Research Center for Medical Sciences
Division of Regenerative Medicine

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General Summary

Regenerative medicine is rapidly moving towards being translated to clinical medicine. However, for regenerative medicine to succeed, the molecular pathways that lead to human diseases must be better understood. To better understand the pathophysiology of neurodegenerative diseases, key roles with be played by studies with good animal models. On the other hand, to study the mechanisms of disease in human cells, differentiated cells of various types can generated and expanded from patient-derived cells via induced pluripotent stem cell (iPSC) technology; these differentiated cells can also be applied to cell therapy. Advances in disease modeling using cells derived from human patients and other primates will have great effects on future opportunities and progress in biomedical research.

Research Activities

Embryonic lethal and abnormal vision gene-like protein 3 regulates neuronal polarity through the alternative splicing

The importance of RNA metabolism in the process of neuronal differentiation and maturation has been widely reported. The RNA-binding proteins modulate RNA metabolism by interacting with target RNAs and regulating the stability, translation, and alternative splicing of RNAs. Neuronal embryonic lethal and abnormal vision gene (*Elav)*-like (Elavl) proteins, composed of Elavl2, Elavl3, and Elavl4, have 3 RNA recognition motifs. A previous study has shown that the long form of Elavl4 localizes in the cytosol and predominantly regulates the stability of RNAs. In contrast, the short form of Elavl4 localizes in the cytosol and nucleus and regulates the alternative splicing of RNAs. The Elavl3 knockout mice exhibited slowly progressive motor deficits and late-onset cerebellar ataxia, and axons of Elavl3 knockout Purkinje cells were swollen (spheroid formation), after which the synaptic formation of axonal terminals was disrupted. A deficit of axonal transport and abnormalities in neuronal polarity were observed in Elavl3 knockout Purkinje cells. We found that the alternative splicing of ankyrin-G (AnkG) exon 34 was misregulated in the cerebella of Elavl3 knockout mice. AnkG localizes at the axon initial segment and nodes of Ranvier in neurons and assembles voltage-gated sodium channels, IV-spectrin, neurofascin, and other related proteins. The clustering of AnkG at the axon initial segment generates the molecular-size-restricted diffusion barrier and substrate-selective filter between the soma and axon and then maintains neuronal polarity. Our study demonstrated that exon 34 of AnkG is alternatively spliced during neuronal development and that misregulation of the alternative splicing of this exon would affect neuronal function. Purkinje cells of Elavl3$^{-/-}$ mice have unique characteristics, and neurons

expressing the "embryonic form" of AnkG, containing exon 34, are observed even in the mature brain. Recently, the existence of immature neurons in adult brains and the abnormal activities of these neurons have been implicated in several psychological disorders. The study of Elavl3$^{-/-}$ mice might also contribute to a better understanding of the relationship between the existence of immature neurons and neurological disorders (Ogawa Y. et al. *Neurosci Res.* 2018).

Cell biological study of an Okinawan large pedigree with bipolar disorder and recurrent depressive disorder

Because bipolar disorder is a psychiatric disease characterized by high genetic heterogeneity, its pathophysiology remains unclear. Despite a genetic component to bipolar disorder, the genomic variations that strongly and directly contribute to this disease have not been specified. A research group of Ryukyu University, our collaborator, found a large family with an autosomal dominant inheritance pattern of bipolar disorder and recurrent depressive disorder in an isolated island of Okinawa. The researchers obtained informed consent from participants and collected blood samples of 8 affected and 8 unaffected individuals. Detected with parametric linkage analysis in a chromosome region was a significant linkage peak, which previous studies have repeatedly reported as a link to bipolar disorder and depression. Whole genomic sequencing-based haplotype phasing determined that a rare haplotype in the chromosomal linkage region was shared among affected individuals of the pedigree. Furthermore, we generated iPSC lines from 3 affected and 4 unaffected individuals of the pedigree and differentiated iPSCs into neurons. An investigation of the phenotype of the iPSC-derived neurons is underway and is expected to uncover the relationship between the genomic variant and the neuronal phenotype.

