Original Article

Morphological Observation of Process of Mouse Temporomandibular Joint Formation

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Received 18 March, 2011/Accepted for publication 24 August, 2011

Abstract

The aim of this study was to clarify the developmental mechanism of the temporomandibular joint (TMJ) cavity, using the relationship between Meckel's cartilage and the mandible to morphologically observe the process of TMJ formation in mouse fetuses. We investigated the involvement of apoptosis in the development of the mouse TMJ cavity. We attempted to 3-dimensionally clarify the developmental process of the mandible and Meckel's cartilage by observing the developmental process optically and reconstructing 3-dimensional images to observe 3-dimensional locations of the mandible and Meckel's cartilage. Formation of the upper joint cavity began on embryonal day 16, and a complete joint cavity was formed on embryonal day 18. Formation of the lower joint cavity began on embryonal day 18, and formation was almost completed on embryonal day 19. Meckel's cartilage adjacent to the mandible decreased with development of the mandible but was vestigial on embryonal day 19. The posterior region of Meckel's cartilage developed toward the posterior direction, and it was 3-dimensionally confirmed that the mandible and Meckel's cartilage were separated. Histological observation by the TUNEL method revealed the presence of solitary and diffuse apoptotic cells not only in the joint cavity, but also around the condyle.

Key words: Temporomandibular joint—Meckel's cartilage—Development—Apoptosis

Introduction

The temporomandibular joint (TMJ) is the only synovial joint in the maxillofacial region, and it plays an important role in jaw movement. The TMJ is a bilateral joint with high flexibility, and is the only joint in the body whose end points are determined by other

hard structures, including the teeth and dental arch. Therefore, to understand not only the function and anatomy of the TMJ, but also TMJ disorders it is important to elucidate its unique structure.

Although a number of reports have investigated the developmental process of the TMJ in various animals^{7,9,11,14}, including human^{2,4,15,16},

This paper was a thesis submitted by Dr. T. Ohshima to the Graduate School of Tokyo Dental College.

no view of the mouse TMJ has been established. In particular, in mouse, although the developmental period of the lower joint cavity showed variation, development occurred after birth in many studies⁵⁻⁷. The ossification point of the mouse mandible is present in mesenchymal tissue near the lateral side of Meckel's cartilage, and mandibular development occurs upon intramembranous ossification of the point^{8,10,18}. The posterior end region of Meckel's cartilage, which bends into a hooked shape, becomes the auditory ossicles, while the rest of the cartilage disappears during the fetal period^{2,10}. The region lost during the fetal period is believed to be occupied by the mandible. However, to our knowledge, no studies to date have reported 3-dimensional observation of the developmental processes of the mandible or Meckel's cartilage. We attempted to 3-dimensionally clarify the developmental process of the mandible and Meckel's cartilage by observing the developmental process optically and reconstructing 3-dimensional images to observe 3-dimensional locations of the mandible and Meckel's cartilage.

There are 2 hypotheses regarding the mechanism of joint cavity formation in synovial joints such as the TMJ. One is the fissure hypothesis, in which mechanical stimulation dilates a fissure and forms the joint cavity^{3,6,12}, and the other is apoptosis-associated formation^{1,6,13,15}. Moreover, to our knowledge, no studies to date have clarified the developmental mechanism of the TMJ cavity. Many *in vivo* and *in vitro* studies have investigated TMJ formation, with some including an analysis of the involvement of homeobox genes^{2,9,11}. The relationship between Meckel's cartilage and the cells involved in jaw bone formation remains to be clarified. Therefore, we investigated the involvement of apoptosis in the development of the mouse TMJ cavity.

Materials and Methods

1. Animals and animal maintenance

Nulliparous female ICR mice and male

mice aged 10–15 weeks were purchased from Clea Japan Inc. and mated; the fetuses produced were used for the study. Animals were fed pellets and given tap water for drinking *ad libitum*. Animals were housed at room temperature ($23 \pm 1^\circ\text{C}$) with approximately 60% humidity and under a 12-hr dark/light cycle. The study was performed in accordance with the Guidelines for Animal Experiments at Tokyo Dental College.

After the animals were acclimatized to the environment and estrus was confirmed by vaginal smear, 2 females and 1 male were mated overnight. When a vaginal plug was noted, the mouse was judged pregnant. For the purposes of this experiment, 0:00 a.m. on the day the mouse was judged pregnant was regarded as 0 hr of embryonal day 0.

