Research Center for Medical Sciences
Division of Regenerative Medicine

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General Summary

Regenerative medicine might soon be 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 be 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.
Magnetic resonance imaging (MRI) is a powerful and flexible imaging tool for diagnosis in clinical practice. Sophisticated MRI hardware enables image assessment from small experimental animals, such as mice, rats and marmosets, at a resolution of several tens of microns. In particular, diffusion tensor imaging (DTI) is a promising method for characterizing structural differences with neuropathology because DTI is highly sensitive to changes at the microstructural level. We use DTI, structural MRI, and MR spectroscopy to investigate brain structure and functions.

Research Activities

Anatomical and surgical evaluation of the common marmoset as an animal model in hearing research
Sensorineural hearing loss (SNHL) is most often associated with impairments of the cochlear hair cells. Cochlear hair cell dysfunction can be caused by aging, infection, exposure to loud noises, and ototoxic drug abuse. Mammalian cochlear hair cells do not spontaneously regenerate, and current treatments for chronic SNHL are ineffective. However, basic research with rodent models has contributed to the understanding of the underlying cause of hearing loss and the development of potentially effective therapies. Direct administration of viral vectors or small compounds to the inner ear might aid in the treatment of SNHL. However, owing to species differences between humans and rodents, translating experimental results into clinical applications remains challenging. Therefore, when evaluating potential interventions for hearing loss, research involving nonhuman primate models is required to minimize the species gap. Moreover, for the successful delivery of potential therapies, the appropriate surgical approach must be accurately defined in such a model. The common marmoset (*Callithrix jacchus*), a New World monkey, is a valuable nonhuman primate model because of its small size, high reproductive efficiency, and the cross-reactivity of its cytokines and hormones with their human coun-

terparts. We describe morphometric data acquired from the temporal bone of the common marmoset to define the routes of topical drug administration to the inner ear. Dissection and DTT were performed on the fixed cadaverous heads of 13 common marmosets. To investigate potential routes for drug administration to the inner ear, we explored the anatomy of the round window, oval window, semicircular canal, and endolymphatic sac. Of these routes, an approach *via* the round window with posterior tympanotomy appeared feasible for delivering drugs to the inner ear without manipulating the tympanic membrane and thereby minimizing the chances of conductive hearing loss. The courses of 4 critical nerves, including the facial nerve, were visualized with 3-dimensional DTT, which might help nerve damage to be avoided during surgery. Finally, to investigate the feasibility of actual drug administration, we measured the volume of the round window niche, which was approximately 0.9 μL. The present findings might help establish experimental standards for evaluating new therapies in this primate model (Kurihara S. et al. Front Neuroanat. 2019).

Cell biological study of hereditary motor and sensory neuropathy with proximal dominant involvement

Hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P) is an autosomal-dominant neurodegenerative disorder characterized by late-onset progressive muscle weakness of proximal limbs followed by distal sensory involvement. A large family affected by HMSN-P has been reported from the Okinawa Islands. Pathological studies of HMSN-P revealed 43-kDa transactivation response DNA-binding protein (TDP-43)-positive cytoplasmic inclusion bodies in the spinal and cortical motor neurons, shared with findings in amyotrophic lateral sclerosis. Furthermore, a heterozygous mutation in Tropomyosin-receptor kinase Fused Gene (TFG) was identified as the responsible gene for HMSN-P. A research group of Okinawa National Hospital, our collaborator, found a large family with HMSN-P in an island of Okinawa. The researcher obtained informed consent from participants and collected blood samples of affected and unaffected individuals in the family. Currently, we generated iPSC lines from 3 affected and 3 unaffected individuals of the pedigree and differentiated iPSCs into motor 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.

Primate brain image analysis using high-field MRI and technological development for comparative neuroscience

The development of nuclear magnetic resonance (NMR) imaging techniques has enabled us to extract macroscopic biological information and achieve three-dimensional analysis of rodent and primate brains. In recent years, there has been a growing momentum to create three-dimensional brain maps (MRI images, tissue images) of human and closely related primate models and to use them to elucidate higher brain functions and psychiatric and neurological disorders in humans. In collaboration with RIKEN, Kyoto University Primate Research Institute, Johns Hopkins University, and Keio University, we have developed a brain imaging database last year by collecting brain anatomy and brain circuit data of primates using our high-field MRI system (9.4T). (Sakai et al, Primates.

2018) (http://www.j-monkey.jp/BIR/index.html). In this fiscal year, we achieved the acquisition of knowledge and technological development to further accelerate the analysis of brain images, such as the development of high-precision segmentation techniques for brain structure using deep learning (Ito R, et al, Neural Networks. 2019), the development of contrast techniques with pathological images (Huo B, et al, Eur J Neursci. 2019), and the development of visualization techniques for biological information and brain function, such as the development of cell type differentiation methods using MRI (Hata J, et al. PLos One. 2019), the evaluation of brain ischemic status by high-precision restrictive tissue structure analysis (Ohki A, et al, Magn Reson Imaging. 2019), and the detection of neural circuit activity disorders in autism (Tsurugizawa T, et al. Science Advances. 2020). This will lower the hurdle for many researchers and experts in medical and biological sciences as well as in mathematical statistics, deep learning, etc. to engage in primate brain science research. It is also expected to bring a new frontier in primate science research as basic and bridging research.

Publications

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