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

Diagnostic biomarkers for amyotrophic lateral sclerosis (ALS) have yet to be identified. One of the causes of neuronal cell death in neurodegenerative diseases is abnormal RNA metabolism, although the mechanisms by which this occurs are unclear. Detection of abnormal RNA metabolism in white blood cells (WBCs) could lead to a new biomarker of ALS onset. TAR DNA-binding protein 43 kDa (TDP-43) is an RNA-binding protein that regulates RNA metabolism. We previously developed a mouse model of ALS that exhibits adult-onset motor dysfunction; these mutant TDP-43 knock in (KI) mice heterozygously express mutant human TDP-43 (A382T or G348C). In the present study, we examined TDP-43 mRNA levels in WBCs of KI mice and found that A382T mutant mRNA is significantly higher than G348C. Our results suggest that each mutant TDP-43 induces distinct RNA metabolism, and that the expression of total TDP-43 alone in WBC is not suitable as an ALS biomarker. To identify additional candidates, we focused on survival and apoptosis-related factors and examined their mRNA metabolism in WBCs. mRNA levels of both Smn1 and Naip5 correlated with TDP-43 levels and also differed between A382T and G348C. Together, TDP-43 and these factors may enable detection of abnormalities in individual ALS pathologies (Hasegawa M. et al. *Neurosci Res*. 2016).

In vivo modeling of human diseases

During rodent experiments, the caudal ventral artery (CVA) is useful for blood pressure (BP) measurement. However, CVA measurements may not reflect the true BP. This study was performed to verify the site-specific accuracy of invasive arterial BP monitoring during surgery in rats. Invasive arterial BP was simultaneously measured in rats via the CVA

and the common carotid artery (CCA). The BP values were analysed while the rats were subjected to cooling of the head or tail. Additionally, the rats underwent digital subtraction angiography and histological examination of these arteries. The pressure difference was more significant in the tail cooling group than in the head cooling group. Digital subtraction angiography revealed that angiospasms occurred more frequently in the CVA than in the CCA upon cooling. This phenomenon was supported by histological analysis, which showed that the tunica media area was significantly larger in the CVA than in the CCA. CVA pressure is susceptible to environmental changes and may not accurately reflect the true BP without a strictly controlled laboratory environment. Therefore, understanding the pitfalls of this method is necessary to avoid cooling of the tail during BP measurement (Ohta H. et al. *Sci Rep.* 2017).

fMRI was conducted to investigate allodynia in mice; allodynia was generated by surgical injury at the L4 spinal nerve root, thus selectively stimulating sensory nerve fibers. In intact mice, only the primary somatosensory cortex (S1) was activated by stimulation of Aβ-fibers. Meanwhile, allodynic mice showed significantly higher BOLD signals in the anterior cingulate area (ACA) and thalamus. Using resting state fMRI, both degree and eigenvector centrality were significantly decreased in the contralateral S1, clustering coefficient and local efficiency were significantly increased in the ACA, and betweenness centrality was significantly higher in the ventral posterolateral nucleus of the thalamus. These results suggest that the observed abnormal BOLD activation is associated with defects in Aβ-fibers when Aβ-fibers in allodynic mice are selectively stimulated（Komaki Y. et al. *Sci Rep.* 2016).

Effects of high-energy particles on neural activity in cortical neurons

"*Light flashes" (LF),* are phosphenes reported by most astronauts in space missions outside the magnetosphere of the Earth. The conditions of occurrence have been thought retinal effects of cosmic ray including heavy ions or protons. A small fraction of the LF might be caused by Cherenkov radiation, while the majority is probably caused by some kind of direct interaction with elements in the retina, as some reports suggest LF could also be sensations of light produced by the activation of neurons along the visual pathway. However cell biological mechanisms of LF have not been addressed yet.

A research group of National Institute of Radiological Sciences (NIRS) in Japan constructed a microbeam facility (named as SPICE) by using our HVEE Tandem accelerator (3.4 MeV proton) and they have shown various biological effects of microbeam irradiation with protons in culture cells. This imaging system, with the position resolution less than 2μm, enables to perform Ca imaging of a single cultured neuron that attach down the bottom of a culture dish. Intracellular calcium is a second messenger that plays important roles in regulating many cell functions and Ca imaging allows real-time analyses of individual cells.

To develop an *in vitro* experimental system, we irradiated cultured mouse cortical neurons with repeated bursts of protons by SPICE and observed neuronal activities by Ca imaging before and after irradiation. The proton irradiation evoked calcium responses in an irradiated single neuron and also it seemed to inhibit the spontaneous calcium oscillation in surrounding cells, which might be a bystander effect of irradiation.

In spaceflight, especially in long travels like Mars mission, the astronauts' brain is exposed to cosmic ray including many protons, from which we are normally protected by the Earth's magnetic shield. Understanding the cell biological bases of the effects of protons to the brain function will be a priority issue in preparations of manned deep space missions.

Publications

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