Department of Anatomy (Histology and Embryology)

Masataka Okabe, *Professor*
Toshiaki Tachinbana, *Assistant Professor*
Hisashi Hashimoto, *Associate Professor*
Yasuyo Shigetani, *Assistant Professor*

General Summary

Our group is interested in the developmental and evolutionary aspects of human organs. By comparing organ development in humans and other 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

Molecular evidence that the lungs and the swim bladder are homologous organs

The air-filled organs of land vertebrates and fish — the lungs and the swimbladder — have long been postulated to be homologous organs. Both of them are air-filled sacs that derive from the digestive tract. On the other hand, the lungs are paired structures, whereas the swimbladder is a single sac. The lungs extend from the ventral side of the digestive tract, whereas the swimbladder extends from the dorsal side. Because of a lack of fossil evidence, determining whether the lungs and the swimbladder are in fact homologous organs has been difficult. We used an evolutionary developmental approach to address this issue. We found that TBX4, fibroblast growth factor (FGF) 10, and NKX2.1, key regulators of mammalian lung development, are also specifically expressed in the developing swimbladder of the zebrafish. Suppression of FGF 10 and TBX4 by antisense morpholino dramatically reduced swimbladder size. These observations show that the development of the swimbladder and that of the lungs employ the same evolutionarily conserved set of genes, strongly supporting the hypothesis that the two organs are indeed homologous.

Acquisition of a novel organ involved in regulation of calcium concentration in the blood during vertebrate evolution

During evolution, tetrapods obtained several new organs, including the parathyroid, metanephros, and placenta, all of which regulate the calcium concentration of the blood to allow tetrapods to live on land. Development of these organs requires transcription factors of the Gcm family. Here we show that Gcm2, which is a key regulator of parathyroid development in tetrapods, is expressed in the chloride cells of teleosts. The chloride cells are specialized cells scattered over the outer skin which are engaged in calcium-ion regulation, in addition to the gills, and are the evolutionary counterpart of the parathyroid gland. This reveals the general role of Gcm2 in the development of calcium-regulating organs, suggesting that changes in their expression pattern may have played an important role in the acquisition of new calcium-regulating organs in different

vertebrate taxa. To identify evolutionary changes in the genome responsible for the differential expression of Gcm2, we searched for enhancer elements driving the expression of Gcm2 in the chloride cells of zebrafish. We identified 2 enhancer regions around the Gcm2 locus which specifically drive expression in the chloride cells.

Differentiation of human mesenchymal stem cells into the collecting duct system in chicken embryos

We attempted to induce human mesenchymal stem cells (hMSCs) to differentiate into cells in the collecting duct system, a derivative of the ureteric bud (UB), by transplanting them into the chicken UB progenitor region. We showed by cell-lineage tracing experiments with DiI that common progenitors of the Wolffian duct (WD) and the UB were both present in the intermediate mesodermal cells of the neck-trunk boundary region of early embryos. We also showed that paired box gene (PAX) 2-expressing hMSCs transplanted into the UB progenitor region migrated caudally with the elongating WD and became integrated into the WD cells. However, hMSCs were rarely able to migrate to caudal end of the WD, where UBs are formed. Therefore, this year, we examined gene expression patterns of PAX2-expressing hMSCs under cell-culture conditions. The reverse transcriptase polymerase chain reaction showed that PAX2-expressing hMSCs did not express *LIM1*, a critical gene involved in WD/UB morphogenesis. The results also raise the possibility that−LIM1-expressing hMSCs differentiate into UB cells, then into cells in the collecting duct system.

Development of the mucosal vascular system in the distal colon of the fetal mouse

The formation of crypts in the distal colon of the mouse was investigated in association with the development of vascular networks. For histological observation, 1-μm cross sections were made from the distal colons of 13- to 18-day fetal mice. The 3-dimensional distribution of vascular networks in the organ was observed after the fetuses had been perfused with rhodamine isothiocyanate-labeled gelatin and immunostained for laminin to examine the boundary between the epithelium and the mesenchyme. At 13 days' gestation, the distal colon and its epithelium had formed a cylindrical tube, and a loose primary plexus of vessels had appeared in the mesenchyme. At 15 days' gestation, the caudal portion of the distal colon began to form crypts, and the vascular plexus constructed from only a few layers had separated from the boundary between the epithelium and the mesenchyme. As development proceeded, crypts were formed in the caudorostral direction. The developing crypts advanced into the vascular plexus, so that few vessels were situated in the mesenchyme between crypts. As the crypts elongated, these vessels formed a small plexus perpendicular to the primary plexus, while the primary plexus became monolayered and loosened. The new plexus was composed of ascending and traversing vessels, but the regular honeycomb-like plexuses around openings of crypts had not been established by 18 days' gestation. The vascular system and the crypts in the distal colon will require several more weeks after birth to be completed.

