Department of Anatomy (Histology and Embryology)

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

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

Sequencing analysis of the novel hereditary ataxic mouse
We had established a novel hereditary mouse line of neurodegenerative disorder which is transmitted by a single autosomal recessive gene locus *hak*, *hindlimb ataxia with kidney iron deposition*. The affected mouse was characterized by heavy hind limb ataxia with gait disorder, which was firstly recognized at about 4 weeks of age and slowly progressed with advancing age. In this study, sequencing analysis of the hak phenotype was performed to reveal the causative gene locus. Linkage analysis had revealed that the hak phenotype had an association peak on chromosome 2. The most significant association was found in rs13476689 located at 2qE3 (chr2:107,305,044 NCBI37/mm9). Sequencing analysis performed on 101,163,197～111,930,241 in chr 2 revealed many single nucleotide polymorphisms (SNPs), insertions and deletions (indels) of single to oligo nucleotides. Affected mouse specific genetic variations were determined by the following conditions: all affected mice are homozygous and all heterozygotic mice are heterozygous. Known SNPs, which had reference SNP ID numbers, were omitted. In consequence, 892 genetic variations were identified as being hak phenotype specific. None of the genetic variations localized in coding sequences. The genetic locus associated for hak phenotype was mapped to 107,305,356～108,637,615 on chromosome 2qE3, in the vicinity of *bdnf* gene. These results suggest that the hak mouse has a tissue-specific impairment in expression of a type of Bdnf transcripts.

Whole exome analysis of the first case complicated with progressive osseous heteroplasia (POH) and Gorlin syndrome (GS)
In this year, we performed the whole exome analysis (WEA) in the first case complicated with POH and GS, as a collaboration with pediatric department. We detected a nonsense GNAS mutation on the maternal allele and a loss of heterozygosity (LOH) containing GNAS gene on the paternal allele. Interestingly, both mutations were somatic, and probably occurred in early embryonic stage before gastrulation. The first hit might be the nonsense mutation on the maternal allele. Soon after the first hit, the LOH on the paternal

allele might occur by gene conversion with the entire maternal long arm of chromosome 20 containing the GNAS nonsense mutation. As the results, the somatic cells carrying these mutations completely lost the GNAS functions. This somatic mosaicism were observed in several tissues from the patient, such as the peripheral blood lymphocytes, the medulloblastoma, the cardiac myofibroma, dermal myofibroma, and cultured dermal fibroblasts.
Our research suggested that the cause of POH might be somatic mosaicism with complete loss of GNAS function, and that GNAS loss of function mutations might become driver mutations of medulloblastom only when the mutations occurred in early embryonic stage.

Lateral line neuromast in Polypterus *appears superficially during development*
Polypterus, the most basal extant actinopterygian fish in molecular phylogeny like a sarcopterygian Coelacanth, possesses enameled scales on the surface of the body, which reminds us of an extinct primitive actinopterygii or teleostei. 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 mechano- or chemosensory 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 placodes or somewhat later as hair cells expressing *Eya1* or *Sfrp1* to form apparent rosette structures in bistratal epidermis, which was observed in the post-hatch larvae. Adjoining the primitive neuromast, an almost acelluler region was present just under the basement membrane. To examine whether the acellular region is the way of neuromast cells passing through or the space for their axons extending as reported in zebrafish, we are analyzing the acellular region by the Maldi-TOF mass spectrometry. The neuromast along the body existed superficially for life and it did not form a distinct canal organ even after mineralization, implying that the lateral line neuromast in the trunk of *Polypterus* is a superficial type pit organ.

Analysis of origins of lungs and gas bladder using polypterus
Although the lung is an important organ in respiration, it has been a mystery of many researchers for many years when it was acquired during the process of evolution. In recent studies of phylogeny and petrology, it is thought that lungs existed before gas bladder. So we focused on Polypurus to understand which is first lung or gas bladder. Polypterus is a lineage that diverged from the actinopterygian at the earliest stage, and unlike other teleost fish, it has a ventral side air-filled organ (lung) was known. We analyzed lung development in polypterus in detail and revealed that lungs of this fish have very similar developmental mechanism to lung animmal such as mammals. We also found that the expression patterns of genes (*Nkx2.1, Fgf10, Tbx4, Tbx5*) important for lung development were also very similar to mammalian lung development. Furthermore, it was

revealed that the *Tbx4* lung enhancer (LME) sequence, which was known to exist only in the sarcopterygian, was also present on the genome of polypterus and conserved. It was confirmed that this arrangement disappears eventually due to insertion / deficiency, etc. in the teleost. Furthermore, this sequence had functional activites in the lungs of chick embryos. From these experimental results it was found that the ventral air-filled organ of polypterus was homologous to our lungs and the genetic program related to its development was conserved. This finding indicate that the lung development program was existed from a common ancestor of the actinopterygii and the sarcopterygii. This gave us real evidence that the lungs existed before the gas bladder.

