# Department of Anatomy (Histology and Embryology)

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## General Summary

Our group is interested in the developmental and evolutional aspects of human body structure. By comparing organ development among vertebrates, we are attempting to reconstitute the evolutionary path that each of our organs has taken, at both the molecular and morphological levels, thus identifying fundamental molecular mechanisms that shape each organ.

## Research Activities

*Mucosal vascular networks in the mouse distal colon*

We have previously demonstrated that leakage of plasma protein and bleeding in the lamina propria in the distal colon occur as primary signs in dextran sulfate sodium (DSS)-induced colitis and have suggested that disturbances and disruptions of colonic circulation, including tissue microcirculation, were involved in the pathogenesis of colitis. However, vascular networks in colonic mucosa have not been studied in detail.

In this study, we have attempted to investigate vascular networks in the mouse colonic mucosa by injecting fluorochrome-labelled gelatin into blood vessels and observing 3-dimensionally the whole mount specimen of the distal colon with a confocal laser scanning microscope. The arterial plexus was observed in the submucosa. Arterioles branched off from the plexus pierced the muscularis mucosa and entered the lamina propria. In the deep part of the lamina propria, the arterioles ramified and anastomosed each other to form a vascular plexus around the base of crypts. Some branches from the plexus run around the crypt or upwards among crypts to enter the subepithelial capillary networks. The subepithelial capillary networks were formed by interconnections of hexagonal capillary rings. Venules emerged from the capillary networks run downward and horizontally at the deep part of the lamina propria, where they received venules from the surroundings, and then pierced the muscularis mucosa to pour into the venous plexus in the submucosa.

There were no reports concerning the arterial plexus and venules in the deep part of the lamina propria and no attention has been paid to them. However, the previous findings that mucosal bleeding in DSS colitis originated at blood vessels running in the deep of the lamina propria and that disturbances in microcirculation in the mucosa occurred prior to the disorganization of the mucosal tissue architecture indicate that vasculatures in the deep part of the lamina propria play a pivotal role in maintaining colonic mucosa. A detailed investigation of these vasculatures may contribute to prevent the DSS induced colitis, a model of the inflammatory bowel disease.

*Regeneration of epidermis basal lamina during posterior lateral line development in* Polypterus

The genus *Polypterus* is the most basal extant actinopterygian fish in molecular phylogeny because of scales covered with dentin and enamel. We focused on the development of the neuromast closely related to the lateral line scale during posterior lateral line development in *Polypterus* to investigate an origin of diversity of the lateral line in bony fishes and found that epidermis basal lamina is regenerated during posterior lateral line development in *Polypterus*.

Initial neuromast cells appeared as the cranial placodes in the neurula and migrated to the caudal side within the lower epidermal layer adjacent to the horizontal septum in the larva. The cell population migrated, depositing a set of neuromast cells to form a rosette-like structure, and finally reached the caudal fin while repeating this process. The basal lamina, as shown with periodic acid-methenamine silver staining and scanning electron microscopy, did not exist just underneath the neuromast, and neurites from the neuron bundle stained with neuron-specific antibodies innervated the neuromast cells within the epidermis. The neuron bundle away from the neuromast was clearly seen underneath the basal lamina, and continuity with the cranial ganglion, suggested that it was the lateral line nerve. Therefore it suggested that the lateral nerve bundles extending from the cranial nerve was innervated to the neuromast cells outside the basal lamina, and that the basal lamina just below the neuromast was regenerated outside the nerve bundles during posterior lateral line development.

*Functional analysis of mouse Glial cell missing 1 gene in kidney*

The glial cell missing (GCM) is a transcription factor conserved from invertebrates to vertebrates and is known to be important for placenta formation in mammals. Deficiency of the glial cell missing 1 (*Gcm1*) in mice causes placental hypoplasia, which is lethal at embryonic day 10. Although *Gcm1* has been reported to be expressed in the kidney, its function remains unclear. We constructed a flox mouse, in which the DNA binding sequence of *Gcm1* was sandwiched between loxP, and crossed it with a mouse in which Wilms tumor 1 (*WT1*)-*Cre* is specifically expressed in the kidney to analyze the kidney of the *Gcm1* conditional knockout mouse. We revealed that *Gcm1* deficiency did not affect renal development and that renal size and function did not differ even after maturation. However, we clarified that fibrosis was significantly reduced in *Gcm1*-deficient kidneys compared with control kidneys when ischemic injury was performed. In addition, we found that the expression of *Tgf-β1*, which is reported to be involved in fibrosis, is decreased in the *Gcm1*-deficient kidney, we finding that suggests that Gcm1 controls the expression of *Tgf-β1* directly or indirectly. We also revealed that cell proliferation was reduced in *Gcm1*-deficient kidney. The analysis of cultured cells showed that *Gcm1* increased the expression of *Tgf-β1*, which might promote cell proliferation. With these experiments, we revealed that *Gcm1* is involved in cell proliferation and fibrosis in renal ischemic injury. This result suggests that controlling *Gcm1* might prevent fibrosis in chronic kidney disease, leading to important results applicable to the treatment of future renal diseases.