2. Observation

The pregnant mice were killed by cervical dislocation under general anesthesia with diethyl ether between 0 hr of embryonal day 15 and 0 hr of embryonal day 19, and fetuses were excised by cesarean section. The excised fetuses were judged alive based on cardiac pulsation. After confirming the absence of external malformation in the fetuses, they were killed by the method described above, the neck was cut, and the head fixed with 4% paraformaldehyde.

1) Optical observation of temporomandibular joint development

Fetuses excised between 0 hr of embryonal day 15 and 0 hr of embryonal day 19 were embedded in paraffin. Sections for paraffin embedding were cut parallel to the plane passing the bilateral external auditory meatus and posterior margin of the cervical vertebrae. Serial anterior frontal cross sections ($5\mu\text{m}$ in thickness) including Meckel's cartilage were prepared and stained with hematoxylin-eosin (H-E stain) and TdT-mediated dUTP nick end labeling (TUNEL).

2) Morphological observation by 3-dimensional imaging

H-E-stained serial sections $5\mu\text{m}$ in thickness were selected every $10\mu\text{m}$ in consideration of the major axis of the TMJ. At each

embryonal age, serial samples were prepared using 40–60 serial sections. The images of these serial samples were inputted using a microscope and a color video camera (KY-M280®, OLYMPUS CO.) attached to a 3-dimensional jaw/oral cavity reconstruction system and stereoscopic imaging reconstruction analysis system (TRI/SRF®, RATOC SYSTEM ENGINEERING CO.) and the contour traced. The contour of the cellular aggregation area in the condyle was traced. Three sets of reference points were located on the head of the mouse for preparation of sections to improve reproducibility. The 3-dimensional image was constructed from the trace data using Windows NT4.0 Workstation and the 3-dimensional reconstruction software TRI/3D-SRF® (RATOC SYSTEM ENGINEERING CO.).

Results

1. H-E preparations

Embryonal day 15: In Meckel's cartilage, cartilage-like cells with central nuclei and bright cytoplasm were arranged in a cobblestone-like pattern, but no vessels were confirmed (Meckel's cartilage in Fig. 1a). A cell proliferation site was observed above the cartilage-like cell layers containing round and oblong cells in the vicinity of cells with large nuclei and bright cytoplasm (Condyle in Figs. 1a, b). Small round cells were observed between this area and the immature woven bone above (Temporal bone in Figs. 1a, b), which was surrounded by connective tissue.

Embryonal day 16: Cells confirmed on embryonal day 15 showed dense proliferation. In the area appearing to be the condyle (Condyle in Fig. 1c), the nuclei had shrunk and the cytoplasm was basophilic in the upper area. Proliferation of round and oblong cells was observed around this area. Above this area was thin connective tissue with scarce cell components, and 4–5 layers of squamous cells (Disk in Fig. 1c); abundant cell components were covered by connective tissue. Dense cellular aggregation observed between the area

above this structure and immature bone (Temporal in Fig. 1c) had disappeared, leaving loose connective tissue.

Embryonal day 17: Cell components were scattered in connective tissue between the condyle (Condyle in Fig. 1d) and immature bone, and a space appearing to be the joint cavity was noted (Upper joint cavity in Fig. 1d). No space was observed in thin connective tissue on the condyle. Compared to embryonal day 16, no change was noted in the 4–5 layers of squamous cells (Disk in Fig. 1d) in the connective tissue above the condyle, but the junction between the cells with bright cytoplasm and surrounding round and oblong cells in the condyle became clear.

Embryonal day 18: A space considered to be the joint cavity was noted in the upper region of connective tissue between the condyle and immature bone (Upper joint cavity in Fig. 1e). Intercellular space in thin connective tissue between the 4–5 layers of squamous cells and the condyle had increased and separated, creating space (Lower joint cavity in Fig. 1e). Cells inside the space were atrophic. To investigate localization of apoptosis in the formation of the joint cavity, TUNEL was performed. TUNEL-positive cells were observed in the space appearing to be the lower cavity and around the condyle in a TUNEL preparation (Fig. 2). Solitary and diffuse TUNEL-positive cells were noted around the joint cavity.

