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JAGGED1 PEPTIDE APPEARING IN MANDIBULAR CONDYLAR CARTILAGE DEVELOPMENT

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Abstract
We investigated the expression pattern of Jagged1 peptide in mandibular condylar cartilage, as a type of secondary cartilage. Mandibular condyle of ddY mice were fixed from embryonic day 15 (E15) through just after birth (equivalent to E19). Serial sections were examined using histological immunohistochemical (IHC) techniques. At E15, the proliferating cells had positive products of Jagged1 in their cytoplasms and cell membrane of almost all coagulating cells. At E17, cytoplasmic and membranuous reactions of Jagged1 factors appeared strongly in the cells just inside the condylar cartilage sheath. At E18, Jagged1 positive products were observed in almost all cells of the layers, and they were mostly distinct in the sheath of the condyle. At just after birth, Jagged1 was observed in a portion of almost all layer cells in their cytoplasm and membrane. These results suggest that Jagged1 plays an essential role for mandibular condylar cartilage morphogenesis and development.

Key words: Jagged1; mandibular condylar cartilage; secondary cartilage; immunohistochemistry

INTRODUCTION

Recently we have reported that Notch1 plays an important role for mandibular condylar cartilage development, in particular that Notch1 is essential for secondary cartilage differentiation [1, 2]. Jagged1 belongs to the DSL (Delta/Serrate/LAG-2) family of ligands for Notch receptor, which control the proliferation and differentiation of various cell lingage [3].

In general, Notch signaling pathway is inducing by DSL family expression which regulates a cell proliferation and differentiation. However, there have been no reports on mandibular condylar cartilage, although there are some reports on the distribution on articular cartilage [4, 5]. Therefore, we focused on Jagged1 in the developing mouse mandibular condylar cartilage.

MATERIALS AND METHODS

ANIMAL EXPERIMENTS

A total of 10 pregnant ddY mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). The mandibular condylar cartilages were removed from the mice under anesthesia with ether. They were sampled at each of the following embryonic days: E15, E16, E17, E18 and just after birth (equivalent to E19). The Matsumoto Dental University Committee for Animal Experimentation approved the study.

HISTOLOGY

The materials were immediately fixed in 4% paraformaldehyde/0.05M phosphate-buffered solution and decalcified in 10% ethylenediamine tetraacetic acid. The materials were then dehydrated by passage through a series of ethanols and embedded in paraffin. Samples were cut at 4μm serial sections. Serial sections were then collected onto silane-coated slides and examined by histological (toluidine blue [TB] (pH 7.0) and immunohistochemistry (IHC) techniques.

IHC

For IHC, deparaffinized sections were prepared after being pretreated with 0.13% pepsin for 30min at 37 °C. Examination was carried out using a Dako EnVision+Kit-K4006 (Dako, Glostrup, Denmark) and two antibodies: anti-human Jagged1(C-20) antibody and anti-rat osteopontin. The Jagged1 polyclonal antibody (Jagged1 : 1/100) was obtained from Santa Cruse Biotechnology, Inc (Santa Cruz California, USA). The Osteopontin (OPN) monoclonal antibody was developed by Solush and Franzen[6]. It was obtained from the Developmental Studies Hybridoma Bank maintained by The University of Iowa, Department of Biological Science, Iowa City, Iowa, under contract NO1-HD-7-3236 from the National Institute of Child Health and Human Development. Samples were then counterstained with hematoxylin. OPN was used as a positive control. Immunohistochemical staining using phosphate buffered saline in place of the primary antibody was included as a negative control.

RESULTS

Histologically, at E15, mandibular condylar cartilage was clearly evident, as TB metacholomasia (Fig. 1A). At E17, perichondral ossification has already started at the periphery of the chondrocytes (Fig. 1B). At E18, endochondral ossification progressed and the mandibular condyle increased in volume (Fig. 1C).

