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 impact on future opportunities and progress in biomedical research.

Research Activities

Disease modeling with iPS cells
We have generated 2 lines of iPS cells derived from patients with metachromatic leukodystrophy, a lysosomal storage disease. Furthermore, we have started to generate iPS cells from patients with familial Parkinson's disease.

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. 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 rescued 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.

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 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. We investigated the causal role of the gene mutation in the ALS/FTLD phenotypes using gene knock-in mice. Two types of mutant TDP-43 knock-in (mTDP-43 KI) mice with mutations in different sites of the gene were generated to investigate the biological effects of each mutation. Considerable differences in phenotypes were observed among mTDP-43 KI mice and wild-type mice: poor weight gain and loss of spinal motor neurons, which is related to ALS symptoms, and a decrease in anxiety levels, which may be related to FTLD. Interestingly, one type of knock-in mouse exhibited predominantly motor dysfunction, and the other type showed behavioral abnormalities. These observations indicate that mutation sites of the *TDP-43* gene are the predominant factor which sink into multimodal or exclusive pathologies. Our results, referencing *TDP-43* mutations, will provide new insights into the pathophysiology of 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. Advances in disease modeling using genetically modified primates will have a huge impact on future opportunities and progress in the research on neurodegenerative diseases.

Publications

Kawagoe S, Higuchi T, Otaka M, Shimada Y, Kobayashi H, Ida H, Ohashi T, Okano HJ, Nakanishi M, Eto Y. Morphological features of iPS cells generated from Fabry disease skin fibroblasts using Sendai virus vector (SeVdp). *Mol Genet Metab.* 2013; **109:** 386–9.

Nishimoto Y, Nakagawa S, Hirose T, Okano HJ, Takao M, Shibata S, Suyama S, Kuwako K, Imai T, Murayama S, Suzuki N, Okano H. The long non-coding RNA nuclear-enriched abundant transcript 1_2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. *Mol Brain.* 2013; **6:** 31.

Seki F, Hikishima K, Nambu S, Okanoya K, Okano HJ, Sasaki E, Miura K, Okano H. Multidimensional MRI-CT atlas of the naked mole-rat brain (Heterocephalus glaber). *Front Neuroanat.* 2013; **7:** 45.

Sawada K, Hikishima K, Murayama AY, Okano HJ, Sasaki E, Okano H. Fetal sulcation and gyrification in common marmosets (Callithrix jacchus) obtained by ex vivo magnetic resonance imaging. *Neuroscience.* 2014; **257:** 158–74.

Fujioka M, Okamoto Y, Shinden S, Okano HJ, Okano H, Ogawa K, Matsunaga T. Pharmacological inhibition of cochlear mitochondrial respiratory chain induces secondary inflammation in the lateral wall: a potential therapeutic target for sensorineural hearing loss. *PLoS One.* 2014; **9:** e90089.