Embryonal day 19: Four to five dense layers of squamous cells were noted between the condyle and immature bone (Upper and Lower joint cavity in Fig. 1f). A narrow space was confirmed above and below the squamous cell layers. The upper space extended outward and narrowed inward. The same finding was observed in the lower space, except the space extended inwards and downwards. Dense layers of squamous basophil cells were arranged between the upper space and bone tissue. No contents were observed in the upper or lower space.

2. 3-dimensional imaging

Embryonal day 15: A concavity was observed

Fig. 1 Micrographs showing H-E preparations of right TMJ

C: Condyle, Me: Meckel's cartilage, D: Disk, U: Upper joint cavity, T: Temporal bone, L: Lower joint cavity. On embryonal day 15, dense proliferation of round and oblong cells was observed around cells with large nuclei and bright cytoplasm (a, $\times 100$). Small round cells were observed between this area and immature woven bone above, which was surrounded by loose connective tissue (b, $\times 200$). On embryonal day 16, nuclei shrank and cytoplasm was basophilic in upper area. Above this area was thin connective tissue with scarce cell components and 4-5 layers of squamous cells with abundant cell components covering connective tissue (c, $\times 200$). On embryonal day 17, cell components were scattered in connective tissue between condyle and immature bone and space appearing to be joint cavity was noted (d, $\times 200$). On embryonal day 18, space considered to be joint cavity was noted in upper region of connective tissue between condyle and immature bone (e, $\times 200$). On embryonal 19, 4-5 dense layers of squamous cells were noted between condyle and immature bone (f, $\times 200$). Narrow space was confirmed above and below squamous cell layers.

Fig. 2 Analysis of apoptosis by TUNEL method (right TMJ)

C: Condyle, U: Upper joint cavity, T: Temporal bone, L: Lower joint cavity, D: Disk
TUNEL-positive cells were observed in space appearing to be lower cavity and around condyle in TUNEL preparation in H-E preparation of embryonal day 18. Space considered to be joint cavity was noted in upper region of connective tissue.

Fig. 3 3-dimensional jaw/oral cavity reconstruction system, stereoscopic imaging reconstruction analysis system TRI/SRF®

Blue: Meckel's cartilage, Green: Mandible, Orange: Disk.
Contour of cellular aggregation area in condyle was traced on embryonal day 15.

in the upper half of the mandible, where Meckel's cartilage was located. The mandible grew, surrounding Meckel's cartilage in the center (Fig. 3).

The width of Meckel's cartilage increased in the posterior region, where it bends into a hooked shape. The anterior margin of the mandible was regarded as the anterior end of the condyle (Fig. 4a).

Embryonal day 16: Compared to embryonal day 15, the concavity in the mandible moved downward. The head and angle of the mandible grew in the postero-upper and

postero-lower direction, respectively, around the concavity in the mandible (Fig. 4b).

Meckel's cartilage near the mandible was located slightly above the concavity in the mandible, becoming thinner and growing in the posterior direction. The hook-shaped posterior region of Meckel's cartilage increased in width. The disk was observed localized in the head of the condyle on embryonal day 16. The disk developed slightly outward.

Embryonal day 17: The head and angle of the mandible grew further postero-upward and postero-downward, respectively, around the concavity in the mandible.

Meckel's cartilage near the mandible was located in the middle of the concavity of the mandible. Compared to embryonal day 16, the width of Meckel's cartilage near the mandible decreased, and the hook-shaped posterior region increased. The disk developed surrounding the condyle (Fig. 4c).

Embryonal day 18: The head and angle of the mandible grew further postero-upward and postero-downward, respectively, around the concavity in the mandible.

Meckel's cartilage adjacent to the mandible

Fig. 4 3-dimensional stereoscopic imaging of right TMJ

On embryonal day 15, mandible grew, surrounding Meckel's cartilage in center. Width of Meckel's cartilage increased in posterior region where it bends into hooked shape (a). On embryonal day 16, head and angle of mandible grew in postero-upper and postero-lower direction, respectively, around concavity in mandible (b). On embryonal day 17, head and angle of mandible grew further postero-upward and postero-downward, respectively, around concavity in mandible (c). On embryonal day 18, head and angle of x mandible grew further postero-upward and postero-downward, respectively, around concavity in mandible. Width of Meckel's cartilage adjacent to mandible further decreased (d). On embryonal day 19, condyle developed upward around concavity in mandible. Head of condyle formed round shape. Width of Meckel's cartilage near mandible further decreased (e).

moved downward under the concavity in the mandible, and the mandible and Meckel's cartilage were separated. The width of Meckel's cartilage adjacent to the mandible further decreased, and the hook-shaped posterior region slightly increased. The disk developed surrounding the head of the condyle (Fig. 4d).

