学位論文抄録

The study on the morphology of embryonic stem cell-derived endothelial cells which is regulated by Foxo1

（Foxo1 によって調節される胚性幹細胞由来内皮細胞の形態に関する研究）

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Abstract of the Thesis

Background and Purpose: The forkhead transcription factors, Foxos participate in both embryonic and adult angiogenesis. The Foxo subgroups regulate the correct organization of the vascular system. One of them, Foxo1 is an important physiological regulator of endothelial morphology in response to VEGF. However, the molecular mechanisms involved in Foxo1-regulated vasculature development are largely unknown. In this study, to elucidate the cellular function of Foxo1, we used a three-dimensional culture system for the differentiation of Flk-1 expressing mesodermal precursor cells derived from ES cells to cord forming endothelial cells and associating vascular smooth muscle cells.

Methods: *Foxo1 (+/+)* or *Foxo1 (-/-)* ES cells were cultured with OP9 stromal cells for 4 days to induce differentiation of $Flk1^+$ mesodermal cells. $Flk1^+$ cells were purified by FACS and allowed to aggregate in suspension culture. $Flk1^+$ cell aggregates were embedded in Type I collagen gel and cultured for 4 days in the presence of 50ng/mL VEGF-A. Dome-like gels were allowed to flatten by liquid adsorption and subjected to immunostaining.

Results: While *Foxo1 (+/+)* endothelial cells organized into long vessel-like structures associated with smooth muscle cells, *Foxo1 (-/-)* endothelial cells could form only short sprouts. *Foxo1 (-/-)* endothelial cells have punctate accumulation of filamentous actin, thick circumferential bundles of microtubules with small spikes at the tip of cells, and no interaction with smooth muscle cells. Our results suggest the involvement of Foxo1 in cytoskeletal remodeling of endothelial cells and recruitment of smooth muscle cells during vascular development.

Conclusions: The forkhead box transcription factor Foxo1 is essential for the formation of ES cell-derived vessel like structures consisting of extending endothelial tubes supported by smooth muscle cells.