Research Center for Medical Sciences
Institute of Clinical Medicine and Research

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General Summary

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 continued to engage in an educational laboratory course program with the assignment of third-year medical students. We also fulfill research support duties for registered researchers of The Jikei University Hospital at Kashiwa campus so that physician-researchers can make the best achievements.

Research Activities

Elucidating the circuitry mechanisms underlying aversive and appetitive learning
Avoiding pain and harm is fundamental for the survival of human and animals. Aversive stimuli, therefore, potently induce adaptive behaviors and memory formation. Clarifying neuronal circuitry mechanisms underlying such adaptive behaviors is fundamental for understanding brain functions. Furthermore, the dysregulation of the neuronal circuitry of such aversive behaviors leads to various anxiety disorders, such as posttraumatic stress disorders, 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 the parabrachial nucleus (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 memory formation, suggesting that the PB-CeA pathway might be involved in some emotional aspects of pain.
As for our research in 2019, 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, and, therefore, that the 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).

Regarding collaborative research, we have contributed to a study of lysosomal storage diseases performed by Professor Toya Ohashi (Division of Gene Therapy, Department of Pediatrics). We found that a mouse model of MGII with lysosomal storage disease knock-out exhibited impaired fear memory formation and that cell-targeted gene therapy with strong preconditioning significantly improved the phenotype to the level comparable to that of wild-type mice (in preparation). These works were supported by a Grant-in-Aid for Scientific Research (B), Strategic Research Program, the Japan Agency for Medical Research and Development (AMED) and Core Research for Evolutional Science and Technology to Professor Watabe; AMED and a Grant-in-Aid for Scientific Research (B) to Professor Ohashi.

Predicted markers of overall survival in patients with pancreatic cancer receiving dendritic cells pulsed with Wilm's tumor protein 1 peptide

We evaluated predictive markers of survival on patients with pancreatic ductal adenocarcinoma (PDA) treated with multiple MHC class I/II-restricted Wilm's tumor protein 1 (WT1) peptide-pulsed dendritic cell vaccines in combination with chemotherapy. The plasma levels of soluble factors (myeloperoxidase, matrix metalloproteinase 9, and transforming growth factor β1) derived from granulocytes in 7 eligible patients with PDA were examined. Compared with the 4 non-super responder patients (overall survival < 1 year), the remaining 3 super responder patients (overall survival ≥ 1 year) showed significantly lower plasma levels of matrix metalloproteinase 9 throughout long-term therapy. Prolonged low levels of a granulocyte-related systemic inflammatory response after the early period of therapy and low WT1 cytoplasmic expression in PDA cells might be predictive markers of survival for patients with PDA receiving WT1-targeting immunochemotherapy. This study was supported by a Grant-in-Aid for Scientific Research (C) to SK.

Development of a qualitative analysis method for high-density lipoprotein capacity and investigation of biomarkers for cardiovascular diseases in diabetic kidney disease

We have established a new method for evaluating high-density lipoprotein-mediated cellular cholesterol efflux capacity with a stable isotope and reported this method and the related study results (J Lipid Res 2019; 60: 1959-67). Our clinical research indicated the significant relevance of uric acid and homocysteine to renal function (estimated glomerular filtration rate), suggesting the possibility that they are markers for the presumption of vascular disorder risk. This research was supported by Grant-in-Aid for Scientific Research (C) to HY.

Mechanism of islet injury and beta cell regeneration in diabetes mellitus

Pancreatic islet β cells have a unique function to secrete insulin depending on blood glucose concentration (glucose-stimulated insulin secretion, GSIS). Under *in vivo* circumstances, this function is finely regulated by the nervous system, the microcirculation system, hormones, and metabolites, whereas the failure of this function causes type 2 diabetes mellitus. Furthermore, insulinoma, in which regulatory functions, such as GSIS, are also lost, shows inappropriate hypersecretion. To identify abnormalities of insulin secretion machinery, in a study of the current fiscal year approved by The Jikei University

Ethics Committee, we extracted genomic DNA, total RNA, and protein components from 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 (1.65 billion reads, 248 billion bases) and in the insulinoma (1.92 billion reads, 287.9 billion bases) and compared to the international standard University of California at Santa Cruz reference sequence human genome 19 generic annotation file (UCSC hg19), mutation of 1.3 million blood cells and insulinomas were found. When analysis was limited to high-precision reads of the sequence, 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.

