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General Summary

Our group is 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

Effect of buffer composition in fixative on immunogenicity
Antibodies do not always show immunopositive reactions on a tissue section prepared from a tissue fixed with 4% paraformaldehyde (PFA) in sodium phosphate buffer (SPB). In this situation, some pretreatment to improve the penetration of the antibodies or retrieve to the antigenicity might be attempted. Moreover, a fixative free of PFA might be challenged. In this study, we have attempted the immunostaining on a tissue fixed with 4% PFA in HEPES buffer and obtained immunopositive reactions with antibodies that had shown no immunoreaction when fixed with 4% PFA in SPB. These antibodies were directed against Ca-binding proteins in the nervous system and membrane proteins. Phosphate ion in 4% PFA in SPB might precipitate in a tissue by binding with calcium ion, and the precipitate might become an obstacle for antibody penetration. Alternatively, structural changes in 3-dimensional conformation of a protein, which might be induced by deprivation of calcium ion by phosphate ion in 4% PFA in SPB, resulted in a loss of antigenicity. Although not all antibodies show immunopositive reactions on tissue fixed with 4% PFA in HEPES, this fixative deserves consideration for some antibodies.

Regeneration of basal lamina associated with neuromast formation during posterior lateral line development in Polypterus
The genus *Polypterus*, the most basal extant actinopterygian fish in molecular phylogeny, is reminiscent of coelacanth because of scales covered by 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*.
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 with periodic acid-methenamine silver staining, also obvious with scanning elec-

tron microscopy, did not exist just underneath the neuromast, and neurites from the neuron bundle stained with neuron-specific antibodies innervated to the neuromast cells within the epidermis. The neuron bundle away from the neuromast was clearly seen underneath the basal lamina and was continuous to the cranial ganglion, suggested as the lateral line nerve. The lateral line nerve thus innervated to the neuromast cells within the epidermis outside the basal lamina, which was regenerated, where is not directly below the neuromast during posterior lateral line development.

Functional analysis of mouse glial cells missing family genes

Mammals have 2 members of the glial cells missing (Gcm) family of transcription factors — Gcm1 and Gcm2 — which are expressed in different regions and play important roles in developmental regions. The gene glial cell missing transcription factor 1 (*Gcm1*) is expressed in mice during placental development, and a deficiency of this gene in mice causes placental hypoplasia and embryonic lethality. The *Gcm2* gene is expressed in the parathyroid developing area, and deficient mice die soon after birth owing to calcium dysregulation. Because deletions of the Gcm family are lethal, to analyze the function of the postnatal Gcm family, a conditional deletion method is essential. We constructed mice in which the DNA binding region was sandwiched between LoxP sequences for functional analysis in Gcm1 and Gcm2 and performed functional analysis of these mice by crossing them with region-specific and time-specific Cre mice.

1. Mouse *Gcm1* has been shown to be expressed in the kidney and the placenta, and in-situ hybridization has confirmed its expression in the renal tubular area of the kidney. Therefore, to clarify the function of Gcm1 in the kidney, we used a mouse (*Wt1-Cre* × *Gcm1*$^{Flox/Flox}$) specifically deficient in *Gcm1* in the metanephric mesenchyme from which the renal tubule was derived. We found that the *Gcm1*-deficient kidney showed no change in size, shape, or function, but when ischemic injury was given, *Gcm1*-deficient kidney showed reduced cell proliferation and fibrosis marker genes. These results suggest that the function of Gcm1 in the kidney is involved in the recovery from ischemic injury.

