Division of Regenerative Medicine

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General Summary

Regenerative medicine is rapidly moving toward translation to clinical medicine. However, a better understanding of the molecular pathways that lead to human diseases is required for regenerative medicine to succeed. Disease models in genetically engineered mice are extremely useful but do not always precisely recapitulate the pathophysiology of human disease, especially neurodegenerative disorders. Good animal models will play a key role in studies leading to a greater understanding of the pathophysiology of neurodegenerative diseases. Recently, we have established a genetically modified primate model of human neurodegenerative disease. On the other hand, induced pluripotent stem (iPS) cell technology has allowed us to generate and expand various types of differentiated cell from patient-derived cells; these differentiated cells can be applied to cell therapy and to the study of the mechanisms of disease in human cells. Advances in disease modeling using patient-derived cells and primates will have huge effects on future opportunities and progress in biomedical research.

Research Activities

Disease modeling with iPS cells

We have generated 3 lines of iPS cells derived from patients with familial Parkinson's disease and optimized differentiation protocols for producing dopaminergic neurons from the iPS cells. Furthermore, we have started to generate iPS cells from patients with chronic renal disease and metachromatic leukodystrophy.

Function of neuronal Elav-like (Hu) proteins in embryonic and adult brain

The Hu proteins (the neuronal Elav-like: nElavl) are the mammalian homologue of *Drosophila* Elav, an RNA-binding protein expressed in the nervous system. In the embryonic brain, Hu family proteins (HuB/C/D) induce neuronal differentiation by binding preferentially to GU-rich sequences with secondary binding to AU-rich sequences in target RNAs (Hayashi et al. *J Neurosci Res.* 2015). To study the function of HuC in mature neurons, we generated HuC-deficient knockout (HuC KO) mice. At 7 months of age, HuC KO mice exhibited intention tremor, gait abnormality, and ataxia. Before the onset of these symptoms, the axons of Purkinje cells underwent the morphological changes of swelling and retraction at the deep cerebellar nuclei, although the pathological changes were not observed during cerebellar development.

Histological analyses showed accumulation of mitochondria and amyloid precursor protein in the swollen Purkinje axons, indicating that the axonal transport system might be impaired in HuC KO mice. To visualize mitochondrial dynamics in the axon, we infected Purkinje cells with a lentivirus encoding the photoconvertible fluorescent protein Kikume

Green-Red, which targets the mitochondrion. Time-lapse imaging of mitochondrial migration revealed a disturbance of axonal transport in HuC KO mice. Furthermore, to identify HuC targets, we performed an RNA-binding protein immunoprecipitation-microarray (RIP-CHIP) assay and high-throughput sequencing-crosslinking immunoprecipitation (HITS-CLIP) assay. Isolated candidate RNAs include Kinesin family members KIF2A, KIF3A, and KIF3C, which are involved in axonal transport. Overexpression of KIF3A or KIF3C in Purkinje cells derived from HuC KO mice partially reversed the swelling of axons. These results indicate that, at least in part, the pathophysiological mechanism of axonal degeneration in HuC KO mice is due to the down-regulation of the kinesin proteins.

Electron microscopic analysis revealed that most of the spheroids of HuC KO mice were filled with mitochondria, smooth endoplasmic reticula, autophagosomes, and highly dense bodies. Amazingly, some of the spheroids were filled with nuclei and ribosomes, which should be in the cytoplasm. Mitochondria, smooth endoplasmic reticula, and autophagosomes are transported normally along axons and are essential for the homeostasis of neurons. However, the nuclei and ribosomes are not transported into axons. These results indicate that the pathological mechanisms of spheroid formation are not simply explained but are rather complicated.