Generating transcriptome analysis from the divided diaphragm

Congenital diaphragmatic hernia is disease that causes dysplasia of the diaphragm, but its pathogenesis mechanism is almost unclear. The diaphragm is formed by gathering different surrounding cells where it is formed, but the details of what kind of region the cells form is not clear yet. Understanding the development of diaphragm is very important for understanding the pathogenesis of congenital diaphragmatic hernia and it is therefore indispensable to identify what kind of cell population the diaphragm is formed from. Last year, we divided the diaphragm into six regions and performed transcriptome analysis. As a result, it was revealed that there was a left / right difference in expressing genes depending on the region. Analysis of these candidate genes revealed that there is a difference in gene expression also in PCR using the regional cDNA of the diaphragm. Furthermore, in situ hybridization of these genes revealed that there is site-specific gene expression. It is thought that further clarification of the cell group expressing these genes will contribute to the understanding of congenital diaphragmatic hernia onset in the future.

Joint formation in zebrafish fins

In zebrafish fins, skeletal elements (called as fin rays) are formed by intramembranous ossification. A fin ray consists of multiple segments separated by joints. The fin ray joint morphogenesis is driven by a transcription factor, even-skipped homeobox 1 (evx1), and zebrafish *evx1* mutants exhibit joint agenesis (Schulte et al, 2011). To understand mechanisms of the orderly bone segmentation in the fin ray formation, we have focused on *evx1*-expressing joint cells *in vivo*.

We have generated a transgenic (TG) fish line, which harbored the insertion of the gene construct with the splice-acceptor and Gal4FF in a coding region of *evx1* gene. In this heterozygous TG fish, all fin ray joints were recognized as the green fluorescence protein (GFP)-positive regions by the Gal4-upstream activating sequence (UAS) genetic system. We have found fin ray joints were not formed in homozygous TG fish, and any aggregations of GFP-positive cells were not observed in the fin ray. These data indicate that this TG fish line is evx1-deficient fish caused by the gene trap construct. By using the confocal laser microscope (LSM880, Zeiss), moreover, we have achieved observation of a GFP-positive joint cell with three-dimensional reconstruction. We therefore take advantage of this novel material and method, and try to elucidate spatiotemporal changes of joint cells in the fin ray formation.

Publications

Shigetani Y*[1]*, Wakamatsu Y*[2]*, Tachibana T*[1]*, Okabe M*[1] *(*[1]Jikei Univ,*** **[2]***Tohoku Univ).*** Conversion of neural plate explants to pre-placodal ectoderm-like tissue in vitro. *Biochem Biophys Res Commun.* 2016; **477:** 807-13.

Tatsumi N, Kobayashi R, Yano T, Noda M, Fujimura K, Okada N, Okabe M. Molecular developmental mechanism in polypterid fish provides insight into the origin of vertebrate lungs. *Sci Rep.* 2016; **6:** 30580.

Uchiyama T, Tatsumi N, Kamejima S, Waku T, Ohkido I, Yokoyama K, Yokoo T, Okabe M. "Hypermethylation of the CaSR and VDR genes in the parathyroid glands in chronic kidney disease rats with high-phosphate diet." *Hum Cell.* 2016; **29:** 155-61.

Nagasawa T, Kawaguchi M*[1]*, Yano T, Sano K*[2]*, Okabe M, Yasumasu S*[1] *(*[1]Sophia Univ,*** *[2]***Josai Univ).*** Evolutionary Changes in the Developmental Origin of Hatching Gland Cells in Basal Ray-Finned Fishes. *Zoolog Sci.* 2016; **33:** 272-81.

Miyake T, Kumamoto M, Iwata M, Sato R, Okabe M, Koie H, Kumai N, Fujii K, Matsuzaki K, Nakamura C, Yamauchi S, Yoshida K, Yoshimura K, Komoda A, Uyeno T, Abe Y. The pectoral fin muscles of the coelacanth Latimeria chalumnae; Functional and evolutionary implications for the fin-to-limb transition and subsequent evolution of tetrapods. *Anat Rec.* 2016 Sep; **299:** 1203-23.