Publications

Matsumura K, Seiriki K, Okada S, Nagase M, Ayabe S, Yamada I, Furuse T, Shibuya H, Yasuda Y, Yamamori H, Fujimoto M, Nagayasu K, Yamamoto K, Kitagawa K, Miura H, Gotoda-Nishimura N, Igarashi H, Hayashida M, Baba M, Kondo M, Hasebe S, Ueshima K, Kasai A, Ago Y, Hayata-Takano A, Shintani N, Iguchi T, Sato M, Yamaguchi S, Tamura M, Wakana S, Yoshiki A, Watabe AM, Okano H, Takuma K, Hashimoto R, Hashimoto H, Nakazawa T. Pathogenic POGZ mutation causes impaired cortical development and reversible autism-like phenotypes. *Nat Commun.* 2020 Feb 26; **11**(1): 859. doi: 10.1038/s41467-020-14697-z. PMID: 32103003; PMCID: PMC7044294.

Hirowatari Y, Yoshida H. Innovatively Established Analysis Method for Lipoprotein Profiles Based on High-Performance Anion-Exchange Liquid Chromatography. *J Atheroscler Thromb.* 2019 Dec 1; **26**(12): 1027-1040. doi: 10.5551/jat.RV17037. Epub 2019 Sep 20. PMID: 31548491; PMCID: PMC6927812.

Yoshida S, Ito Z, Suka M, Bito T, Kan S, Akasu T, Saruta M, Okamoto M, Kitamura H, Fujioka S, Misawa T, Akiba T, Yanagisawa H, Sugiyama H, Koido S. Clinical Significance of Tumor-Infiltrating T Cells and Programed Death Ligand-1 in Patients with Pancreatic Cancer. *Cancer Invest.* 2019; **37**(9): 463-477. doi: 10.1080/07357907.2019.1661427. Epub 2019 Sep 18. PMID: 31490702.

Yoshida H, Tada H, Ito K, Kishimoto Y, Yanai H, Okamura T, Ikewaki K, Inagaki K, Shoji T, Bujo H, Miida T, Yoshida M, Kuzuya M, Yamashita S. Reference Intervals of Serum Non-Cholesterol Sterols by Gender in Healthy Japanese Individuals. *J Atheroscler Thromb.* 2020 May 1; **27**(5): 409-417. doi: 10.5551/jat.50187. Epub 2019 Sep 5. PMID: 31484845; PMCID: PMC7242229.

Shimizu T, Miyazaki O, Iwamoto T, Usui T, Sato R, Hiraishi C, Yoshida H. A new method for measuring cholesterol efflux capacity uses stable isotope-labeled, not radioactive-labeled, cholesterol. *J Lipid Res.* 2019 Nov; **60**(11): 1959-1967. doi: 10.1194/jlr.D086884. Epub 2019 Aug 27. PMID: 31455616; PMCID: PMC6824490.

Yanai H, Yoshida H. Beneficial Effects of Adiponectin on Glucose and Lipid Metabolism and Atherosclerotic Progression: Mechanisms and Perspectives. *Int J Mol Sci.* 2019 Mar 8; **20**(5): 1190. doi: 10.3390/ijms20051190. PMID: 30857216; PMCID: PMC6429491.

Yoshida H. Clinical Impact and Significance of Serum Lipoprotein (a) Levels on Cardiovascular Risk in Patients With Coronary Artery Disease. *Circ J.* 2019 Apr 25; **83**(5): 967-968. doi: 10.1253/circj.CJ-19-0221. Epub 2019 Apr 4. PMID: 30944264.

Ito Z, Kan S, Bito T, Horiuchi S, Akasu T, Yoshida S, Kajihara M, Hokari A, Saruta M, Yoshida N, Kobayashi M, Ohkusa T, Shimodaira S, Okamoto M, Sugiyama H,Koido S. Predicted Markers of Overall Survival in Pancreatic Cancer Patients Receiving Dendritic Cell Vaccinations Targeting WT1. *Oncology.* 2019; **97**(3): 135-148. doi: 10.1159/000500359. Epub 2019 Jun 19. PMID: 31216557.