Embryonal day 19: The condyle developed upward around the concavity in the mandible. The head of the condyle formed a round shape. Little change was confirmed in mandibular angle.

No change was confirmed in the location of Meckel's cartilage near the mandible, being

located slightly below the center of the concavity in the mandible. No marked change was noted in the location of the mandible or Meckel's cartilage. The width of Meckel's cartilage near the mandible further decreased. Compared to embryonal day 18, the disk was thinner and had grown toward the exterior direction (Fig. 4e).

Discussion

Regarding the development of the upper joint cavity, Uemura¹⁷⁾ and Frommer⁷⁾ reported that the joint cavity in mouse appeared on embryonal day 16 and 19, respectively; in rat, Nagasawa¹⁴⁾ reported its appearance on embryonal day 16, while Cunat *et al.*³⁾ reported its appearance on day 19. In this study, a space appearing to be the upper joint cavity developed on embryonal day 16, and was completed on embryonal day 18.

Uemura¹⁷⁾ reported that the lower joint cavity developed on embryonal day 19 in mouse and was completed on postnatal day 2, and Frommer⁷⁾ reported that the lower joint cavity developed on embryonal day 19. Nagasawa reported that the lower joint cavity developed on embryonal day 20 and expanded on postnatal day 0 in rat¹⁴⁾. While the developmental period of the lower joint cavity showed variation among studies, development occurred after birth in many studies. However, in our study, a space appearing to be the lower joint cavity developed on embryonal day 18 and was completed on embryonal day 19, the day before birth, suggesting that the TMJ in mouse fetuses is mostly formed in the uterus. This would allow the jaw movement necessary for breast feeding immediately after birth²⁾.

There are 2 hypotheses regarding joint cavity formation. According to one hypothesis, the early joint cavity is partially formed in the region between the 2 primordial bones of the mandible, and mechanical stimulation of joint movement expands the cavity. In the other hypothesis, the joint cavity is formed by the disappearance of cells in the region between the 2 primordial bones due to cell

death. Matsuda suggested the involvement of apoptosis in the formation of the joint cavity by confirming apoptotic bodies with an electron microscope during the developmental process of the TMJ in human^{16,18)}. Solitary and diffuse TUNEL-positive cells were noted around the joint cavity on embryonal day 18. This suggests the involvement of apoptosis in the formation of the mandibular joint cavity.

Meckel's cartilage is different from other cartilages of long bone primordium in that it is a temporary structure appearing during the development of the mandible. Meckel's cartilage is not present after formation of the mandible. It does not become a primordium of the mandible, and disappears later, which is unique. To our knowledge, no studies to date have reported 3-dimensional observation of the location and developmental process of Meckel's cartilage and the mandible.

In this study, the mandible surrounded Meckel's cartilage on embryonal day 15, but the width of Meckel's cartilage near the mandible decreased with embryonal age, and it was confirmed 3-dimensionally that the mandible increased in width, occupying the entire region.

After embryonal day 16, it was revealed that the width of Meckel's cartilage near the mandible decreased, and the posterior region increased, showing development in the posterior direction. Meckel's cartilage was vestigial on embryonal day 19, suggesting that Meckel's cartilage disappears after birth.

We took a 3-dimensional approach to study the relationship between the development of the TMJ space and apoptosis in an experiment using mice. This 3-dimensional visualization enabled us to identify the backward movement of Meckel's cartilage and the separation of the mandible and Meckel's cartilage. TdT-mediated dUTP nick end labeling also enabled us to observe apoptosis not only in the joint space, but also around the mandibular condyle. To our knowledge, no studies to date have described how Meckel's cartilage disappears 3-dimensionally. The appearance of positive cells on the 18th day of fetal life demonstrated the formation of joint space as

a result of the disappearance of cells between two primitive bones due to apoptosis. Whether apoptosis plays a role in the formation of the mandibular joint cavity remains to be determined, and this hypothesis will be explored in future study.

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