学 位 論 文 の 要 旨

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学 位 論 文 名　Histomorphometric Analysis of the Epithelial Lumen, Mesenchyme, Smooth Muscle Cell Layers, and Mesentery of the Mouse Developing Duodenum in Relation With the Macroscopic Morphogenesis

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論 文 内 容 の 要 旨

INTRODUCTION

Vertebrate embryonic guts consist of the endodermal epithelium, the mesenchyme from splanchnic mesoderm, and the later joining neuronal component. The gut is initially a simple tube when it starts formation in mice at around E10.5, but during development it undergoes differential morphological and histological changes, including elongation, looping, and rotation, and forms the mature gastrointestinal tract with different organs including the stomach, duodenum, and jejunum. The primitive gut tube is connected with the dorsal body wall by the dorsal mesentery, through which blood and nerve supplies are delivered to the developing gut. It has been reported that the superior mesenteric artery along with its branches in the dorsal mesentery contributes to the formation of the midgut and is considered a very stable landmark. In the present study, we found that the duodenal epithelial lumen at E13.5 was oval rather than round, whereas the contour of the section of the entire duodenum was round, indicating the oval lumen was not due to an artifact. Previous studies also have suggested that the changes in epithelial cell shape and mesenchymal cell density in the dorsal mesentery may regulate the developing midgut for subsequent gut looping and rotation. Given the oval lumen shape, it may be possible that some form of mechanical stress generated among the luminal epithelium, mesenchyme, or smooth muscle cell (SMC) layers affected the overall “C”-shaped loop of the duodenum. The elongation and clustering of the mesenchymal cells is the first sign of their differentiation into SMCs. We recently reported that there are regional differences in the

development of the orientation of the SMC layers in relation to the mesentery which strongly suggest the essential role of the possibly regionally differential interaction between the component layers as well as the essential role of the relation between the components and the mesentery in the regionally differential macroscopic morphogenesis of the gut. In the present study, we observed the detailed morphological and histological changes in the epithelial lumen along with the mesentery and surrounding mesenchyme at E13.5. We focused on the duodenum, since it is characterized by a stable and reproducible "C"-shaped loop in the proximal part of the duodenum both in humans and in mice. Thus the duodenum is suitable for analyzing the relation among the components of the wall and their relation to the mesentery, and their possible roles in the macroscopic morphogenesis.

MATERIALS AND METHODS

C57BL/6J mice (total number of mouse, n = 14) (CLEA Japan, Tokyo) between 8 and 20 weeks of age were used. Dams were sacrificed under deep anesthesia at E13.5 (n = 8), when the "C" loop of the proximal duodenum is established, as well as at E15.5 (n = 3) and E17.5 (n = 3). The embryos were collected and kept at 4°C in physiological saline. The entire digestive tube of the embryo was very carefully dissected out and straightened by removal of the adjacent mesentery under a dissection microscope (n = 5) for histomorphometric and immunohistochemical analysis. Histomorphometric analysis was done by 4 procedure; 1. Measurement of the epithelial lumen angle and orientation against the mesentery. 2. Measurement of the mesenchymal cell density. 3. Measurement of the epithelial nuclear shape ratio. 4. Number of SMC layers and arrangements against the mesentery.

All experiments with animal in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

Measurement of the intestinal lumen angle against the mesentery axis at E13.5: In the present study, we observed the detailed morphological and histological changes in the epithelial lumen along with the mesentery and surrounding mesenchyme at E13.5. We observed that the epithelial oval lumen against the mesentery exhibited a general clockwise rotation along the long axis of the proximal duodenum at E13.5. Since this luminal rotation in the proximal duodenum was reproducible both in the intact (unstraightened) and straightened samples, it was not due to an artifact. On the other hand, while the angle was ranged from 180° to 600° in the distal duodenum including the duodenojejunal flexure (DJF), the angle at the distal part remains constant between 180° and 300° except for sample 2 in which the angle increased up to 600°. Thus, the involvement of the luminal angle, if any, may be different between the "C" loop and

DJF, and various mechanisms may play different roles in the regionally specific loop/rotation. The epithelial cell shape and mesenchymal cell density change along the oval lumen: We found that the mesenchymal cell density was higher in the area along the short axis of the oval lumen than in the area along the long axis, and the epithelial cell nuclei shape (cell shape) changes correspondingly to become longer and thinner in the area at the long axis and shorter and broader in the area at the short axis, respectively. These findings suggest an interaction between the epithelium and the surrounding mesenchyme. Thus the difference in the density of the mesenchymal cells may affect and regulate the shape and height of epithelial cells/nuclei and cause the oval luminal shape. Since the oval epithelial lumen rotates along the long axis of the proximal duodenum, the epithelial shape and mesenchymal density should also change/rotate correspondingly along the long axis. How this is made possible, including whether or not the mesentery plays a role, and whether the primary event occurs in the epithelium, the mesenchyme, or somewhere else, are among the many unanswered questions that warrant further molecular and experimental analyses. Number of SMC layers and arrangements against the mesentery: In the study, at E13.5 we observed that the duodenum inner circular layer (IC) was irregularly arranged in 2-3 (average 2.26) SMC layers. Interestingly, at E15.5 mostly arranged in 2 (average 2.11) SMC layers, and at E17.5 the number of SMC layers in the IC was 3-4 (average 3.17), indicating that there is a slight and transient decrease in the number of SMC layers at E15.5. The outer longitudinal layer (OL) first appeared at E15.5 and continued to consist of only one SMC layer at E17.5. At E13.5, E15.5 and E17.5, IC arrangement showed a regional difference against the mesentery position, but there was no clear regional difference along the long axis of the duodenum in either IC or OL. Therefore, it is unlikely that the SMC layers are directly involved in the luminal winding along the long axis of the duodenum. Previous studies have reported that at E13.5 mesenchymal cells undergo differentiation and form a separate layer of αSMA-expressing circular cells, thereby initiating the IC, and after 48 hours the OL develops by involving the signaling molecules and secondary induction of the peripheral mesenchyme. The actual origin of the OL layers is still not completely understood, but these chronological changes suggest that the SMC in OL is not the progeny of the SMC in IC but is formed de novo from the outer mesenchymal cell.

CONCLUSION

In conclusion, the present findings suggest that rotation of the oval epithelial lumen based on the epithelial-mesenchymal interaction in the surrounding SMC layers without change along the long axis may be involved in the formation of the “C” loop in the proximal duodenum, and raise a novel question as to whether or not a similar mechanism may be involved in the other regionally specific looping/rotation patterns of the gut tube.