By IHC, at E15, the proliferating cells had positive products of Jagged1 in their cytoplasms and

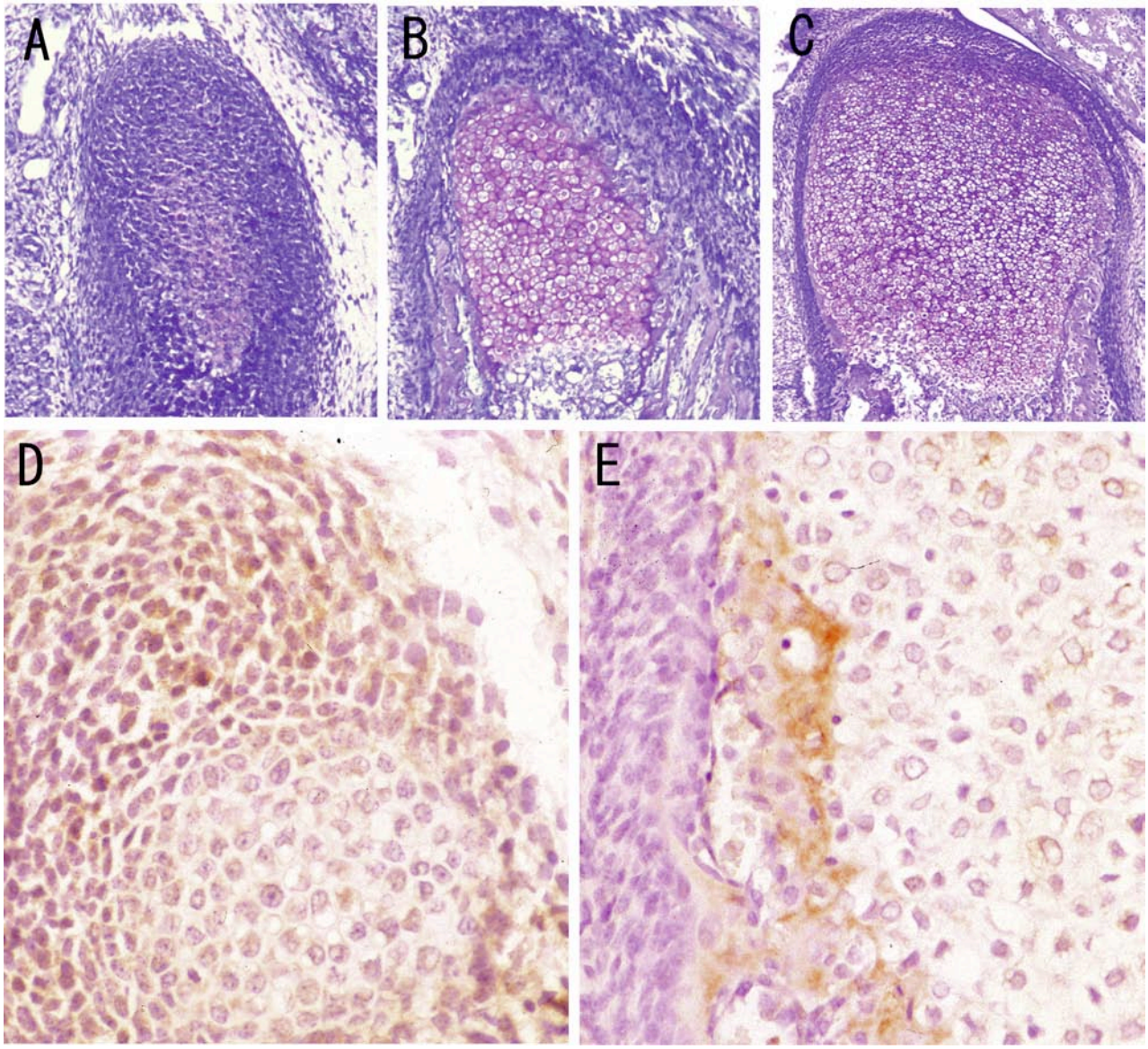

Fig. 1. Histological and immunohistochemical findings of mouse mandibular condylar cartilage. (A) Mandibular condylar cartilage is clearly evident. The middle portion shows metachromasia (E15, TB magnification x55). (B) Direct bone formation occurs (E17, TB magnification x55). (C) Mandibular condyle increases in volume (E 18, TB magnification x35). (D) Jagged1 is observed at coagulating cells membrane and cyteplasm (E15, IHC magnification x500). (E) Jagged1 factors appeared strongly in the cells just inside of the condylar cartilage sheath (E17, IHC magnification x500).

