Department of Anatomy (Histology and Embryology)

Masataka Okabe, *Professor*
Hideaki Suzuki, *Assistant Professor*
Hisashi Hashimoto, *Professor*
Yasuyo Shigetani, *Assistant Professor*

General Summary

Our group is interested in the developmental and evolutionary aspects 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, thus identifying fundamental molecular mechanisms that shape each organ.

Research Activities

Genomic analysis of congenital ataxia mouse
The genomic analysis of the congenital ataxic mouse has revealed the deletion of 7048 bps in the second intron of predicted gene 13912 (*Gm13912*). The relationship between the ataxic phenotype and genotype of *Gm13912* was investigated in the progeny of intercross mice generated by backcrossing B6ICRF1 to C57BL/6N mice 6 times. Most mice showed a clear relationship between the ataxic phenotype and the genotype of *Gm13912*. However, several phenotypically normal mice showed genotypically ataxic homozygosity for the *Gm13912* gene. Interestingly, these phenotypically normal mice also showed genotypically ataxic homo on rs13476689. The other alterations in the genome of ataxic mice are presently under investigation.

Whole exome analysis of Japanese mosaic variegated aneulpoidy syndrome
Mosaic variegated aneuploidy type I (MVAI) is an autosomal recessive disorder characterized by mosaic aneuploidies and involving multiple different chromosomes and tissues. Common clinical manifestations are prenatal onset growth failure, mental retardation, and microcephaly. The cause of the mosaic aneuploidies is the premature onset of anaphase by a defect of the spindle assembly checkpoint (SAC). In several cases cancers or sarcomas or both developed. More than 10 cases of MVAI in Japanese patients have been reported. They showed unique and homogeneous clinical features, such as severe microcephaly and hypoplasia of cerebral hemisphere, Dandy–Walker malformation, and Wilms' tumor. These features suggested that this syndrome contains cilia/centrosome dysfunctions in addition to the SAC dysfunction. Non-Japanese patients with MVAI carry biallelic mutations of the SAC of the BUB1 mitotic checkpoint serine/threonine kinase B gene, *BUB1B*. All Japanese patients had a nonsense mutation of *BUB1B*-coding regions on 1 allele but no mutations on the coding region of another allele. Recently, the intergenic single nucleotide mutation at 44 kb upstream from transcription start site of *BUB1B* was reported as a hypomorphic mutation on another allele. However, the mouse models carrying hypomorphic *BUB1B* or other SAC gene mutations did not show any brain

anomalies or Wilms' tumors. We believed that the hypomorphic mutation could not be explained by clinical features in Japanese cases. Thus, we performed trio whole exome analysis of our case. We found a Japanese-specific nonsynonymous single nucleotide variation of the cilia/centrosome-associated gene on 15q near *BUB1B*. We are evaluating this single nucleotide variation with culture system.

Morphogenesis of the lateral line in the primitive fish Polypterus

Polypterus, the most basal extant actinopterygian fish in molecular phylogeny, possesses ganoid (enamel) scales on the surface of its body, which reminds us of an extinct primitive actinopterygii or teleostei, such as *Psarolepis* or *Lophosteus*. Fossil records of these extinct genera reveal that there are no apparent openings on the surface of the scale for the lateral line neuromast, and in existing bony fishes a wide variety of shapes are known in the lateral line. The lateral line neuromast is the mechanosensory or electrosensory receptor, which is distributed to the cranial and lateral body regions from a part of the cranial nerves. All aquatic animals have the neuromast, despite the lateral line being thought to be a vestigial organ for a terrestrial tetrapod, such as *Xenopus*, that undergoes 3 rounds of whole-genome duplication, as do most actinopterygians. Therefore, we investigated the morphogenesis of the lateral line neuromast in *Polypterus* as a representative model of the primitive actinopterygians.