Primates brain imaging repository for comparative neuroscience

High-resolution magnetic resonance imaging (MRI) and computational analysis technology enable various primate brains to be compared in a 3-dimensional electronic format and to provide precious information about common features across primates and species-specific features in neuroanatomy. To facilitate scientific discoveries in the field of comparative neuroanatomy and brain evolution, we launched a collaborative project to develop as an open resource a repository of nonhuman primate brain images with *ex vivo* MRI. In an initial attempt, we released a collection of structural MRI and diffusion tensor images obtained from 12 species: pygmy marmoset, owl monkey, white-fronted capuchin, crab-eating macaque, Japanese macaque, bonnet macaque, toque macaque, Sykes' monkey, red-tailed monkey, Schmidt's guenon, De Brazza's guenon, and Lar gibbon. Sixteen postmortem brain samples from the 12 species, stored in the Japan Monkey Centre (JMC), were scanned with a 9.4-T MRI scanner of The Jikei University and made available through the JMC collaborate research program (http://www.j-monkey.jp/bir/index.html). The JMC Primates Brain Imaging Repository would contribute as a resource for comparative neuroscience research, an optimizing method to scan large fixed brains, and a reference for veterinary neuroradiology and would preserve various primate brains, including those of endangered species, in a permanent digital form (Sakai T. et al. *Primates.* 2018).

Publications

Ogawa Y, Yamaguchi J[1], Yano M[2], Uchiyama Y[1], Okano HJ ([1]Juntendo Univ, [2]Niigata Univ). Elavl3 regulates neuronal polarity through the alternative splicing of an embryo-specific exon in AnkyrinG. *Neurosci Res.* 2018 Oct; **135:** 13-20.

Maruyama T[1], Baba T[1], Maemoto Y[1], Hara-Miyauchi C, Hasegawa-Ogawa M, Okano HJ, Enda Y[1], Matsumoto K[1], Arimitsu N[1], Nakao K[2], Hamamoto H[2], Sekimizu K[2], Ohto-Nakanishi T[3], Nakanishi H[4], Tokuyama T[1], Yanagi S[1], Tagaya M[1], Tani K[1] ([1]Tokyo Univ of Pharm Life Sci, [2]Univ of Tokyo, [3]Japan Lipid Tech, [4]Akita Univ). Loss of DDHD2, whose mutation causes spastic paraplegia, promotes reactive oxygen species generation and apoptosis. *Cell Death Dis.* 2018 Jul **23; 9:** 797.

Saito S[1], Ozawa H[1], Fujioka M[1], Hikishima K[2], Hata J, Kurihara S, Okano HJ, Ogawa K[1] ([1]Keio Univ, [2]OIST). Visualization of nerve fibers around the carotid bifurcation using a 9.4 T microscopic magnetic resonance diffusion tensor imaging with tractography. *Head Neck.* 2018 Oct; **40:** 2228-34.

Tajiri S, Yamanaka S, Fujimoto T, Matsumoto K, Taguchi A[1], Nishinakamura R[1], Okano HJ, Yokoo T ([1]Kumamoto Univ). Regenerative potential of induced pluripotent stem cells derived from patients undergoing haemodialysis in kidney regeneration. *Sci Rep.* 2018 Oct 8; **8:** 14919.

Sakai T[1], Hata J, Ohta H, Shintaku Y[2], Kimura N[3], Ogawa Y, Sokabe K[4], Mori S[1], Okano HJ, Hamada Y[2], Shibata S[5], Okano H[5], Oishi K[1] ([1]Johns Hopkins Univ, [2]Kyoto Univ, [3]JPN Monkey Ctr, [4]Tokyo Metrop Univ, [5]Keio Univ). The Japan Monkey Centre Primates Brain Imaging Repository for comparative neuroscience: An archive of digital records including records for endangered species. *Primates.* 2018 Nov; **59:** 553-70.