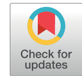
Archives of Oral Biology 89 (2018) 84–92

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Developmental characteristics of secondary cartilage in the mandibular condyle and sphenoid bone in mice



Hidetomo Hirouchi[a,*], Kei Kitamura[b], Masahito Yamamoto[a], Kento Odaka[a], Satoru Matsunaga[a], Koji Sakiyama[c], Shinichi Abe[a]

[a] Department of Anatomy, Tokyo Dental College, 2-9-18 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan
[b] Department of Histology and Developmental Biology, Tokyo Dental College, 2-9-18 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan
[c] Division of Anatomy, Meikai University School of Dentistry, 1-1 Keyakidai, Sakado, Saitama 350-0283, Japan

ARTICLE INFO

Keywords:
Hypertrophic chondrocytes
Secondary cartilage
3-Dimensional stereoscopic reconstruction
Maxillofacial region
Mice

ABSTRACT

Objective: Secondary cartilage develops from osteochondral progenitor cells. Hypertrophic chondrocytes in secondary cartilage increase within a very short time and then ossify rapidly. In the present study, we investigated the sequential development process of osteochondral progenitor cells, and the morphology and size of hypertrophic chondrocytes in secondary cartilage.

Design: ICR mice at embryonic days (E) 14.5–17.5 were used. The mandibular condyle and the medial pterygoid process of the sphenoid bone were observed as secondary cartilage, and the cranial base and the lateral pterygoid process of the sphenoid bone, which is primary cartilage, were observed as a control. Thin sections were subjected to immunostaining and alkaline phosphatase (ALP) staining. Using a confocal laser microscope, 3D stereoscopic reconstruction of hypertrophic cells was performed. To evaluate the size of hypertrophic chondrocytes objectively, the cell size was measured in each cartilage.

Results: Hypertrophic chondrocytes of secondary cartilage first expressed type X collagen (Col X) at E15.5. SRY-box 9 (Sox 9) and ALP were co-expressed in the fibroblastic/polymorphic tissue layer of secondary cartilage. This layer was very thick at E15.5, and then rapidly became thin. Hypertrophic cells in secondary cartilage were markedly smaller than those in primary cartilage.

Conclusions: The small hypertrophic cells present in secondary cartilage may have been a characteristic acquired in order for the cartilage to smoothly promote a marked increase in hypertrophic cells and rapid calcification.

1. Introduction

The maxillofacial region contains several cartilages with characteristics different from those of primary cartilages in the limbs and cranial base (CB), and which develop through a process different from general endochondral ossification for the following reasons: (1) delayed timing of development (Shibata, Suzuki, Tengan, Ishii, & Kuroda, 1996; Yamamoto et al., 2017), (2) origin of chondrocytes from the periosteum of existing bone (Beresford, 1981; Miyake, Cameron, & Hall, 1997; Shibata et al., 1996), and (3) absence of a secondary ossification center (Jing et al., 2015). The mandibular condylar cartilage (CC), the mandibular angular cartilage, the mandibular coronoid process cartilage, and the medial pterygoid process cartilage of the sphenoid bone (MP) have these characteristics, and are defined as secondary cartilage (Shibata et al., 1996; Yamamoto et al., 2017).

Many researchers have investigated secondary cartilage focusing on the CC. Silbermann et al. (1987) maintained that the CC develops from already differentiated progenitor cells known as 'skeletoblasts', which function as osteochondral progenitors. The CC arises from progenitor cells that are positive for alkaline phosphatase (ALP) and express type I collagen mRNA (Fukada, Shibata, Suzuki, Ohya, & Kuroda, 1999; Miyake et al., 1997; Shibata et al., 1996, Shibata, Fukada, Suzuki, & Yamashita, 1997), being derived from progenitor cells expressing both Sox9 and Runx2 RNA (markers of the chondrogenic and osteogenic pathways, respectively) (Shibata, Suda, Suzuki, Fukuoka, & Yamashita, 2006). These cells differentiate into secondary cartilages, and become distinguishable into three distinct portions along the main axis during the fetal period: a fibroblastic/polymorphic tissue layer containing progenitor cells, a zone of flattened chondrocytes, and a zone of hypertrophic chondrocytes (Shibukawa et al., 2007). During the postnatal period, the CC is divided into four distinct portions: a fibrous cell layer, a polymorphic progenitor cell layer, a zone of flattened chondrocytes, and a zone of hypertrophic chondrocytes (Luder, Leblond, & Von Der Mark, 1988). However, there have been few attempts to explore the

