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General Summary

Our group is interested in the developmental and evolutional aspects of structure of the human body. 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, and, thus, are attempting to identify fundamental molecular mechanisms that shape each organ.

Research Activities

Histological analysis of the congenital ataxic mouse
Anterior horn cells in the spinal cord of the congenital ataxic mouse were examined with fluorescence Nissl staining. The L4 to L5 segments of the spinal cord were removed from congenital ataxic mice and phenotypically normal mice aged 4, 10, and 20 weeks. The spinal cord segments were serially sliced at a thickness of 150 μm and stained with an aqueous solution of cresyl violet. The sections were mounted with a polyester resin and observed throughout the whole thickness with a laser scanning microscope. The number of anterior horn cells in ataxic mice was comparable to that in phenotypically normal mice. No degenerative changes were found in the anterior horn cell of either ataxic or normal mice. These results indicate that the peripheral motor neuron is not affected in the congenital ataxic mouse.

Primitive actinopterygian Polypterus *has superficial neuromasts in the lateral line*
Polypterus, the most basal extant actinopterygian fish in molecular phylogeny, possesses ganoid (enamel) scales on the surface of the body, which reminds us of an extinct primitive actinopterygii or teleostei, such as *Psarolepis* or *Lophosteus*. Fossil records of these extinct genera reveal no apparent openings on the surface of the scale for the lateral line neuromast, and in the present day a wide variety of shapes are present in the lateral line of existing bony fishes. The lateral line neuromast is the mechanosensory or electrosensory receptor, which is distributed to the cranial and the lateral body regions from a part of the cranial nerves. All aquatic animals (except for marine mammals) have a neuromast despite the lateral line being considered a vestigial organ for a terrestrial tetrapod that underwent 3 rounds of whole-genome duplication, as did most actinopterygians. We thus investigated the morphogenesis of the lateral line neuromast in *Polypterus* as a representative model of a primitive actinopterygian.
Initial neuromast cells appeared in neurula as the placode and the neural crest cells to coalesce into apparent rosette structures in bistratal epidermis, which was observed 5 days after fertilization. At the hatching larval stage, the lateral line neuromasts, which bulged

out from the surface of the epidermides, projected their axons inward toward the lateral line nerve. The neuromast will finally be buried in the adult under the superficial epidermis with a small pore in the surface, which was reported last year.
Therefore, the lateral line neuromasts along the body existed superficially for life and did not form a distinct canal organ, even after mineralization; this finding indicates that the lateral line neuromast in the trunk of *Polypterus* is a superficial organ. Moreover, the development pattern of the neuromast in *Polypterus* resembles that in the zebrafish, as previously reported.

Transcriptome analysis of the developing diaphragm
Congenital diaphragmatic hernia (CDH) caused by a defect of diaphragmatic development. However, the pathogenic mechanism of CDH is still unknown. Because the diaphragm consists of several tissues from around its area of development, understanding the cells' origin and location in the diaphragm can be difficult. Therefore, determining which tissues comprise which part of the diaphragm is important to understand diaphragmatic development. To investigate this issue, we performed several studies and found that some tissues were asymmetrically distributed in the diaphragm. We hypothesized that this asymmetrical distribution of some tissues contributes to the occurrence of CDH. This year, we attempted a more detailed analysis of gene expression to understand these asymmetrical tissues. We divided the developing diaphragm into 6 parts, in each of which a transcriptome was constructed. From the gene expression patterns of each part, we found that some genes were expressed only on the right side and are well known to be responsible for CDH. We believe that these asymmetrical tissues indicate that different genes are expressed in left-right asymmetry and might contribute to the occurrence of CDH. Our future studies will investigate the pathogenic mechanism of CDH.

Region-specific heparan sulfate proteoglycan function in the zebrafish fin development
Proteoglycans, which are proteins with glycosaminoglycan chains, act as the extracellular matrix responding to the biochemical and mechanical cues. Heparan sulfate proteoglycans (HSPGs) are synthesized by exostosin genes (*EXT*), which encode glycosyltransferases. The protein exostosin-like glycosyltransferase 3 (EXTL3) is an N-acetylglucosamine transferase in the initial step of HSPG synthesis, and in embryogenesis, *EXTL3* gene is expressed ubiquitously. Interestingly, *extl3*–mutant zebrafish do not form the distal part of the pectoral fin in development. To investigate functions of other *ext* genes in pectoral fin development, we have generated gene knockout fish with the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9) system. We have found some malformations (abnormal chondrogenesis and loss of the distal part of the fin) of the pectoral fin primordia with double knockout of the genes for ext1b and ext1c. Previous studies suggest that ext2 is required for the proximal part of the fin, which is homologous to limbs of tetrapods. Our data suggest that the genes *ext1b, ext1c*, and *extl3* are required for the distal part of the fin in development and that the fish-specific structure, which is called the fin-ray, is formed by the region-specific HSPG function.

Publications

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