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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

Polypterus, a model animal for studying tetrapod evolution

The evolutionary transition from aquatic vertebrates to terrestrial tetrapods occurred around 370 million years ago in the late Devonian period. What changes in genome functions caused such evolution and led to the acquisition of new organs remains uncertain. We have been investigating the evolution of the tetrapod body plan using a primitive actinopterygian fish, *Polypterus*, with analyses of comparative anatomy, comparative developmental biology, and comparative genomics. Because a large-scale analysis of the *Polypterus* genome has not been performed, preparations have been made to obtain the basic information. First, construction of a bacterial artificial chromosome library of *Polypterus* has been started. Genomic DNA was prepared with nucleated blood cells extracted from an adult male. The genome size was found to be 2.8 GB. This initiative is a collaborative project with Professor Asao Fujiyama of the National Institute of Genetics. In collaboration with Professor Shinichi Aizawa of the RIKEN Center for Developmental Biology, expressed sequence tag (EST) analyses have also been started using *Polypterus* embryos at stages up to gastrulation. Complementary DNAs were constructed using RNAs extracted from *Polypterus* embryos, resulting in a determination of nucleic acid sequences of 10,000 clones. Of the genes obtained, 80.1% have 1 clone. Therefore, another 10,000 clones will be sequenced. For embryos at stages later than neurulation, a complementary DNA library is now under construction, and EST analyses will be performed at the National Institute of Genetics.

Immunohistochemical detection of intravascularly transplanted mesenchymal stem cells

Recently some attempts have been made to treat damaged tissues and organs by transplanting functional cells differentiated from bone marrow-derived stem cells or induced pluripotent stem cells. However, revealing the behavior of the transplanted cells in a host is difficult. To trace the transplanted cells, they should have a maker that: 1) is cell-autonomous and localized in the cell; 2) is not secreted and transferred to the surrounding cells; 3) does not affect the differentiation of transplanted cells or their

blending with other cells; 4) is taken over by the descendant cells; and 5) can be detected with a light microscope.
The cells of BALB/cA^{-CSA} mice have such a marker. This strain of mouse has the genetic background of the BALB/cA stain and an Hspa9 variant derived from the C3H strain. The antibody against the C3H-specific antigen, CSA, reacts with the Hspa9 variant and not with Hspa9 of BALB/cA strain.
In this study, we have investigated whether intravascularly transplanted cells derived from BALB/cA^{-CSA} mice in a host mouse of the BALB/cA strain can be detected in the host tissue. Fone marrow–derived mesenchymal stem cells were obtained from femurs of BALB/cA^{-CSA} mice; they proliferated and were transplanted into a BALB/cA mouse via the femoral vein. The host mouse was killed, and each organ was fixed in Bouin's solution, dehydrated, embedded in paraffin, and sectioned. The mesenchymal cells from BALB/cA^{-CSA} were immunohistochemically detected on each section with an anti-CSA antibody.
A number of CSA-positive cells were detected in blood vessels of the host mouse but were not detected in any organ other than the lung. The CSA-positive cells were present in small numbers in the lung 4 days after transplantation but were not found 1 month after transplantation.
These results indicate that mesenchymal cells transplanted into the blood vessel cannot intrude into and survive in the tissue. This study suggests that the behavior of transplanted mesenchymal stem cells, induced pluripotent stem cells, and their derivative cells can be traced by using the combination of mice of the BALB/cA and BALB/cA^{-CSA} strains.

Novel method for genome-wide analysis of tissue-specific epigenetic memory
In addition to genetic variation, epigenetics provides an added layer of phenotypic variation that might mediate the relationship between genotype and internal and external environmental factors. This finding suggests that examining the tissue-specific epigenome might help clarify tissue-specific adaptive states due to chronic exposure to internal and external environmental factors. We designed a new method to understand the cellular pathophysiology in chronic diseases by examining tissue-specific epigenomes. This method is combined with tissue-specific addition of a tag to H3.3, the histone H3 variant localized specifically in transcriptionally active regions, and chip-on-chip or chip-seq assay using specific antibodies for the tag.
This year, we constructed a conditional knock-in vector expressing tagged H3.3 dependent on Cre recombinase. We confirmed that this vector worked as expected.

Molecular mechanism of trigeminal placode and ganglion development in the vertebrates
The trigeminal nerve is the largest cranial nerve, containing both sensory and motor neurons responsible primarily for sensation in the face and the movements for mastication. The trigeminal ganglion comprises cells from 2 distinct origins: placode and neural crest cells. Mechanisms of its development have been well studied in the chick; however, the molecular mechanism remains unknown. We investigated the role of

fibroblast growth factor (FGF) 8 signaling and unknown genes from head ectoderm EST analysis in trigeminal nerve development. Implantation of an FGF8-soaked bead beneath the trigeminal placode of the chick suppressed expression of *Brn3a,* the earliest trigeminal placode marker, around it. Electroporation of the dominant negative type of *Sprouty2*, a repressor of FGF8 signaling, produced a similar effect. Removal of the isthmus, the source of FGF8 in the neural ectoderm, enhanced expression of *Brn3a* and of *Pax3*, another trigeminal placode marker, implying that FGF8 signaling has a negative effect on trigeminal placode induction. Genes of morphology, causable factors of diseases, and their related genes were isolated as EST clones, which may shed light upon the molecular mechanism that could bridge the gap between FGF8 signaling and known trigeminal placode markers.

Anatomical research on diaphragm development

The diaphragm is a muscular membrane found only in mammals which separates the thoracic cavity from the abdominal cavity. In birds, the thoracic and abdominal cavities are separated by 2 membranes, the pulmonary diaphragm and the septum obliquum, which are thought to be homologous to the mammalian diaphragm.

To investigate diaphragm development and morphogenesis processes, we observed 4 genes (*Wt1*, *Gata4*, *Slit3*, and *Raldh2*) known to be responsible for diaphragmatic hernia by means of in situ hybridization in the mouse and chicken embryo. We found that all of these genes were expressed in the primordial diaphragm of the mouse embryo. Moreover, these genes expressed in the homologous tissues of the mouse were also observed in the chicken embryo. These results suggest the membrane that separates the thoracic and abdominal cavities was acquired from the amphibian ancestor of mammals and birds. We are now preparing the Pax3 mutant mouse to study the migration pathway of myoblasts. The same experiment is being performed with 3,3′-dioctadecylindocarbocyanine iodide to label somites in the chicken embryo.

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