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Spatio-temporal expression patterns of Wnt signaling pathway during the development of temporomandibular condylar cartilage

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ABSTRACT

There is a growing body of evidence supporting the involvement of the Wnt signaling pathway in various aspects of skeletal and joint development; however, it is unclear whether it is involved in the process of temporomandibular joint development. In order to clarify this issue, we examined the spatio-temporal distribution of mRNAs and proteins of the Wnt family during the formation of the mandibular condylar cartilage at the prenatal and postnatal stages. An in situ hybridization test revealed no mRNAs of β-catenin and Axin2 during early mesenchymal condensation; the ligands surveyed in this study (including Wnt-4, 5a, and 9a) were clearly detected at various ranges of expression, mainly in the condylar blastema and later distinct cartilaginous layers. Apart from β-catenin and Axin2, the Wnt family members surveyed in this study, including Lef-1, were found to be immunopositive during early chondrogenesis in the condylar cartilage at E14.5. After distinct chondrocyte layers were identified within the cartilage at E16.5, the expression of the Wnt signaling members was different and mainly restricted to proliferating cells and mineralized hypertrophic chondrocytes. In the adult mandibular condylar cartilage, the Wnt-4 mRNA, as well as the Wnt-4 and Wnt-9a proteins, was not observed. Our findings demonstrated that the Wnt signaling pathway was associated with the development of mandibular condylar cartilage.

1. Introduction

The diarthrodial temporomandibular joint (TMJ) is the only synovial joint in the craniofacial region. It not only serves as a site for articulation with the glenoid fossa and disc, but also acts as an engine for mandibular growth. Previous studies have correlated dysplasia of TMJ with many craniofacial malformations, asymmetry, and chondromatosis (Kengaku et al., 1998; Behrens, 2000; Gu et al., 2008). Although various regulators and signaling pathways have been shown to participate in the morphogenesis of TMJ, very little information is available regarding the underlying mechanisms.

The Wnt signaling pathway has been conserved across species (Behrens, 2000) and helps in regulating the homeostasis of different tissues during embryonic development and adult life. In previous studies, different Wnt signaling components have been detected during the early morphogenesis of the facial region in chicken and mouse embryos (Loganathan et al., 2009; Vendrell et al., 2009; Mani et al., 2010). Knockout mouse models targeting the Wnt genes have shown malformed craniofacial phenotypes (van Amerongen and Berns, 2006). Additionally, Wnt signaling has been shown to play a pivotal role in the morphogenic determination of articular cartilages and synovial joints (Akiyama et al., 2004; Guo et al., 2004). Although extensive studies have been performed on Wnt signaling, none has focused on TMJ development (Yan et al., 2014; Winkler et al., 2014; Kinsley et al., 2015). The aim of this study was to investigate the distribution of Wnt signaling molecules in the developing condylar cartilage to understand their potential roles in the morphogenesis of TMJ.

2. Results

2.1. Histological observation

Toluidine blue (TB) staining was performed to test specific proteoglycans secreted by the cartilage matrix. When proteoglycans are exposed to toluidine blue, metachromasia occurs within the cartilage matrix. Therefore, the first detection of

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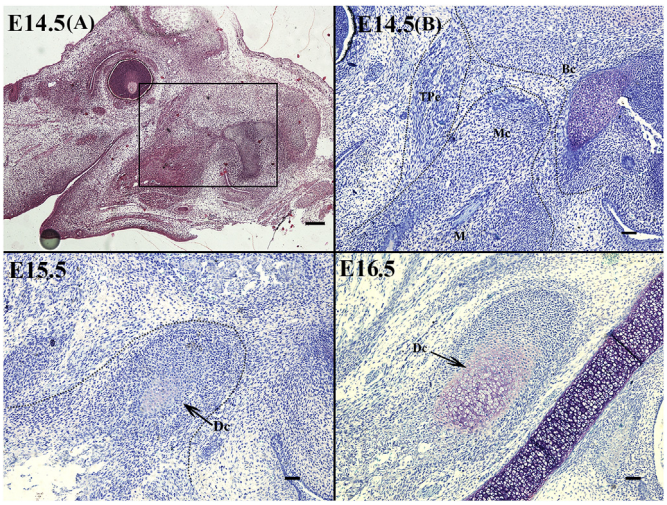

Fig. 1. Results of hematoxylin-eosin (H&E) staining and toluidine blue (TB) staining. E14.5(A): H&E staining at E14.5 (scale bar = 100 μm). E14.5(B): TB staining for the boxed part of E14.5(A). Mesenchymal cells aggregated at the distal upper portion of the developing mandible. No metacholomatic reactions to TB were observed during the mesenchymal condensation. E15.5: Condylar primordium became enlarged. TB metachromatically stained matrix was faintly visible; it punctuated the differentiated chondrocytes in the middle of mesenchymal condensation. E16.5: All major anatomical features of TMJ could be recognized, and all four layers of condylar cartilage could be distinguished. The condylar cartilage was elongated, and the differentiated chondrocytes increased in number and occupied the central part of the cartilage. (E14.5(B), E15.5, and E16.5 scale bar = 50 μm). Mc: mandibular condyle (condylar primordium); M: mandible; TPe: temporalis muscle; Bc: basis cranii; Dc: differentiated chondrocytes.

