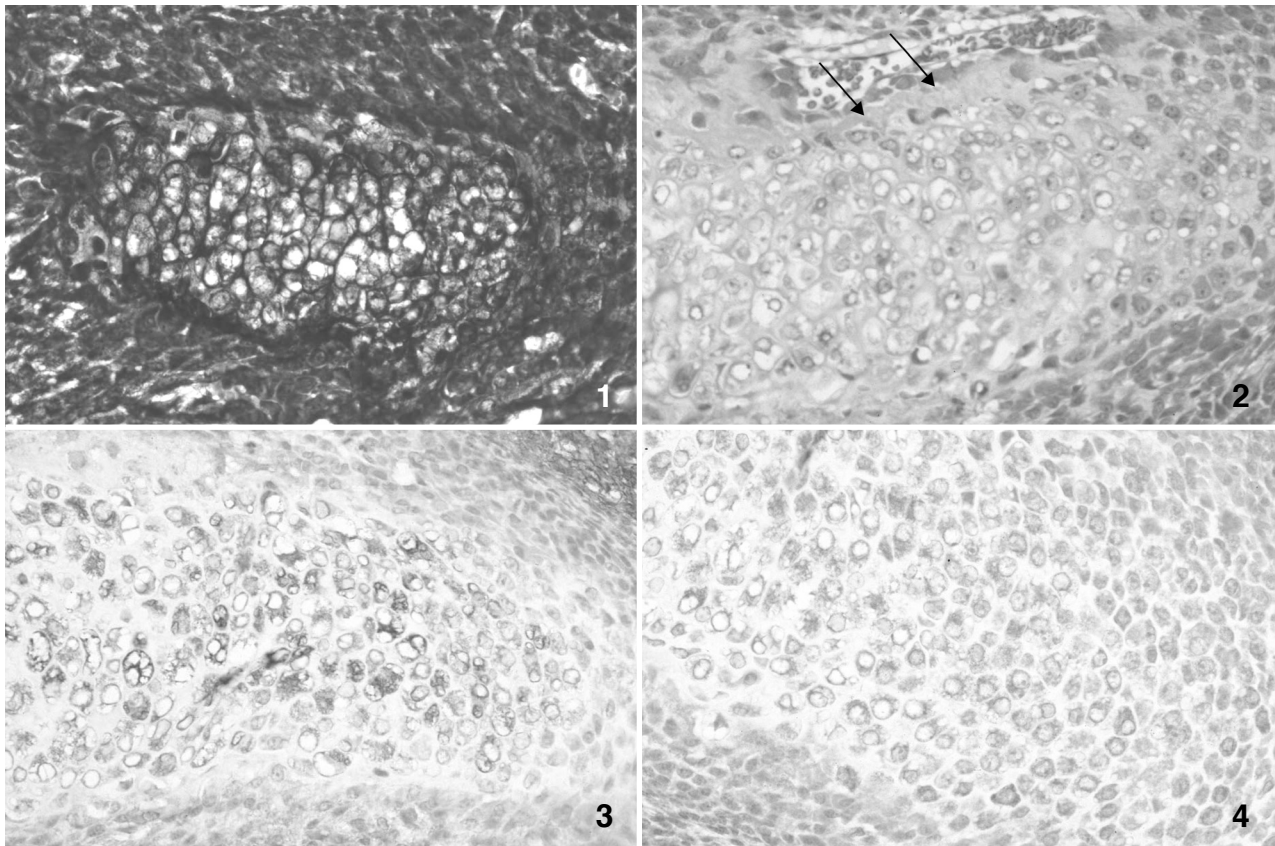


Fig. 1. Cell coagulation showing metachromasia to TB (16th fetal day, magnification x 200)

Fig. 2. Pericondral ossification (arrows) at the surface of chondral coagulation (17th fetal day, magnification x200)

Fig. 3. Positive reactions to type 1 collagen (18th fetal day, magnification x200)

Fig. 4. Positive reaction to OPN (17th fetal day, magnification x200)

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 mandibular angular cartilage showed positive reactions to type 2 collagen, as well as to type 1 collagen and OPN. In an examination of mandibular condylar 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 mandibular angular cartilage show that the characteristics of proliferating mandibular angular cartilage are nearly the same as mandibular condylar cartilage, and slightly different from normal physiological articular cartilage.

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