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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

Temporal and spatial cellular distribution of neural crest derivatives and alpha cells during islet development

Recent studies have shown that signals from neural crest (NC) derivatives regulate the mass, proliferation, and maturation of beta cells in the developing fetal pancreas. However, little is known about the cellular distribution of NC derivatives during pancreatic development or the process whereby the developing islets are enclosed. We studied the temporal and spatial distribution of NC derivatives and endocrine cells at each developmental stage. At embryonic day 10.5 (E10.5) of the mouse embryo, NC derivatives that migrated to the prospective pancreatic region were distributed near pancreatic epithelial cells. As development proceeded, most NC derivatives surrounded endocrine rather than exocrine cells and were distributed nearer to alpha cells than to beta cells. At E20, approximately 70% of the NC derivatives enclosing endocrine cells were distributed near alpha cells. Moreover, the expression of synaptic cell adhesion molecule (SynCAM), a $Ca2^{+}$–independent homophilic transcell adhesion molecule, was confirmed from E16.5 and was more prevalent at the cell boundaries of alpha cells and NC derivatives. These findings suggest that NC derivatives are distributed near alpha cells as a result of homophilic binding of SynCAM expressed by alpha cells and NC derivatives during islet development.

Molecular biological and histopathological analysis of the novel ataxia mouse

In the progressive ataxic gait mouse (the ataxic mouse), which was developed in our laboratory, a difficulty in exercising the hind limbs manifests at about 4 weeks of age and becomes more severe with age. This disorder is inherited in an autosomal recessive manner, but the responsible gene has not been identified. A linkage analysis of this disorder and single nucleotide polymorphisms (SNPs) in crossbreds of the ataxic Institute for Cancer Research (ICR) line and the C57BL/6J strain revealed a SNP that was conclusively linked to the onset of this disorder. The SNP was located on chromosome 2, and

the causative gene for this disorder was estimated to be localized in a gene segment of 10 million base pairs.
Neuropathological changes in the cranial nerves of the ataxic mouse were investigated. Many vacuoles were found in the trigeminal nerve, the facial nerve running in the temporal bone, and in the trigeminal ganglion. These vacuoles showed intense immunoreactions for neurofilaments, parvalbumin, and calbindin. However, symptoms caused by this vacuolar degeneration of neurons have not been clarified.

The development of a novel transgenic mouse for in-vivo ribosome profiling
Cardiomyocytes comprise at least 3 types of cell with different origins: the first heart-field origin, the second heart-field origin, and the proepicardium origin. Each type of cell might have a different gene regulatory network. To clarify differences in physiologic function by analyzing the comprehensive and cell-type-specific gene regulatory network, we plan to develop a new transgenic mouse in which ribosomal protein RPL10a might be expressed with either Halo7-tag or 3xFlag-tag in a cell-type-specific manner. The tagged RPL10a might be able to purify the ribosome with messenger RNAs under translation.
First, we constructed cytomegalovirus-promoter vectors expressing each tagged RPL10a and examined the intracellular localization of the tagged proteins in HEK293 cells. As expected, both of the tagged proteins localized at ribosomes and rough endoplasmic reticulum. Next, we constructed a vector expressing the tagged proteins exchanged in a Cre-recombinase-dependent manner. In expression experiments, the 3xFlag-RPL10a proteins were exchanged for the Halo7-RPL10a proteins when Cre-recombinase was co-expressed.
To express the tagged RPL10a specifically in striated muscles, we examined the function of the myosin heavy chain 6 (Myh6) promoter. The 5.8 kb upstream from the start codon of Myh6 was inserted into the luciferase-reporter vector by means of the Red/ET system. Promoter activity was measured with luciferase activity in C2C12 cells. The luciferase activity was weak in the logarithmic growth phase but strongly increased after induction of differentiation in a serum-starvation culture in 0.5% serum for 6 days. To complete the transgenic vector, the cytomegalovirus promoter of the tag-exchange vector replaced the Myh6 promoter.
Furthermore, we established MCF7 cell lines stably expressing Halo7-RPL10a to examine experimental conditions for performing ribosomal profiling. Using these cell lines, we will examine a ribosomal extraction method, a nuclease treatment method, a subtraction method for ribosomal RNA removal, a library-producing method for next-generation sequencing and a data-treatment method. In the future, we plan to develop a transgenic mouse.

The vertebrate-specific structures the neural crest and the placode arise from the neural plate border: Development of a new culture method for a possible precursor of exterior epithelium of the neural plate
Previous studies have shown that the neural crest is induced in the neural plate–the embryonic ectoderm border by the action of bone morphogenetic protein (BMP) 4, which

is derived from the embryonic ectoderm, and that the neural plate explant is likewise transformed to the neural crest cells by BMP4. We described a new culture method we developed and used to find that the additive effects of BMP4 and fibroblast growth factor (FGF) 2 on the neural plate explant results in morphological change to the simple squamous epithelium, which characteristically expresses Dlx5, which is a neural plate border specifier that positions the neural crest and the future epidermis. We then examined the effect of Dlx5 downstream genes that are expressed in the neural plate and its border region on the induced epithelium. The expression levels of epidermis specific markers *GATA3/keratin19* and neural crest markers *Slug/Msx1* in the induced epithelium were increased at the expense of the neural plate marker *Sox2*. The preplacodal ectoderm or ridge (PPE or PPR), which was recently reported by Streit et al and other groups, arises in the anterior border of the neural plate in the form of an inverted-U shape and is regarded as a presumptive placode area, which gives rise to the hypophyseal, nasal, lens, trigeminal, otic, and epibranchial placodes at the late neurula-early pharyngula stage. The expression levels of the PPE-specific genes *Six1/Eya2*, known as the direct downstream genes of *Dlx5*, and of some placode-specific markers were also increased, albeit only slightly. A heterogeneity test with an antibody against Dlx5 on the induced epithelium showed that it uniformly expressed Dlx5. This study thus suggests that neural plate cells have a latent ability to be transformed into exterior epithelium of the neural plate, such as the neural crest, the PPE, and the embryonic ectoderm, through the action of BMP4 and FGF2. The induced epithelium might be a precursor of all exterior epithelium of the neural plate. We are now investigating molecular cascades and cell differentiation in both the epithelium induced by the explant culture and the neural plate border in the embryo.

The study of the diaphragm: Development and acquisition

The diaphragm is a muscular membrane that developed only in mammals. It separates the body cavity into the thoracic cavity and the abdominal cavity and has important roles in respiration. The development of the diaphragm is not completely understood, but research on diaphragm development would be useful for elucidating the pathogenesis of congenital diaphragmatic hernia.

We used in situ hybridization to compare the genes responsible for diaphragmatic development in the mouse, which has a diaphragm, and the chick, which does not. We found that Sim2 was important in the muscle differentiation of the diaphragm and was not expressed in the chick embryo. This finding suggests that Sim2 is the key molecule for acquisition of the diaphragm in mammals.

Publications

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