学 位 論 文 審 査 の 要 旨

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学 位 論 文 題 名

Study on three-dimensional deformation of epithelial sheets
in lumen and dome morphogenesis with *in vitro* cell culture model
（インビトロ細胞培養実験系を用いた管腔形成及びドーム形成における
上皮細胞シートの三次元変形に関する研究）

博士学位論文審査等の結果について（報告）

In development, cells form complicated three-dimensional (3D) shapes. It has been a long-standing question that how the cells develop the 3D shapes accurately. Recent studies have demonstrated that the deformation of epithelial cell sheet plays important roles in the shape forming (morphogenesis). *In vitro* epithelial sheets are reported to deform for lumen and dome formation, however, the factors to lead the deformation have not been fully explained yet. The aim of this study is to explore the epithelial sheet deformation in lumen and dome morphogenesis with *in vitro* experiments.

Lumen formation is an important event in morphogenesis; however, an unanswered question is whether it involves the collective cell migration which is one of the important cues to cause the morphogenesis. Here, with the use of collagen gel overlay culture method, we show that Madin-Darby canine kidney cells (MDCK cells) collectively migrated and formed a luminal structure in a collagen gel. Immediately after the collagen gel overlay, MDCK sheets folded from the periphery, migrated inwardly and formed a luminal structure. The inhibition of integrin-β1 or Rac1 activity delayed the folding for the lumen formation. Moreover, the lumen formation was prevented by the disruption of apical-basolateral polarity induced by transforming growth factor-β1. These results indicate that cell migration and cell polarity play an important role in the folding of MDCK cell sheet for the lumen formation.

Dome formation is also one of the fundamental processes of morphogenesis, but many parts of the mechanisms have been unveiled. Previous *in vitro* studies showed that osmotic gradient is the driving factor of the dome formation. However, these investigations were performed without extracellular matrix (ECM), which supports the 3D structures in morphogenesis. With the use of ECM, we observed that basal hypertonic stress induced stable dome structures *in vitro* that have not been seen in previous studies. The stable domes appeared simultaneously with the bending of flat MDCK sheets. These domes appeared with the ECM swelling induced by aquaporin water transport activity. Uneven water transport occurs in cell

height dependent manner, leading the local ECM swelling. The local ECM swelling stretched the cells. The positive feedback between extending cell and enhanced water transport resulted in dome formation. These results indicate that osmotic gradient induces dome-shaped deformation to MDCK sheets via both enhanced water transport activity and subsequent ECM swelling.

In conclusion, the author has new findings that deformation of epithelial sheet played a key role in both lumen and dome morphogenesis with *in vitro* experimental systems. The lumen formation after gel overlay showed collective cell migration with the folding of epithelial sheets. And the osmotic gradient induced the appearance of domes with bending of flat epithelial sheet. These findings will contribute to gain the importance of sheet deformation during 3D shape morphogenesis.

Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Life Science from Hokkaido University.