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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

Acquisition of glial cells missing 2 enhancers contributes to a diversity of ionocytes in zebrafish

Glial cells missing 2 (gcm2) encoding a GCM-motif transcription factor is expressed in the parathyroid gland in amniotes. In contrast, *gcm2* is expressed in pharyngeal pouches (a homologous site of the parathyroid), gills, and H^+-ATPase-rich cells (HRCs), a subset of ionocytes on the skin surface of the teleost zebrafish. Ionocytes are specialized cells that are involved in osmotic homeostasis in aquatic vertebrates. Here, we show that *gcm2* is essential for the development of HRCs and Na^+-Cl^- cotransporter-rich cells (NCCCs), another subset of ionocytes in zebrafish. We also identified *gcm2* enhancer regions that control *gcm2* expression in the ionocytes of zebrafish. Comparisons of the *gcm2* locus with its neighboring regions revealed no conserved elements between zebrafish and tetrapods. Furthermore, we observed *gcm2* expression patterns in embryos of the teleost fishes medaka (*Oryzias latipes*) and fugu (*Fugu niphobles*), the extant primitive ray-finned fishes polypterus (*Polypterus senegalus*) and sturgeon (a hybrid of *Huso huso*×*Acipenser ruhenus*), and the amphibian African clawed frog (*Xenopus laevis*). Although *gcm2*-expressing cells were observed on the skin surface of medaka and fugu, they were not found in polypterus, sturgeon, or the African clawed frog. Our results suggest that the acquisition of enhancers for the expression of *gcm2* contributes to the diversity of ionocytes in zebrafish during evolution.

Novel ataxia mouse shows extensive neuropathological changes in the dorsal root ganglion neurons

We have investigated the neuropathological changes in the peripheral nervous system of our novel ataxia mouse, which has a severe gait disorder of the hind legs. On transverse sections of the lumbar spinal nerves, many vacuoles were found in nerve fibers. Longitudinal sections showed swellings and vacuoles in both the spinal nerves and the nerve roots. In the lumbar dorsal root ganglion, the perikarya of some of the larger neurons

showed autolysis, whereas those of the smaller neuron appeared to be intact. The shape of the lysed neurons was preserved, and some satellite cells encircled the neuron. No inflammatory cell infiltration was observed. The autolysis of the larger neuron was also found in the cervical and thoracic dorsal root ganglia. These results suggest that the gait disorder of the hind legs of our novel ataxia mouse is caused by a neuropathy of proprioception.

A novel method for analyzing tissue-specific epigenetic memory

This year we attempted to develop recombination-induced tag exchange (RITE), a genetic method that induces a permanent epitope-tag switch in the C-terminal coding sequence of H3.3, a histone H3 variant working as an active epigenetic memory, after a tissue-specific induced activation of causes recombination (Cre) recombinase.

We constructed a vector that ubiquitously expresses hemagglutinin (HA)-tagged H3.3, under a cytomegalovirus promoter without Cre recombinase. The existing Cre recombinase HA-tag at H3.3 C-terminal was exchanged for a 3xFlag-tag.

First, this vector was applied to 293 cells with or without a Cre-expression vector. As expected, HA-tagged H3.3 was incorporated in chromatin without Cre recombinase, whereas Flag-tagged H3.3 was incorporated in chromatin with Cre recombinase.

Next, we constructed a RITE vector working under a muscle-specific creatine kinase promoter by recombination with a bacterial artificial chromosome. We applied this muscle-specific expression vector to the C2C12 cell line. After 7 days of differentiation following transfection, HA-tagged H3.3 was incorporated in chromatin without Cre recombinase, whereas Flag-tagged H3.3 was incorporated in chromatin with Cre recombinase.

Development of a new culture method for a precursor to the neural crest and pre-placodal ectoderm

The vertebrate-specific structures neural crest and sensory placodes arise from a region of the embryonic ectoderm that lies between the neural plate and the future epidermis. We developed a new culture method for a precursor to both the neural crest and the pre-placodal ectoderm; the cultured cells characteristically express Dlx5, which is a neural plate border specifier that non-cell-autonomously positions the neural crest shown by Slug/SOX2/3 and the future epidermis shown by keratin19/GATA3. The original method for producing the neural crest cells is to add bone morphogenetic protein (BMP) 4 into the medium of grafts, which are dissected from the neural plates of stage 6 to 7 chicken embryos. An additional recombinant protein, fibroblast growth factor (FGF) 2, leads to increased expression of neural plate border-specific molecular markers *Dlx5*, and moderately increased expression of *Six1* and *Eya2*, following morphological change to the simple squamous epithelium. The graft-derived cells also express *Pitx2*, *Pax6*, *Msx1/2*, *Pax3*, and *Brn3a*, which are cranial placode markers in Hox-negative regions. When we superadded FGF8 into the medium instead of FGF2, the graft transformed into the neural crest cells. Addition of FGF8 into the medium on condition specific for producing the pre-placodal ectoderm proved ineffective. The epithelial-like cells, therefore, are a precursor to the neural crest and the pre-placodal ectoderm in Hox-negative regions, and

they can conditionally be formed upon BMP4 and FGF2. The neural plate cultural system would help clarify the complex array of genes involved in the programming of the placode-specific identity.

Study of the diaphragm: Development and acquisition

The diaphragm is a muscular membrane present only in mammals. The diaphragm separates the mammalian body cavity into the thoracic cavity and the abdominal cavity and plays an important role in respiration. How the diaphragm develops is not clearly understood, and research into its development would be useful for clarifying the cause of congenital diaphragmatic hernia. We marked muscle cells present in the diaphragm and tried to understand how muscle precursor cells migrate into the diaphragm and the timing of cell differentiation. In mouse embryo on embryonic day (E) 10.5, the muscle precursor cells were detected from the upper part of the forelimb to the region of the heart. On E12.5, muscle precursor cells had invaginated into a primitive diaphragm. On E14.5, muscle cells were observed through the diaphragm, as is shown in textbooks of mouse development. Muscle differentiation started from the dorsolateral side of the muscle precursor and had been completed by E14.5. These results might be important for our future studies to understand the development of the diaphragm.

Publications

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