General Summary

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

Histopathological changes in the peripheral nervous system of the novel ataxia mouse before the onset of ataxia

The progressive ataxic gait mouse (Ataxic mouse) developed in our laboratory has hind limb ataxia that manifests from about 4 weeks of age and becomes more severe with age. Histopathological investigations of the Ataxic mouse showed vacuolar degeneration in the medulla of the cerebellum, the spinal nerves, and the trigeminal nerve. These disorders are inherited in an autosomal recessive manner, but a responsible gene has not been identified. A linkage analysis of these disorders and single nucleotide polymorphisms (SNPs) in crossbreeds of the ataxic ICR line and the C57BL/6J strain revealed a SNP that was completely linked to the onset of these disorders. Therefore, we were able to predict the phenotype of crossbred infants by analyzing polymerase chain reaction (PCR) products including the SNP, before the phenotype emerged.

In this study, we examined histopathological changes in the spinal and trigeminal nerves and ganglia of genotypically homozygous Ataxic and wild-type infants of the crossbreed before the onset of disorders.

The homozygous (Ataxic) mice exhibited an ataxic gait on postnatal day (PD) 30, and many vacuoles accumulating neurofilaments (NFs) were found in the nerve fibers and ganglion cells of spinal nerves and the trigeminal nerve. On PDs, 13, 17, and 21 homozygous Ataxic mice appeared phenotypically normal, but NFs had accumulated in the vacuoles of nerves and ganglia. In the peripheral nerves on PD 9, small swellings containing accumulated NFs were found in nerve fibers but not in ganglion cells. No swellings or accumulations of NFs were found in the nerves of the homozygous Ataxic mice on PD 6, as in the wild-type mice of the same age.

These results suggest that the peripheral nerves develop normally but any impairment may appear in the growth or maturation of some neurons in the spinal nerves and the trigeminal nerve.
The impairment of homotetramerization of fructose-1,6-bisphosphatase owing to 2 Japanese founder mutations

We performed biochemical analyses of 2 candidate missense mutations (S164F and F194S) of the fructose-1,6-bisphosphatase gene (FBP1), found in a Japanese patient with episodic hypoglycemia and lactic acidosis. Using the Flp-In system (Invitrogen/Life Technologies Corp., Carlsbad, CA, USA), we established 293 cell lines expressing the wild-type or the mutant FBP1. These cell lines had one copy of an FBP1-expressing construct at the same site of the genome. Therefore, each cell line might express wild-type or mutant FBP1 messenger (m) RNA in almost the same amounts. In fact, the expression of FBP1 mRNA did not differ markedly among these cell lines. As opposed to the amount of mRNA, the amount of FBP1 protein was markedly decreased in the cell lines expressing mutant FBP1 than in those expressing wild-type FBP1. When the mutant FBP1-expressing cell lines were cultured with MG132, a proteasome inhibitor, the amount of mutant FBP1 protein by each increased in an MG132-dosage-dependent manner. Next, we examined whether the small amount of residual mutant FBP1 protein had enzymatic activity. An in-vitro assay for enzymatic activity revealed that the 2 mutant proteins had null activities. The formation of the homotetramer is necessary for the activity of FBP1. Using native polyacrylamide gel electrophoresis, we examined the multimerization of mutant FBP1 proteins. Thus, the two mutant FBP1 proteins, S164F and F194S, never formed homotetramers.

The 2 mutant FBP1 proteins S164F and F194S were previously and independently reported in Japanese cases of FBP1 deficiency. Our data provide new evidence that these 2 Japanese founder mutations impair the enzymatic activity of FBP1 by preventing the formation of homotetramers.

The vertebrate-specific structures of the neural crest and 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-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 into 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 2 on the neural plate explant result 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.

The induced epithelia were first tested with reverse-transcription (RT)-PCR for GATA3/keratin19 as epidermis-specific markers, for Sox1/Sox3/Neurogenin1/NCAM as neural plate markers, for Slug/Snail1/Msx1/AP2/Zic1 as neural crest markers, and for Dlx5/Six1/Six4/Eya2 as neural plate border markers using cells collected from neural plate explants and the corresponding control cells to evaluate the validity of RT-PCR testing. We found that the expression levels of epidermis, the neural crest, and the neural plate border markers were all increased in the induced epithelia, in contrast to those in control cells.
We next examined the effect of Dlx5 downstream genes that are expressed in the neural plate and its border region on the induced epithelium by using RT-quantitative PCR. 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 expression of neural plate marker Sox2. The preplacodal ectoderm (PPE) or preplacodal ridge 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 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, such as Pax3/Brn3a, were also increased, albeit only slightly. 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 fibroblast growth factor 2. The induced epithelium might be a precursor of all exterior epithelia 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.

Establishment of a method for constructing a 3-dimensional gene expression model of inner-ear development

We attempted to establish a method for constructing 3-dimensional (3D) models of gene-expression patterns related to inner-ear development. To construct a 3D model, we prepared samples of whole mounts of in-situ hybridization, made cryosections, and captured images of each slice. The images were then imported into Amira (FEI Visualization Sciences Group, Bordeaux, France, and Zuse Institute Berlin, Berlin, Germany), a software program for 3D models, and reconstructed into a 3D model. From this model, we found differences in expression patterns between our data and previous reports. These differences were due to the intensity of gene expression, because our models detect expression at extremely low levels to allow detailed analysis. We also attempted to distinguish the intensity of gene expression with different colors, which indicated the center of the inducing area on the 3D models. Our 3D reconstruction models indicate gene expression in great detail.

Publications


