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

HuC (Elavl3) KO mice, unique animal models that constantly develop slowly progressive axonal degeneration

Neuronal Elav-like (nElavl or neuronal Hu) proteins are RNA-binding proteins that regulate RNA stability and alternative splicing, which are associated with axonal and synaptic structures. nElavl proteins promote the differentiation and maturation of neurons via their regulation of RNA. The functions of nElavl in mature neurons are not fully understood, although Elavl3 (HuC) is highly expressed in the adult brain. Furthermore, possible associations between nElavl genes and several neurodegenerative diseases have been reported. We investigated the relationship between nElavl functions and neuronal degeneration using Elavl3 knockout (KO) mice. Elavl3 KO mice completely lost the expression of nElavl proteins in the Purkinje cells. Elavl3 KO mice exhibited slowly progressive motor deficits and late-onset cerebellar ataxia, and axons of Elavl3 KO Purkinje cells were swollen (spheroid formation), followed by the disruption of synaptic formation of axonal terminals. Deficit in axonal transport and abnormalities in neuronal polarity was observed in Elavl3 KO Purkinje cells. These results suggest that nElavl proteins are crucial for the maintenance of axonal homeostasis in mature neurons. Moreover, Elavl3 KO mice are unique animal models that constantly develop slowly progressive axonal degeneration. Therefore, studies of Elavl3 KO mice will provide new insight regarding axonal degenerative processes (Ogawa Y. et al. *Sci Rep.* 2018).

Neuronal RNA binding protein Elavl3 regulates neuronal polarity through the alternative splicing of an embryo-specific exon in AnkyrinG

Alternative splicing of RNAs diversifies the functionalities of proteins, and it is optimized

for each cell type and each developmental stage. nElavl proteins are the RNA-binding proteins that is specifically expressed in neurons, regulate the alternative splicing of target RNAs, and promote neuronal differentiation and maturation. We found that the alternative splicing of AnkyrinG exon 34 was misregulated in the cerebella of Elavl3 KO mice. AnkyrinG is an essential factor for the formation of neuronal polarity and is required for normal neuronal functions. We revealed that exon 34 of AnkyrinG was normally included in immature neurons and was mostly excluded in mature neurons; however, it was included in the cerebella of Elavl3 KO mice even in adulthood. In the Purkinje cells of adult Elavl3 KO mice, the length of the AnkyrinG-positive region shortened and somatic organelles leaked into the axons. These results suggested that exon 34 of AnkyrinG is an embryonic-stage-preferential exon that should be excluded from mature neurons and that Elavl3 regulates neuronal polarity through alternative splicing of this exon (Ogawa Y. et al. *Neurosci Res.* 2018).

Pathophysiological consequences of retromer dysfunction in neurological disorder
Retromer is a heteromeric protein complex that plays a critical role in endosome-to-Golgi retrieval of membrane proteins. In neurons, retromer is known to mediate retrograde trafficking from endosomes located in neuronal dendrites to Golgi elements located in the cell body. Recent studies demonstrate that retromer dysfunction is pathogenically linked to several brain disorders including Parkinson's disease (PD). Mutations of the retromer component Vacuolar Protein Sorting-35 (VPS35) is linked to autosomal dominant forms of familial PD, PARK17. However, the precise biological mechanisms by which the mutation causes the neurological disorder is not clear. To understand pathophysiological consequences of the mutations, we analyzed two independent iPS cell lines from PD patients heterozygous for the VPS35 mutation. After induction to dopaminergic neurons (DN), the movement of the retromer and early endosomes are monitored by using newly generated retromer-reporters which enable fluorescent live imaging. We found that the fluorescent-labeled retromer rapidly moved around within the cytoplasm and dendrites together with early endosomes in control DN, however the movement of early endosome was slower in PD group compared with healthy control. Our results suggest VPS35 mutation affects the normal delivery of lysosomal enzymes to the endosomal-lysosomal system. Our result indicates that VPS35 mutation causes mistrafficking and mislocalization of endosomes in PARK17 iPSC-derived neural cells. Recent studies have shown that retromer dysfunction is also linked to Alzheimer disease, indicating a pathogenic role in two of the most common neurodegenerative diseases. Retromer is a potential target in drug discovery and strengthening its functional activity would be a strong therapeutic promise for these neurological disorders.

Publications

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