Multimodal and exclusive pathology between amyotrophic lateral sclerosis and frontotemporal lobar degeneration caused by mutations of TDP-43

The 43-kDa transactive response DNA-binding protein (TDP-43) gene (*TDP-43*) has been identified as a causative gene of both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Ubiquitin-positive cytoplasmic inclusion bodies containing TDP-43 are observed in neurons of patients with ALS or FTLD, and point mutations of the *TDP-43* gene have recently been found in both familial and sporadic cases of ALS and FTLD. However, the relationship between the pathogenesis of these conditions and the accumulation of inclusions is not clear, and even the multimodal/exclusive linkage of ALS with FTLD has not been revealed.

Here we generated 2 lines of mutant human *TDP-43* knock-in mice (mTDP-43 KI) and investigated the causal role between the gene mutation and ALS/FTLD phenotypes. We chose 2 mutants of the *TDP-43* gene, which were found in patients with either familial or sporadic ALS, by observing cultured cells holding multimodal structures of inclusions caused by overexpression of each mutant. Interestingly, considerable differences were observed in the phenotype and pathology between the 2 lines of mTDP-43 KI. Poor weight gain, decrease of motor function, and loss of motor neurons — phenotypes related to ALS — were more significant in 1 of the mTDP-43 KI lines. In this line, *TDP-43*-positive inclusions and cystatin C-positive Bunina bodies appeared in spinal cord motor neurons. In the other line, decreases in anxiety levels, which may be related to FTLD, were observed, but not in another mTDP-43 KI mouse. These observations indicate that the mutation site is the predominant factor that causes multimodal or exclusive pathologies. Our results based on reference of *TDP-43* mutations are useful information for developing therapeutic strategies for ALS and FTLD.

A transgenic nonhuman primate model of neurodegenerative diseases

Medical research based on animal models serves as a bridge between basic and clinical research. Among various experimental animals, a nonhuman primate model is of growing importance for research in neuroscience and related fields, including pharmacology, genetics, reproductive biology, and social behavior. The common marmoset (*Callithrix jacchus*), a small New World primate, is becoming increasingly popular in biomedical research, because of its advantage for translation to genetically close human systems. We used a lentiviral vector to generate a transgenic marmoset carrying a mutant form of human alpha-synuclein (SNCA) and TDP-43. The *SNCA* gene is responsible for PARK1- and PARK3-type Parkinson's disease with an autosomal dominant pattern of inheritance. On the other hand, *TDP-43* is thought to be a causative gene of ALS. Lentivirus-transduced embryos were transferred to surrogate mothers, and founder animals were obtained. The founders were analyzed with minimally invasive methods, such as positron emission tomography and magnetic resonance imaging. In collaboration with Keio University, we developed a motion capture system capable of reconstructing 3-dimensional hand positions of common marmosets and evaluated hand kinematics during food retrieval movement in both healthy and hemispinalized animals (Takemi et al. *Behav Brain Res.* 2014).
Advances in disease modeling using genetically modified primates will have a huge impact on future opportunities and progress in the research on neurodegenerative diseases.

A 9.4-tesla magnetic resonance imaging research system for magnetic resonance histology

Magnetic resonance histology is a powerful technology finely visualizing internal body structure and organs by dissecting at any plane in 3-dimensional imaging. Magnetic resonance imaging (MRI) has greatly extended the exploration of neuroanatomy, especially the wiring of nerve fibers in the brains of humans and animals. The quality of its images is guaranteed by high magnetic field MRI. Diffusion-tensor imaging is used to evaluate the anisotropic nature of water diffusion in tissues, and tractography might be used to evaluate 3-dimensional fiber structures.
Using a 9.4-T MRI, we visualized fine structures of human temporal bone, including Reissner's membrane; scala tympani, media, and vestibule; and nerve fibers. With diffusion-tensor imaging analyzed with an algorithm for tracking fibers, we successfully distinguished 4 separate nerves and tracked individual fibers to the end organ (cochlea, vestibules, and main trunk of facial nerve at the mastoid tip). This study was conducted in collaboration with Keio University and the Central Institute for Experimental Animals.

Publications

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