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GENE EXPRESSION OF JAGGED2 IN MANDIBULAR CONDYLAR CARTILAGE DEVELOPMENT

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Abstract
Expression pattern of Jagged2 gene in mandibular condylar cartilage was examined by means of *in situ* hybridization (ISH) technique. At E14, Jagged2 mRNA signals appeared in cytoplasm of proliferating chondrocytes. From E15 to E19, Jagged2 mRNA was detected throughout almost all cytoplasm in all layers. However, the distribution pattern was not uniform. These results suggest that Jagged2 plays an essential role for mandibular condylar cartilage morphogenesis and development.

Key words: Jagged2 gene; mandibular condylar cartilage; secondary cartilage; in situ hybridization

INTRODUCTION

In general, Notch-ligands interactions play important vital roles in cell fate decisions. Although there have been some reports on the distribution on articular cartilage [1, 2], there have been no reports on mandibular condylar cartilage. We have reported that Notch signaling plays an important role for mandibular condylar cartilage development. In particular, we considered that the signaling is essential for secondary cartilage differentiation [3-5]. Jagged2 belongs to the DSL (Delta/Serrate/LAG-2) family of ligands for Notch receptor, which also control the proliferation and differentiation of various cell lingage [6]. Therefore, we focused on Jagged2 mRNA in the developing mouse mandibular condylar cartilage.

MATERIALS AND METHODS

ANIMAL EXPERIMENTS

A total of 12 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: E14, E15, E16, E17, E18 and just after birth (equivalent to E19). The Matsumoto Dental University Committee for Animal Experimentation approved the study.

IN SITU HYBRIDIZATION (ISH)

The mandibular condylar cartilage was 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.

Digoxigenin (DIG)-labeled single strand RNA probes of Jagged2 and OPN were prepared using a DIG Labeling Kit (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) according to the manufacturer's instructions. Sense and anti-sense DIG-11-UTP-labeled RNA probes were constructed. For Jagged2, cDNA fragments (Jagged2 ca. 0.89 kb 2025-2915) was obtained by RT-PCR and subcloned into pCR21 (Invitrogen, Tokyo, Japan). For OPN, OPN ca. 1.2 kb was kindly provided by Associate Professor Shintaro Nomura of Osaka University School of Medicine. For ISH, the 4μm sections were deparaffinized in xylene, rehydrated in ethanol and incubated with 3mg/ml of proteinase K (Roche Diagnostic GmbH, Penzberg, Germany) in 10mM Tris-HCl (pH 8.0) and 1mM EDTA for 15min at 37 °C. Acetylation of the sections was performed by incubation with freshly-prepared 0.25% acidic anhydride in 0.1M triethanolamine-HCl buffer (pH 8.0) for 10min at room temperature. The hybridization solution contained 50% deionized formamide, 10% dextran sulfate, 1xDehardt's solution, 600mM NaCl, 0.25% SDS, 250 mg/ml of Escherichia coli tRNA (proteinases treated) 10mM dithiothreitol, and 0.1 to 2.0 mg/ml of DIG-UTP labeled RNA probe. The probe was placed on the sections and covered by parafilm and incubated at 50°C overnight in a moist chamber. After hybridization, the slides were washed with a series of SSC at 50°C and then incubated with 1.5% blocking reagent in DIG 1 buffer for 60 min. Anti-DIG-AP Fab fragment (1:800) in DIG 1 buffer was applied to the sections and incubated for 1hr at room temperature. Coloring solution containing 337.5mg/ml of nitro blue tetrazolium and 165mg/ml of 5-bromo-4-chloro-3-in-