* Corresponding author.
E-mail address: hirouchihidetomo@tdc.ac.jp (H. Hirouchi).

https://doi.org/10.1016/j.archoralbio.2017.12.027
Received 29 August 2017; Received in revised form 22 December 2017; Accepted 28 December 2017
0003-9969/ © 2018 Elsevier Ltd. All rights reserved.

sequential process of progenitor cell development during the fetal period.

It has been demonstrated that chondrocytes initially appearing in the CC in mice have already differentiated into hypertrophic chondrocytes simultaneously expressing collagen types II and X, and that the hypertrophic chondrocyte layer extends rapidly (Fukada et al., 1999; Shibata et al., 1997). In addition, we have shown that hypertrophic chondrocytes in the MP increase within a very short time and then ossify rapidly (Yamamoto et al., 2017). However, many points remain unclear with regard to the morphology and size of the hypertrophic chondrocytes in secondary cartilage.

In this study, we investigated (1) the sequential process of molecular transformation of osteochondral progenitor cells during the fetal period, and (2) the morphology and size of the hypertrophic chondrocytes in secondary cartilage.

2. Materials and methods

ICR mice at E14.5, 15.5, 16.5, and 17.5 were used in this study. This mouse is an albino strain that originated in Switzerland and was selected by Dr. Hauschka to create a fertile mouse line. Tests were conducted in accordance with the Guidelines for Animal Experiments at Tokyo Dental College (No. 290104). Ten fetuses at each embryonic stage were utilized, giving a total of 40 specimens. A female mouse was housed with a male overnight, and noon of the day when the vaginal plug was observed was designated as E0.5. Fetal tissues were then fixed in 4% phosphate-buffered paraformaldehyde. Specimens were decalcified with 10% EDTA for 7 days at room temperature. Paraffin blocks were prepared using standard methods, and a series of 5-μm-thick tissue sections were cut using a sliding microtome. Frontal sections including the lateral pterygoid process and cranial base (LP&CB), CC and MP were prepared, and then stained with 0.05% toluidine blue (TB) (0.05 M phosphate buffer) for morphological observation. During the fetal period, secondary cartilages became distinguishable into three distinct portions: a fibroblastic/polymorphic tissue layer containing progenitor cells, a zone of flattened chondrocytes, and a zone of hypertrophic chondrocytes (Fig. 1).

2.1. Immunohistochemical analysis

To analyze the expression of protein in the samples, immunolocalization of type X collagen (Col X) and SRY-box 9 (Sox 9) was investigated. The slides for Col X and Sox 9 were incubated and digested with 25 mg/ml testicular hyaluronidase (Sigma Chemicals, St Louis, MO, USA) in PBS for 1 h at 37 °C. After several additional washings in PBS, both sets of sections were incubated in 3% hydrogen peroxide with methanol for 30 min. The sections were then subjected to several additional washings in PBS and incubated in 3% bovine serum albumin for 1 h to block nonspecific binding. Subsequently, the sections were treated with primary antibodies against Col X (1/400, LSL, Tokyo, Japan) and Sox 9 (1/1000, Abcam, Cambridge, UK) and incubated for 1 h at 37 °C in a moist chamber. The secondary antibody was then applied using an ABC staining kit (Funakoshi, Tokyo, Japan) at room temperature. Next, after several more washings in PBS, the sections were treated with impact DAB (Funakoshi) to detect any reaction and then inspected after counterstaining with hematoxylin.