Initial neuromast cells appeared at neurula as the placode and the neural crest cells to coalesce into apparent rosette structures in the bistratal epidermis, which was observed soon after hatching. At the larval stage, the lateral line neuromasts, hair cells of which bulged out from the surfaces of the epidermides, projected their axons inward lateral line nerve. In a young larva, mineralization starts in a few underlying scales in the rear and advances toward a row of frontal scales. Interestingly, the posterior margin of the lateral line scale transiently appeared, and the neurogenesis appeared to be avoiding calcium deposits for a short period. Scanning electron microscopy of the surface of a juvenile revealed that the epidermis on the lateral line scale had several pores that were nearly covered with mucous substances and were surrounded by the epidermis in a concentric fashion. The neuromasts in the adult fish were macroscopically seen where pigment cells gathered, and the axon bundle from the neuromast threaded the vascular cavities of the lateral line scale inwards and finally pieced it.

Therefore, the lateral line neuromasts along the body existed superficially for life and did not exhibit a distinct feature of the canal structure, even after mineralization, which indicates that the lateral line neuromast in the trunk of *Polypterus* is a superficial pit organ.

Distribution of Wt1*-positive cells in diaphragm development*

Because the diaphragm consists of several tissues from around the area in which it develops, understanding where the cells come from and which part of the diaphragm they consist of can be difficult. To understand the development of the diaphragm, we focused on the Wilms tumor 1 gene (*Wt1*), which is related to the congenital diaphragmatic hernia (CDH). We had previously found that *Wt1*-positive cells are restricted to the left-posterior area of the adult mouse diaphragm. This year, we performed a more detailed analysis of the developmental stages of the mouse embryo and observed an asymmetrical distribution

of the *Wt1*-positive cells more on the left side than on the right side. This finding suggests a relationship between the distribution of *Wt1*-positive cells and CDH. We will continue to analyze the mechanism of the distribution of *Wt1*-positive cells to the left side and hope to determine the mechanism of CDH.

Bone formation of vertebrate appendages (limbs and fins)

The paired fins of fish (pectoral and pelvic fins) consist of 2 types of bones, which are derived from lateral plate mesoderm (LPM). In the proximal part of the fin, skeletal elements are formed by endochondral ossification. In the distal part, skeletal elements are formed by intramembranous ossification. To understand the mechanisms of fin bone development, we have established a genome-editing technique with the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system. Several guided (g) RNAs, which bound target sites of the fish genome, were synthesized, but some gRNAs did not work well. However, there were few differences in base sequences in the 3′ terminal part. We will collect specimens that form the abnormal skeleton and will raise founder fish and offspring fish to maintain genome-edited fish lines. Moreover, we have established a precise cell transplantation technique for zebrafish embryos by using a micromanipulator. *Boxer* fish, which are mutants of the exostosin-like glycosyltransferase 3 gene (*extl3*), do not form the distal part of the fin during embryogenesis. To investigate adult bone phenotypes of *boxer* fish, *boxer* LPM cells were transplanted into the wild-type LPM region at gastrulation stages. *Boxer* LPM cells differentiated, not into LPM derivatives (pectoral fins and heart), but into several mesodermal tissues (blood vessels, muscles and tail structures), because we could not define the host LPM region at earlier embryonic stages. We will improve this transplantation method and will make a fin-specific mutant fish.

Publications

Izuhara L, Tatsumi N, Miyagawa S, Iwai S, Watanabe M, Yamanaka S, Katsuoka Y, Nagashima H, Okano HJ, Yokoo T. Generation of a felinized swine endothelial cell line by expression of feline decay-accelerating factor. *PLoS One*. 2015; **10:** e0117682.

Takeuchi-Igarashi H, Kubota S, Tachibana T, Murakashi E, Takigawa M, Okabe M, Numabe Y. Matrix remodeling response of human periodontal tissue cells toward fibrosis upon nicotine exposure. *Odontology*. 2014 Oct 15. Epub ahead of print.

Reviews and Books

Yano T, Matsubara H[1], Egawa S[1], Onodera K[1], Tamura K[1] ([1]Tohoku Univ). Chapter 22: Fins and limbs: emergence of morphological differences. In: Kondoh H, Kuroiwa A, editors. New principles in developmental processes. Tokyo: Springer; 2014. p. 291-302.