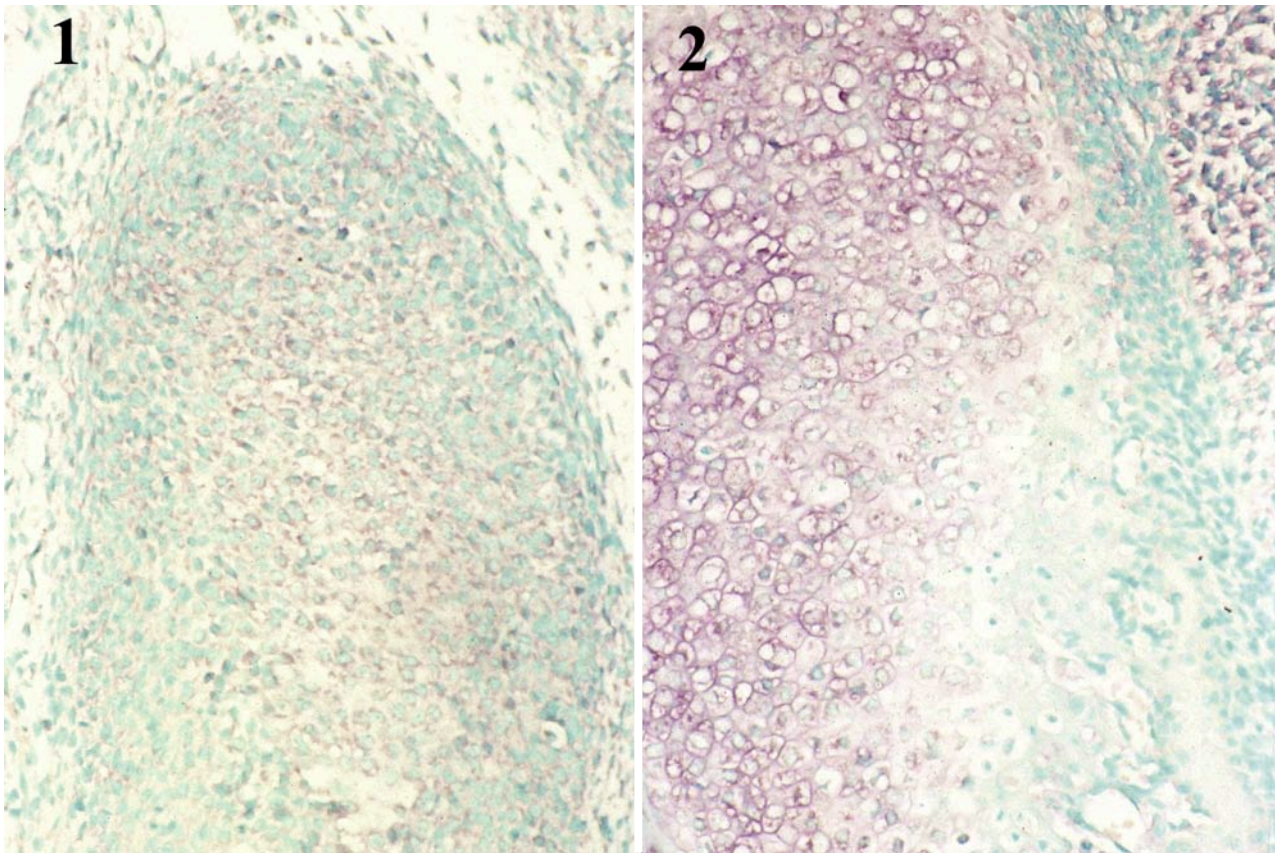

Fig.1. Proliferating chondrocytes express Jagged2 gene in their cytoplasm (E15, magnification x270).

Fig.2. Positive reactions to Jagged2 mRNA were reduced at the rim of condyle (E17, magnification x270).

dolyl phosphate in DIG 3 buffer (100mM tris-HCl, pH 9.5, 100mM NaCl, 50 mM $MgCl_2$) was mounted on the sections and incubated at 37 °C until the signal-noise ratio was maximum. The slides were mounted after counterstaining with methyl green. The negative controls included hybridization with sense (mRNA) probe. OPN was used as a positive control.

Results

At E14, expressions of Jagged2 mRNA appeared in cytoplasms of proliferating chondrocytes. Their distribution intensity was not uniform. At E15, cytoplasmic and membranous reactions of Jagged2 gene appeared in the cells of chondrocytes (Fig. 1). After E16 and up to just after birth, cytoplasmic positivities for Jagged2 mRNA were detected throughout almost all condylar cells. However, positive signals for Jagged2 mRNA were reduced at the rim of condyle (Fig. 2). Furthermore, their distribution pattern was not uniform.

OPN mRNA was detected in almost all cells of all layers at E15 and E17. Signals were localized in their cytoplasms. At just after birth, OPN signals were restricted in the cytoplasm of maturative and proliferative layers.

Discussion

There are some published data on mandibular condylar cartilage as a secondary cartilage [7-13]. In this literature, the regulation factors are known for a number of morphogenesis and/or development features, especially for onset on secondary cartilage development. [3-5].

In General Jagged2 is an important positive regulator of Notch activity. It has been reported that Jagged2 is a Notch ligand, which control the proliferation and differentiation of various cell lingage [6]. 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 [14, 15]. Notch1 and Notch ligands are expressed in the developing limbs, and Notch signaling has been implicated in the formation of a variety of tissues that comprise the limb, such as skeleton, musculature and vasculature. In particular, Notch1 signals through Jagged2 to regulate apoptosis in the apical ectodermal ridge of the developing limb bud [16]. Hayes et al. [1] discussed that Notch signalling is closely related to the articular cartilage formation and ossification in the growth plate in mice.

In this examination, expression of Jagged2 gene might relate to cell-to-cell intercommunication through the examination period. After E15 and up to just after birth, cytoplasmic positivities for Jagged2 mRNA were detected throughout almost all condylar cells. This agrees with our past research which explained that the Notch1 expression of ISH is present in the almost all layers [3]. The results of the present study support that the Jagged2 expression leads to secondary chon-

drocyte differentiation, especially in morphogenesis during embryonic stage. After E16 up to E19, our examination results showed Jagged2 gene was expressed at weak levels at the cartilage inside of the sheath of mandibular condylar cartilage. We demonstrated the Notch1 mRNA distribution pattern in our previous research [3]. Furthermore, in this examination results showed Jagged2 mRNA distribution pattern, and the patterns were very similar. This phenomenon means Notch signaling is activated by Jagged2 ligands. Notch1 and Jagged2 gene distribution intensity is not uniform, thus it is suggested that there is some relationship with mandibular condylar cartilage development.

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