2. Mouse *Gcm2* is expressed in the parathyroid gland throughout life; this gene is involved in the development of the parathyroid gland developmental stage, but its postnatal function is unknown. Therefore, to clarify the Gcm2 parathyroid function in adult mice, we performed tamoxifen-induced time-specific *Gcm2* deletion analysis. When tamoxifen was administered to 8-week-old mice (*Tm-Cre* × *Gcm2*$^{Flox/Flox}$) to make *Gcn2* deficiacy in parathyroid. At 1 month of *Gcm2* deficiency in the parathyroid gland, changes were not seen in the size, expression of related genes, calcium concentration in the blood, but some follicular structures were observed. Seven months after the deletion of *Gcm2*, the parathyroid gland had shrunk, resulting in decreased expression of related genes and a decreased calcium concentration. The follicular structures seen at 1 month of *Gcm2* deficiency parathyroid were more marked, and the shape had also significantly changed. When cell proliferation and cell death were observed, the cell proliferation decreased from 1 month after the defect, and at 7 months cell proliferation decreased and cell death increased. These results clearly show that Gcm2, which is expressed in the parathyroid gland throughout life, is involved in maintaining cell proliferation, shape, and function of the parathyroid gland even after birth.

Analysis of the origin of the gas bladder

To investigate how fish acquired a gas bladder, we analyzed sturgeon, gar, zebrafish, and *Polypterus*. The comparative analysis showed that the branching region of the gas bladder from the esophagus is different in each fish. The gas bladder of gar was branched from the entrance of the esophagus (larynx in mammals), the same as the lung of *Polypterus*. The gas bladder of zebrafish branched from the central part of the esophagus, and the sturgeon gas bladder was branched from the gastric entrance. In analysis of enhancers related to lung development, while this region is conserved in *Polypterus*, insertion and deletion of sequence have already been observed in gar, is hardly conserved in zebrafish, and is completely defective in sturgeon. These results show that the gas bladder can be acquired in various ways. The gar gas bladder seems to use the lung mechanism. Zebrafish and sturgeons have lost lung developmental mechanisms, which might have caused the development of gas bladders in the posterior region of the esophagus. This is an important finding for understanding the relationship of lungs to the gas bladder in regard to gas bladder acquisition.

Functional analysis of microRNA-155 in dextran sulfate sodium-induced mouse model of colitis

Ulcerative colitis is a refractory disease for which maintaining long-term remission improves quality of life. However, long-term remission has been maintained in few cases with the current treatment method, and a new treatment method is greatly desired. At this time, C57BL (wild-type; WT) mice, which are more sensitive to dextran sulfate sodium (DSS), and microRNA (miR)-155 knockout mice, which are less sensitive, were prepared as mouse models of DSS-induced colitis, which are commonly used as mouse models of ulcerative colitis, to clarify the key molecular mechanisms of preventing the occurrence and recurrence of colitis. When the mice were analyzed, exfoliation of epithelial cells, loss of crypts, and significant inflammatory cell infiltrate in the lamina propria were observed in WT mice as a pathological change in large-intestinal mucosa due to DSS administration. However, no tissue damage was observed in miR-155 knockout mice.

Next, the real-time polymerase chain reaction method was used to measure the expression level of miR-155 before and after colitis was induced in WT mice. An increase in the miR-155 expression level was observed after DSS was administered. As a result, a correlation between miR-155 and the occurrence and worsening of colitis was indicated. Currently, miR-155 expression was verified by using in situ hybridization before and after DSS administration in WT mice has been verified using the in-situ hybridization method. Based on data obtained with this experiment, we would like to clarify the homeostatic mechanism of large-intestinal mucosa and to verify whether inhibition of miR-155 can be achieved with a new treatment method to maintain remission of ulcerative colitis.

Joint morphogenesis in zebrafish fins

In tetrapod limbs, synovial joints are important structures for locomotion. Fish fins are thought to be homologous to tetrapod limbs, but in the fin ray region are unique joints, which are filled with ligament-like connective tissues. To elucidate the mechanism of the fish joint formation, we have observed a series of transverse sections with hematoxylin,

Alcian blue, and periodic acid–Schiff stain. We found that an articular cavity, which was the region positive for hematoxylin and Alcian blue, was in the joint architecture. Moreover, the proteoglycan 4a gene (*prg4a*) was expressed at the fin joint. These findings suggest that the fin joint is classified into a diarthrosis, although it consists of the articular capsule and a mucin-containing cavity but not cartilage.

Publications

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