Research Center for Medical Sciences
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General Summary

The research group run by Professor Sasaki (applied molecular medicine) continued to work on regenerative medicine for pancreatic islets. The group also collected and accumulated clinical samples to find novel volatile biomarkers for inflammatory and metabolic diseases in humans and performed multivariate analysis with a gas chromatography technique. The research group run by Professor Watabe (molecular and behavioral neurosciences) focuses on the neuronal mechanisms regulating aversive and affective memory formation and adaptive behaviors, using molecular, cellular, electrophysiological, and behavioral techniques.
In addition to performing our own research activities, we continue to engage in an educational laboratory course program with the assignment of third-year students from the School of Medicine. We also fulfill research support duties for registered researchers from the University Hospital at Kashiwa campus so that physician-researchers can work efficiently.

Research Activities

Mechanism of islet injury and beta cell regeneration in diabetes mellitus
Pancreatic islet β cells have a unique function to secrete insulin depending on the blood glucose concentration (glucose-stimulated insulin secretion, GSIS). Under *in vivo* circumstances, this function is finely regulated by the nervous system, the microcirculation system, and hormones and metabolites, whereas the failure of this function causes type 2 diabetes. Furthermore, insulinoma, in which regulatory functions such as GSIS are also lost, shows inappropriate hypersecretion. To identify abnormalities of the system of insulin secretion, in a project of the current fiscal year approved by The Jikei University Ethics Committee, we extracted genomic DNA, total RNA, and protein components from the surgical specimens of insulinoma tumor tissue. In this study, we referred to the genome from peripheral blood nucleated cells as the germline of the same person (a patient with insulinoma). When genomes were analyzed in the germline (1,650 million reads, 248 billion bases) and in insulinomas (1,920 million reads, 287.9 billion bases) and compared with the international standard University of California at Santa Cruz reference sequence human genome 19 generic annotation file (UCSC hg19), mutations of 1.3 million blood cells and insulinomas were found. When analysis was limited to high-precision reads of the sequences, 540,000 sites (hereinafter referred to as "PASS") were found. Of the PASS, 67 genes were found to be mutated in insulinomas but not in the germline, and 92 genes were mutated in blood cells but not in insulinomas. Furthermore, of the mutations in

PASS, 90,787 were in the exon region, of which 41 were definitely pathogenic and 7 were likely pathogenic (they differed from UCSC hg19 in both the germline and insulinomas, suggesting that these originated from the germline genome). A study of whether these 48 exonal variations are responsible for the dysregulation of insulin secretion in insulinoma cells could lead to a better understanding of insulin secretion failure in diseases, including diabetes. This research was supported by a Grant-in-Aid for Scientific Research, with Professor Sasaki as the principal researcher.

Search with gas chromatography for novel volatile biomarkers in breath and skin gas
Substances derived from volatile organic compounds (VOCs) are detected as 400 or more types of volatile components in human breath, and many of them are attributable to metabolism in the body. The types of VOCs may differ depending on the pathological condition, and detection of these differences can be applied to early, noninvasive diagnosis and preemptive medicine. The VOCs exhaled by patients with inflammatory conditions were analyzed in a research study in collaboration with Professor Masami Nemoto of The Jikei Katsushika Medical Center and with the approval of the Ethics Committee of this university. Gas was analyzed with gas chromatography/mass spectrometry in collaboration with Professor Takeo Iwamoto (Division of Molecular Cell Biology), and VOCs that were quantitatively and qualitatively different from those of healthy people were searched for. The detected VOCs were identified with a mass spectrum database compiled by the National Institute of Standards and Technology, and multivariate analysis was performed of associations with diseases. The results so far have clearly shown a specific pattern of VOCs (a profile formed by a plurality of VOCs) in patients with inflammatory diseases. In the future, we will consider using artificial intelligence to identify the specific pattern for each disease. This research was performed at The Jikei University, where Professor Sasaki was the research leader, and with the support of the pioneering joint research promotion fund.

Elucidating the circuitry mechanisms underlying fear memory learning
Avoiding pain and harm is fundamental for the survival of humans and other animals. Aversive stimuli, therefore, potently induce adaptive behaviors and memory formation. Clarifying neuronal circuitry mechanisms underlying such adaptive behaviors is fundamental to understanding brain functions. Furthermore, the dysregulation of the neuronal circuitry of such aversive behaviors leads to various anxiety disorders, such as posttraumatic stress disorder, and other psychiatric diseases.
The amygdala is acknowledged as a critical brain region to attach the aversive valence of nociceptive stimuli onto various sensory stimuli. This association is considered to be mediated *via* synaptic plasticity, which underlies certain forms of learning paradigm, such as fear conditioning. Although neuronal networks and plasticity mechanisms for fear conditioning have been intensively studied, not much is known about how the emotional value of pain itself is regulated at the circuitry level.
In previous studies, we have identified one such nociceptive pathway: neurons in parabrachial nuclei (PB) of the pons form a direct monosynaptic projection on the central amygdala (CeA). We found that the PB–CeA pathway is necessary and sufficient for fear mem-

ory formation, suggesting that the PB–CeA pathway might be involved in some emotional aspects of pain.

Our research in 2018, with real-time conditioned place avoidance in a Y-maze test, found that the optogenetic activation of the PB–CeA pathway is perceived by mice as an aversive signal (in preparation for publication). Also, we have reported in a review article that the PB serves as an integration site for multimodal information, including pain, hunger, taste, and general metabolism; therefore, synaptic plasticity at the PB–CeA pathway might contribute to the modification of the emotional valence of sensory information (Nagase et al., Curr Opin Behav Neurosci., 2019). We have contributed to a study of lysosomal storage disease conducted by Professor Ohashi (Division of Gene Therapy, Department of Pediatrics).

We found that a mouse model of MGII with LSD knock-out exhibited impaired fear memory formation and that cell-targeted gene therapy with strong preconditioning significantly improved the phenotype to a level comparable to that of wild-type mice (in preparation). These works were supported by a Grant-in-Aid for Scientific Research (C), the Strategic Research Program, and Core Research for Evolutional Science and Technology to Professor Watabe and by the Agency for Medical Research and Development and a Grant-in-Aid for Scientific Research (B) to Professor Ohashi.

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