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General Summary

Our group are interested in the developmental and evolutional aspects of human body structure. By comparing organ development in 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

Production of a congenic strain of the congenital ataxic mouse line

We have attempted to produce a congenic strain of the congenital ataxic mouse line. The ataxic mouse line was originally developed in a closed colony of ICR. A heterogenic ICR male mouse was backcrossed with a female of C57BL/6J and pups of the 1st generation were obtained. Each male pup was screened for genotypic heterogenicity. The genotypically heterogenic male was crossed with a heterogenic female ICR. If phenotypically ataxic animals were found in their pups, the genetically heterogenic male was regarded as heterogenic. By repeating these processes, the 10th heterogenic male was obtained. Since the genotyping was based on a neighborhood locus of the responsible gene of ataxia, genotypically heterogenic but normal males were found in the processes.

Reanalysis of Single-Cell RNA-seq Data from Intestinal Epithelium

Recently, it is possible to obtain genome-wide transcriptome *data* from single cells using high-throughput sequencing (*scRNA-seq*). The scRNA-seq is an indispensable tool for dissecting the cellular heterogeneity and decomposing tissues into cell types and/or states. Although an entire *scRNA-seq* data is too large to be published in the paper, it is obligatory to disclose the data on the public database, such as Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). We can reanalyze such data ourselves for exploring novel knowledges.

In this year, we attempted to reanalyze the scRNA-seq data derived from 672 cells of mouse intestinal epithelium, (GEO accession #GSE76408). We used RaceID3_StemID2 (https://github.com/dgrun/RaceID3_StemID2), R program developed by Grün D et al. for clustering and for exploring both rare cells and stem cells in scRNA-seq data. The 672 cells were partitioned into 18 clusters. Especially, 6 of them were grouped as enteroendcrine-related clusters. The cluster containing the cells expressing Neurog3 and Smarcd2 was probably thought as an early enteroendcrine progenitors. After passing through the cells expressing Nkx2,2 and Neurod1 and further the cells expressing Isl and

Arx, the early enteroendcrine progenitors might differentiated into the late enteroendcrine progenitors expressing Ghrelin.

Regeneration of basal lamina during posterior lateral line development
Polypterus, the most basal extant actinopterygian fish in molecular phylogeny, has a superficial type of posterior lateral line neuromast in the epidermis covering body scales. We this time focused on development of the posterior lateral line during cell migration.
An epidermal cell population that constitutes the lateral line neuromast migrated from a head region to the caudal side within the lower epidermal layer adjacent the horizontal septum in the larva. This cell population migrated, depositing a set of neuromast cells in the epidermal layer, and it finally reached the caudal fin while repeating this process. During the process, a cell-less region was observed at first outside of the basal lamina, and it gradually became located to the inside of it. The cell-less region was positive with anti-neurofilament associated protein antibody, and it was supported by a result of MALDI-TOF mass spectrometry analysis of the region. Thus, a temporary destruction of the basal lamina occurred during posterior lateral line migration, which is because the afferent cranial nerve fibers contacted the neuromast in the epidermis, resulted in formation of the lateral line nerve in the dermis after regeneration ended.

Varidation of GONAD method
In recent years, Genome-editing using CRISPR / Cas9 became possible, and along with that, many new experimental method were developed. Among them, GONAD method (Takahashi et al. Sci Rep. 2015; 5: 11406) made it possible to produce genome-edited mice quickly with less cost, more conveniently. Therefore, our laboratory also verified the GONAD method using the guide RNA of Fgf10. As a result, it was possible to obtain limbs defect similar to the report. We are planning to produce knock-in mouse by CRISPR / Cas 9 in the future.

The analysis of the influence of the microenvironmental changes on intestinal inflammation in Dextran sulfate sodium-induced colitis
Inflammatory bowel disease is typified by ulcerative colitis and Crohn disease, and it repeats relapse and remission. In a past study, there are various examinations about the mucosal epithelium disorder, but there is not the report regarding the relationship between the change of the microcirculation and the mucosal epithelium disorder in a mucosa of a colon. To clarify involvement of the microcirculation in the enteritis onset, we analyzed the mucosal circulation system and changes in the microenvironment during the onset of enteritis using DSS (Dextran sulfate sodium)-induced colitis mice used as a model mouse of UC. As a result, microangiopathy to precede the epithelium disorder appeared mainly on the descending colon located in the distal part from superior and inferior mesenteric arteries. And then, expression of iNOS which caused a tissue disorder by overexpression increased in nerve cells of the Auerbach's plexus.
Therefore, as to DSS-induced colitis, it was suggested that the epithelium damage was caused by the microcirculation disorder due to the vascular damage, and iNOS derived from nerve cells aggravated enteritis. Particularly, the vascular damage occurred at the

descending colon which was poor of the bloodstream anatomically, so it was looked like ischemic colitis of the person. We think that the attention of the microenvironmental changes enables early detection of inflammation, and it helps new clarification of pathology in ulcerative colitis of person.

Appendage skeletogenesis in zebrafish (Danio rerio)

Zebrafish is a popular model organism in vertebrates; an embryo finishes to form most organs within five days and adult organs (fins, heart and central nerves system) regenerate morphological and functional organization of these tissues after traumas.

In adult, a fin ray joint is formed between dermal bones. We have done the SBF-SEM (Serial Block-Face SEM) analysis to confirm three-dimensional organization of the fin ray joint and found that the fin ray joint was covered with joint cells often projecting many cellular processes into collagen fibers of joint ligaments. We are planning to examine morphological and molecular differences between fin ray joints and mammalian joints (diarthrosises and synarthrosises).

Publications

Noda M, Miyake T, Okabe M. Development of cranial muscles in the actinopterygian fish Senegal bichir, Polypterus senegalus Cuvier, 1829. *J Morphol.* 2017; **278:** 450-63.

Hashimoto H, Kawabe T, Fukuda T, Kusakabe M. A Novel Ataxic Mutant Mouse Line Having Sensory Neuropathy Shows Heavy Iron Deposition in Kidney. *Neurodegener Dis.* 2017; **17:** 181-98.