metachromatically stained matrix around cells in the anlage of the articular process was considered the initial indication of cartilage formation (Shibata et al., 1996). In a previous study, mesenchymal cells were found to aggregate at the distal upper portion of the developing mandible, and no metacholomatic reactions to toluidine blue (TB) staining were detected at E14.5. At E15.5, the condylar blastema was clearly enlarged. The TB metachromatically stained matrix was faintly visible, indicating that the condylar chondrocytes had started forming (Fukada et al., 1999). Such findings were slightly different from the results of a previous study, which showed that condylar cartilage formation started on day 14.5 of pregnancy when metachromatic TB staining was first detected (Shibata et al., 1996). The use of different mice stains or method of testing might be responsible for these different outcomes. At E16.5, layers within the condylar cartilage became distinct, and the number of hypertrophic chondrocytes increased. At E17.5, the condylar cartilage had further increased in size. From E18.5 to the post-natal stage, the condylar cartilage continuously increased in volume as the endochondral ossification progressed (Figs. 1 and 2).

2.2. In situ hybridization

The mRNAs of β-catenin and Axin2 were not observed in the mesenchymal cell aggregates at E14.5 and E15.5. At E16.5, β-catenin and Axin2 mRNAs were detected for the first time at the center of the developing condylar cartilage in the differentiated chondrocytes. The β-catenin mRNA was expressed only in differentiated cells, while the Axin2 mRNA was observed in both differentiated and peripherally undifferentiated cells. At day P0, β-catenin and Axin2 were found to be expressed in the mineralized hypertrophic chondrocytes near the subchondral bone, except in the mature chondrocytes. This expression pattern lasted for 1 week after birth at P7, but gradually became weaker in the mineralized hypertrophic layer. In the adult mandibular condyles, β-catenin and Axin2 mRNAs were expressed in the mature and calcified chondrocytes (Fig. 3a and b).

The Wnt-4, 5a, and 9a mRNAs were consistently expressed from early mesenchymal condensation. Wnt-4 mRNA was mainly found in the central mesenchymal cell at E14.5 and E15.5, while Wnt-5a and 9a mRNAs were detected in almost all coagulating mesenchymal cells. At E16.5, Wnt-4, 5a, and 9a mRNAs were detected in

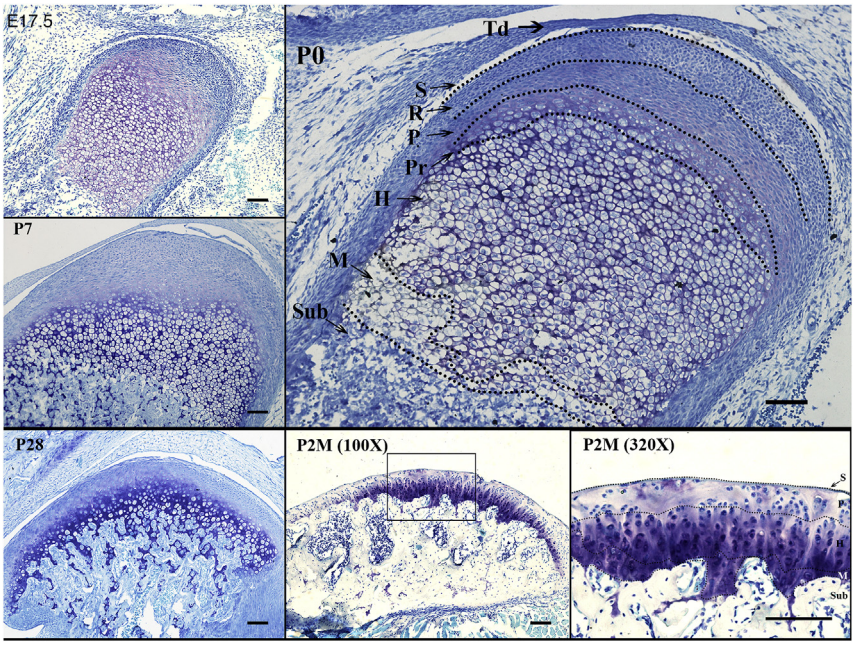

Fig. 2. E17.5: The condylar cartilage had further increased in size. P0: Different layers of developing mandibular cartilage. P7 and P28: Post-natal stage; the hypertrophic chondrocyte layer shortened in the longitudinal direction. The mandibular condyle increased in volume as the endochondral ossification progressed (scale bar = 50 μm). P2M (100 ×): Adult condylar cartilage, TB metachromatically stained was observed in mature chondrocytes (scale bar = 50 μm). P2M (320 ×): Different layers of adult condylar cartilage (scale bar = 40 μm). Td: temporomandibular joint disk; S: surface articular layer; R: resting chondrocyte layer; P: proliferative chondrocyte layer; Pr: pre-hypertrophic chondrocyte layer; H: hypertrophic chondrocyte layer (non-mineralized hypertrophic chondrocyte zone); M: mineralized hypertrophic chondrocyte layer; Sub: subchondral bone.