*Functional analysis of tenascin C in the induction of DSS enteritis*

Ulcerative colitis (UC) is a diffuse nonspecific inflammation of the large intestine, and abnormalities in intestinal mucosal barrier function are thought to be involved in the disease. Mucosal epithelial cells maintain homeostasis by interacting with stromal cells and the extracellular matrix. We believed that to elucidate the intestinal mucosal barrier mechanism, the extracellular matrix supporting mucosal epithelial and interstitial cells should be analyzed. Therefore, we focus on the extracellular matrix glycoprotein tenascin C (TNC), analyze its relationship with mucosal epithelial damage when intestinal inflammation is induced, and attempt to verify its involvement in the intestinal mucosal barrier mechanism. In this study, we used the DSS-induced colitis mouse, which is a frequently used mouse model of UC, to observe TNC expression during the induction of colitis by immunohistochemical staining. We found that in the normal large intestinal mucosa, TNC is expressed around microvessels in the lamina propria just below the mucosal epithelium and that the distribution of TNC expression changes from the superficial to the deep lamina as inflammation progresses. These findings suggest that TNC functions to suppress inflammation. Currently, the distribution of TNC expression in human UC samples is being verified by immunohistochemical staining. Based on these data, we have clarified the relationships of epithelial cells, stromal tissues, and extracellular matrix and elucidated the homeostatic maintenance mechanism of colonic mucosa.

*Organ size regulation in the lives of zebrafish*

The caudal fin of zebrafish develops in a fan-like shape, grows in a different (bi-lobed) shape during juvenile stages, and becomes larger throughout adult stages. To investigate the mechanisms of fin shape regulation, we measured bone lengths of caudal fins and standard body lengths (from the tip of the mouth to tail vertebrae) at several growing periods. We found the growth-changing point: there was positive-allometric growth until an early juvenile stage (approximately 7.0 mm standard length), and isometric growth occurred after the 7.0 mm standard length stage. To analyze messenger RNAs and microRNAs expression we collected fin tissues around the point of time when growth patterns changed and found that the muscle segment homeobox gene (*msxb*) and 2 microRNAs were highly expressed molecules at an allometric growth stage and that the TTK family protein kinase gene (*mps1*) and 2 other microRNAs were highly expressed molecules at an isometric growth stage.

*The role of chorion-specific transcription factor* GCM1*in* Polypterus

Glial cells missing 1 (GCM1) is a transcription factor that is required for development of the trophoblast cells of chorion in mammals. GCM1 is a remarkable trigger for placental evolution, but the functions and spatial expression patterns of *Gcm1* in other vertebrae is unknown. We recently found that glial cells missing 1 gene (*Gcm1*) is conserved in the genome of the extant actinopterygian fish, *Polypterus*. This finding suggests that the origin of cells expressing *Gcm1* go back from the early branched group of ray-finned fishes to mammals. Therefore, we investigated the gene expression of *Polypterus* by whole-mount *in situ* hybridization with a *Gcm1* RNA probe. We found that *Gcm1* is expressed in scattered cells in the skin of external gills and in the yolk sac membrane. We also revealed

with transmission electron microscopy that these cells contain characteristic large vacuoles in the cytoplasm. These new findings suggest that cells expressing *Gcm1* might be ionocytes, which are present in most fishes to maintain body fluid ionic and osmotic homeostasis. Further analyses, such as mass spectrometry, will reveal the function of *Gcm1*-expressing cells in *Polypterus*.

## Publications

***Hirasaki Y, Seino Y, Okabe M.*** The "Handmade" Heart Model as a Learning Tool to Facilitate Understanding of the 3-Dimensional Cardiac Anatomy. *J Cardiothorac Vasc Anesth.* 2019 May; **33**(5): 1483-1485. doi: 10.1053/j.jvca.2019.01.031. Epub 2019 Jan 11. PubMed PMID: 30737121.

***Kamejima S, Tatsumi N, Anraku A, Suzuki H, Ohkido I, Yokoo T, Okabe M.*** Gcm1 is involved in cell proliferation and fibrosis during kidney regeneration after ischemia-reperfusion injury. *Sci Rep.* 2019 May 27; **9**(1): 7883. doi: 10.1038/s41598-019-44161-y. PubMed PMID: 31133638; PubMed Central PMCID: PMC6536531.

***Shono T, Thiery AP, Cooper RL, Kurokawa D, Britz R, Okabe M, Fraser GJ.*** Evolution and Developmental Diversity of Skin Spines in Pufferfishes. *iScience.* 2019 Sep 27; **19**: 1248-1259. doi: 10.1016/j.isci.2019.06.003. Epub 2019 Jul 25. PubMed PMID: 31353167; PubMed Central PMCID: PMC6831732.