Study of skeletal muscle — specific manganese superoxide dismutase — deficient mice
To investigate the pathological significance of oxidative stress in the skeletal muscle, we generated skeletal muscle — specific manganese superoxide dismutase — deficient mice. The mutant mice showed severe disturbances of physical activities but no atrophic changes in skeletal muscles. On histological and histochemical analyses, the mutant mice showed centralized nuclei in muscle fibers and selective loss of enzymatic activity in mitochondrial respiratory chain complexes. In addition, the mutant mice displayed increased oxidative DNA damage and the reduced ATP content in muscle.

Molecular mechanisms for development of the trigeminal ganglion
The trigeminal nerve is the largest cranial nerve, containing both sensory and motor neurons responsible primarily for sensation in the face and movement for mastication. The trigeminal ganglion comprises cells derived from two distinct origins: placode and neural crest cells. The mechanism of trigeminal ganglion development has been well-studied in the chick; however, the molecular mechanism remains unknown. We investigated the roles of secreted factors, such as FGF8, in trigeminal nerve development and performed expressed sequence tag (EST) analysis of unknown genes from head ectoderm. Implantation of FGF8-soaked beads underneath the trigeminal placode suppressed expression of *Brn3a,* the earliest trigeminal placode marker. Electroporation of the dominant negative type of *Sprouty2*, a repressor of FGF8, had the same effect. Genes for morphology and causable factors of diseases and related genes were isolated as EST clones, which may shed light upon the molecular mechanism that bridges the gap between the FGF8 pathway and *Brn3a*.

How to make figures and presentations that are friendly to color-blind persons
In scientific presentations and publications, color has become a significant vehicle for information and presentation effect. However, color perception varies greatly among individuals; in particular, red-green color blindness is found in 4% to 9% of males in various populations, a frequency comparable to that of the AB blood type. Thus inappropriate color choices can cause unexpected difficulty in understanding color figures. We are examining how color and color combinations are perceived by persons with various types of color vision, to develop a method for presenting color information that can convey maximal information to all persons, including those with color blindness. We introduced this method on our web site: http://www.nig.ac.jp/color

Publications

Ito T, Ohi S, Tachibana T, Takahara M, Hirabayashi T, Ishikawa H (Nihon Dental Univ), Kusakabe M (Tokyo Univ), Hashimoto H. Development of the mucosal vascular system in the distal colon of the fetal mouse. *Anat Rec (Hoboken)* 2008; **291:** 65–73.
Hashimoto H, Kusakabe M (Tokyo Univ), Ishikawa H (Nihon Dental Univ). A novel method for three-dimensional observation of the vascular networks in the whole mouse brain. *Microsc Res Tech* 2008; **71:** 51–9.
Ohi S. Characterization, anticancer drug susceptibility and atRA-induced growth inhibition of a novel cell line (HUMEMS) established from pleural effusion of alveolar rhabdomyosarcoma of breast tissue. *Hum Cell* 2007; **20:** 39–51.
Jin S. Novel method for the establishment of cardiomyocytes derived from rat embryonic

stem cells *in vitro*. *Hum Cell* 2007; **20:** 111–8.
Matsuno M[1], Kose H[1], Okabe M, Hiromi Y[1] ([1]Nath Inst Genet). TFIIH controls developmentally-regulated cell cycle progression as a holocomplex. Genes to cells: devoted to molecular & cellular mechanisms. *Genes Cells* 2007; **12:** 1289–300.
Shigetani Y, Itasaki N (MRC Natl Inst Med Res, UK). Expression of Wise in chick embryos. *Dev Dyn* 2007; **236:** 2277–84.
Ohi S, Takahashi N, Ninomiya K, Nakajima M, Hashimoto H, Tachibana T, Yanaga K, Ishikawa H (Nihon Dental Univ). Establishment and characterization of a cisplatin-resistant cell line (IGSK-1) from a poorly differentiated gastric adenocarcinoma. *Hum Cell* 2007; **20:** 15–22.
Ohi S, Takahashi N, Hashimoto H, Tachibana T, Hirabayashi T, Sugiyama K, Yanaga K, Ishikawa H (Nihon Dental Univ). Establishment and characterization of an IGSK-2 cell line derived from ascitic fluid of recurrent hCG and somatostatin secreted adenocarcinoma of the stomach. *Hum Cell* 2007; **20:** 52–61.
Ninomiya K, Ohi S, Tabei I, Jin S, Tachibana T, Yamashita S, Yanaga K, Hashimoto H. Establishment and characterization of a cell line (BTIC) including HER-2-positive cells derived from pleural effusion of recurrent breast invasive ductal carcinoma, scirrhous type. *Hum Cell* 2007; **20:** 85–90.

Reviews

Yokoo T, Fukui A, Matsumoto K, Okabe M. Stem cells and kidney organogenesis. *Front Biosci* 2008; **13:** 2814–32.
Shimizu H (Nath Inst Genet), Okabe M. Evolutionary origin of autonomic regulation of physiological activities in vertebrate phyla. *Journal of comparative physiology. A, Sensory, neural, and behavioral physiology* 2007; **193:** 1013–9.