Research Center for Medical Sciences
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. Good animal models will play a key role in studies leading to a greater understanding of the pathophysiology of neurodegenerative diseases. On the other hand, induced pluripotent stem cell (iPSC) 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-related RNA binding proteins

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.

Electron microscopic analyses showed the accumulation of mitochondria, endoplasmic reticulum and cytosolic organelles, such as nuclei and ribosomes, in the swollen Purkinje axons, indicating that a diffusion barrier system between soma and axons might be impaired in HuC KO mice. When the data of electron microscopy and the previous analysis of HuC target RNAs were combined, AnkyrinG was identified as a responsible factor for these pathological phenomena. In neurons, AnkyrinG is distributed to the axon's initial segment and forms the size-dependent diffusion barrier between soma and axons. This system is needed to define the delicate protein distribution in neurons. Our studies have shown that HuC regulates the alternative splicing of AnkyrinG and that the splicing pattern of AnkyrinG is disrupted in HuC KO mice. Intriguingly, the expression level of the embryo-specific variant of AnkyrinG was increased in HuC KO. Furthermore, the embryonic and adult variants of AnkyrinG exhibited differential binding affinity to spec-

trin. This data indicates that HuC maintains the homeostasis of axons by controlling the alternative splicing of AnkyrinG.

The TAR DNA-binding protein of 43 kDa (TDP-43) gene (*TDP-43*) has been identified as a causative gene of both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), based on the pathological findings as ubiquitin-positive cytoplasmic inclusions containing TDP-43 protein. Point mutations of *TDP-43* have been found in patients with ALS or FTLD. A cause of neuronal cell death in neurodegenerative diseases is abnormal RNA metabolism, although the mechanisms are unclear. 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. Interestingly, considerable differences in the phenotype and pathology between the 2 lines of mTDP-43 KI were observed. Poor weight gain, decrease of motor function, loss of motor neurons, and 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.

Furthermore, to identify diagnostic biomarkers for ALS, we determined abnormal RNA metabolism in white blood cells of mTDP-43 KI lines. We found that messenger RNAs of both Smn1 and NLR family, apoptosis inhibitory protein 5 (*Naip5*) were candidate targets for further studies.

A primate model of human diseases

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. To investigate the use of magnetic resonance imaging diffusion-tensor imaging (DTI) to detect denervation of the nigrostriatal pathway in a marmoset model of Parkinson disease (PD) after treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The DTI and the tractography showed the loss of fiber structures of the nigrostriatal pathway in the PD model. Our study provides a potential basis for the use of DTI in the clinical diagnosis of PD (Hikishima K et al. *Radiology* 2015). Furthermore, decreases in the volume of the substantia nigra in a marmoset model of PD were detected with voxel-based morphometry and magnetic resonance imaging and confirmed with histologic findings as the degeneration of dopaminergic neurons (Hikishima K et al. *Neuroscience* 2015).

Disease modeling with iPSCs

Pallidopontonigral degeneration, part of frontotemporal dementia with parkinsonism related to chromosome 17, is caused by mutations in the microtubule associated protein tau gene (*MAPT)* encoding tau protein. We generated iPSCs from a patient with an N279K mutation of *MAPT* to investigate the underlying disease mechanism. In iPSC-derived neural stem cells, we observed accumulation of endosomes and exosomes and a reduction of lysosomes, which displayed impaired endocytic trafficking. Our experiments demonstrate that alterations of intracellular vesicle trafficking in neural stem cells and neurons likely contribute to neurodegeneration (Wren et al. *Mol Neurodegener* 2015*).*

Publications

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