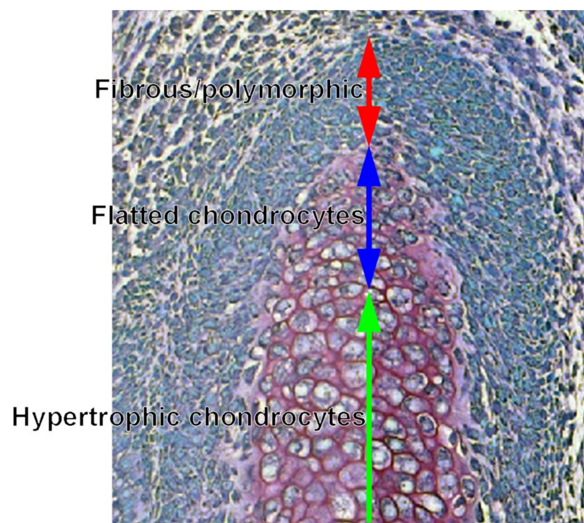


Fig. 1. Histology of the growing (prenatal) mouse mandibular condylar cartilage (CC). Frontal section, toluidine blue stain. The cell population was divided into a fibroblastic/polymorphic tissue layer containing progenitor cells, a zone of flattened chondrocytes, and a zone of hypertrophic chondrocytes based on the degree of cell differentiation at E16.5.

For confocal laser scanning microscopy analysis of hypertrophic chondrocytes, we prepared sections with a thickness of 30 μm from fetuses at E16.5. The sections were deparaffinized and washed in PBS, and then incubated and digested with 25 mg/ml testicular hyaluronidase (Sigma Chemicals) in PBS for 1 h at 37 °C. After several additional washings in PBS, the sections were incubated in 3% hydrogen peroxide with methanol for 30 min. The sections were then subjected to several additional washings in PBS and incubated in 3% bovine serum albumin for 1 h to block nonspecific binding. Subsequently, the sections were treated with primary antibody against Col X (1/400, LSL) and incubated overnight at 4 °C in a moist chamber. The secondary antibody was then applied using Alexa Fluor 488 (1/1000, Invitrogen, Carlsbad, CA) for 1 h at room temperature. Cell nuclei were counterstained with DAPI-Fluoro-KEEPER (Nakarai Tesque, Kyoto, Japan). The stained specimens were observed by confocal laser scanning microscopy using a LSM5 DUO microscope (Carl Zeiss MicroImaging Inc., Göttingen, Germany) with a 40 × /1.40 oil-immersion objective. A series of z-stack images were scanned in increments of 0.38 μm at excitation wavelengths of 489 and 532 nm. The images were analyzed using Zen 2009 maximum intensity projection (Carl Zeiss MicroImaging Inc.).

2.2. Alkaline phosphatase (ALP) staining

Sections were stained using an ALP staining kit (Primary Cell, Hokkaido, Japan) in accordance with the manufacturer's instructions. Briefly, sections were rinsed in running distilled water for 1 min, after which 50 mL of staining solution was dropped on each section. The sections were incubated at room temperature for 3 h until the ALP turned dark blue, and they were then washed in PBS and inspected after counterstaining with nuclear fast red stain.

2.3. Cross-section measurement and statistical analysis of hypertrophic chondrocytes

Ten fetuses at E16.5 were utilized for measurement of the area of hypertrophic chondrocytes in MP, CC, and LP&CB. The cross-sectional area of the Col X-positive hypertrophic chondrocytes immediately before replacement of the cartilage with calcified bone was measured in sections. Using photographs taken under a ×20 objective lens, the chondrocytes were traced manually and scanned using Adobe Illustrator CC. To calculate the cross-sectional area of the traced hypertrophic chondrocytes in the visual field, the scanned images were processed using IMAGE-PRO®PLUS (Media Cybernetics, Inc., USA). Tukey's multiple comparison test was performed after calculating the cross-sectional area of the traced hypertrophic chondrocytes. P values of < 0.05 were considered to indicate high statistical significance. Data were analyzed with the SPSS 18.0 statistical software package (SPSS, Chicago, IL).

Download English Version:

https://daneshyari.com/en/article/8696482

Download Persian Version:

https://daneshyari.com/article/8696482

Daneshyari.com