The Role of miRNA-200a in The Early Stage of The Development of Primary and Secondary Cartilages in Mandible in Mice.

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（マウス下顎骨一次、二次軟骨早期発達におけるm.RNA-200aの役割）
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論　文　内　容　の　要　旨

MicroRNAs are non-coding small RNAs, which regulate various cellular functions, growth and development of various organs through interfering the translation from messenger RNAs to proteins. The present research is aimed to analyze the function of microRNA-200a (miR-200a) during the development of primary and secondary cartilage in the mandibular process. It was hypothesized that miR-200a has different regulatory effects on the ectomesenchymal cell differentiation related to the formation of both mandibular condylar and Meckel's cartilages anlagen. The hypothesis was tested through studying the effect of miR-200a on the mandibular undifferentiated ectomesenchymal cells of Meckel's cartilage as an example of primary cartilage and undifferentiated mesenchymal cells of mandibular condylar and angular cartilages as examples of secondary cartilage.

Timed pregnant ICR mice were used on gestational day (E) 9, 10, 11, 12, 13 and 14 were used in the present study to analyze the expression levels and patterns of miR-200a. The miR-200a expression analysis in the mandibular process during normal development was assessed by qRT-PCR and *in situ* hybridization. The qRT-PCR analysis showed a gradual increase of miR- 200a expression level in the mandibular process from E9 to E14. The *in situ* hybridization analysis revealed that miR-200a signal was noticed at Meckel's cartilage, mesenchymal condensation of mandibular condylar cartilage and tooth germ in E13 and 14.

Mandibular organ culture system using modified Trowel method of E10 ICR mouse was implemented. The microdissected mandibular processes were cultured for 9 days. DiI-tracing with microinjection in the cultured mandibular process was performed to determine the injection site that targets the initial ectomesenchymal condensation of condylar and Meckel's cartilages anlagen. DiI labeling indicated that the

initial location of cell aggregation for Meckel's cartilage is located in the middle anterior part of mandibular process, while the site of the mandibular condylar cartilage is located in anterior lateral part.

Using microinjection and electroporation, miR-200a mimic was transfected in the determined sites of the mandibular explant on the first day of culture. Control small interfering RNA (siRNA) was also transfected as a negative control. After nine days culture, Alcian blue analyasis was performed to evaluate the effect of miR-200a on the formation of condylar and Meckel's cartilages. The transfection of miR-200a mimic decreased the formation of condylar cartilage, while no effect was noticed for Meckel's cartilage.

In conclusion, these results imply that miR-200a could negatively regulate the formation of secondary cartilage during the early mandibular development. miR-200a could have different role between the development of the mandibular primary and secondary cartilages.