cell membrane of almost all coagulating cells (Fig. 1D). At E16, Jagged1 positive reactions were detected in cells of the proliferative, maturative and hypertrophic layers, and weakly labeled in cells of the fibrous layer. Furthermore, cytoplasmic and membranous reactions were observed in the cells just inside of the condylar cartilage sheath. At E17, cytoplasmic and membranous reactions of Jagged1 appeared strongly in the cells just inside the condylar cartilage sheath (Fig. 1E). At E18, Jagged1 immunohistochemical positive products were observed in almost all cells of the layers, and they were mostly distinct in the sheath of the condyle. At just after birth, Jagged1 peptide was observed in a portion of almost all layer cells in their cytoplasm and membrane. Through the examination period, the pattern of distribution and intensity of expression of Jagged1 peptides were not uniform.

Proliferating chondrocytes showed positive reactions to OPN-antigen through the examination periods, particularly in the cytoplasms of the proliferating chondrocytes.

Discussion

Mandibular condylar cartilage is regulated by a number of morphogenesis regulation factors and/or their signaling [1, 7-13].

Generally, Jagged1 is an important positive regulator of Notch activity. It has been reported that Jagged1 belongs to the DSL (Delta/Serrate/LAG-2) family of ligands for Notch receptor, which control the prolifer-

ation and differentiation of various cell lingage [3]. Genetic and biochemical studies have identified a number of proteins that may function to influence the activity of the Notch receptor proteins. Genetic interaction and homology screens identified the Jagged1 ligands that activate Notch receptor protein, and interaction between the ligands and their receptors have been demonstrated [14]. Disruption of Notch ligands and receptors, as well as downstream signaling components of the Notch pathway, have been implicated in many developmental defects and pathological conditions [15, 16]. Furthermore, Hayes *et al.* [4] described that Notch signalling is closely related to the formation of articular cartilage and allows for co-oriented ossification in the growth plate in mice experiments.

Concerning our recent paper [1], Notch1 expression at E14 leads to mesenchymal cells further differentiating into chondrocytes as a secondary cartilage. Furthermore, NICD translocates from the cell membrane to the nucleus, which act as a transcriptional activator and regulating gene expression through the examination period.

In this examination, expression of Jagged1 at the cell membrane might relate to cell-to-cell intercommunication through the examination period. After E16 and up to E19, Jagged1 expression appeared in hypertrophic cartilage in IHC specimens. This agrees with our past research which explained that the Notch1 and Runx2 expression of IHC and ISH is present in the hypertrophic layer and also takes part in the endochondral ossification mode [1, 9]. The results of the present study support that hypertrophic chondrocytes further differentiate into osteoblasts. After E16 up to E18, Our examination results showed the distribution of Jagged1 peptide expression at the cartilage inside of the sheath of mandibular condylar cartilage, where direct bone formation occurs. Jagged1 expression might relate to perichondral ossification in which mesemchymal cells differentiate into osteoblasts. Jagged1 was detected by means of IHC examination, which indicates that the Jagged1 expression leads to secondary chondrocyte differentiation, especially in morphogenesis during embryonic stage. Our result demonstrated that NICD and Jagged1 were both detected by IHC, and their distribution pattern was very similar. This phenomenon means Notch signaling is activated by Jagged1 ligands [1]. Furthermore, Hayes *et al.* have reported that Jagge1 peptide were absent from developing articular cartilage but were present in deeper layers from after one month [4]. Therefore, the expression patterns of mandibular condylar cartilage and articular cartilage were different. It is strongly suggested that mandibular condylar and articular cartilage differ slightly from physiological articular cartilage.

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