Kagawa School of Pharmaceutical Sciences
Tokushima Bunri University

Measurement and functional analysis of proteins

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Research

Theme 1. Quantification of trace amount of proteins (Etsuro Ito, Mika Morikawa, Mugiho Kaneda)

We are developing the method to detect and quantify very tiny amount of a given protein in a small volume, such as in a single cell. We aim at establishing an enterprise to commercialize protein quantification reagents and kits based on this technique. Using our "enzyme cycling method", it is now possible to detect protein with ten thousands as high sensitivity as ever. The improved method with more sensitivity and easier to handle will be available in the near future.

Grant Support: Grants from Ministry of Education, Culture, Sports, Science and Technology, Japan Society for the Promotion of Science, Japan Science and Technology Agency, and some foundations.

Collaborations: With private companies and other universities.

Theme 2. Molecular mechanisms of learning and memory (Etsuro Ito, Miki Yamagishi, Mika Morikawa, Mugiho Kaneda)

We are attempting to clarify the molecular mechanisms of learning and memory using 3xTg-Alzheimer disease mice and the pond snail *Lymnaea stagnalis*. We made clear the effect of insulin in the brain on long-term synaptic changes and long-term memory.

Grant Support: Grants from Ministry of Education, Culture, Sports, Science and Technology, Japan Society for the Promotion of Science, Japan Science and Technology Agency, and some foundations.

Collaborations: With private companies and other universities.

Theme 3. The Study of the structure and the dynamics of proteins by means of site-directed spin-labeling ESR (Shoji Ueki)

The spin label reagent attached to cysteine residue tells us its environment and the structural information of the protein through the ESR spectrum. In other words, the spin label acts as a reporter. In the case of two spin labels introduced into a protein, we can get the distance between two labels by the spectrum. The merit of this method is that we can monitor only the spin label whatever the sample condition is. So we can measure the membrane protein in lipid for example, that is difficult to measure by other spectroscopic method.

Publications (2010~2015)

[Original papers]

2014

1. Okada, R., Ikeno, H., Kimura, T., Ohashi, M., Aonuma, H., and Ito, E. (2014) Error in the honeybee waggle dance improves foraging flexibility. Sci Rep 4, 4175.
2. Mita, K., Yamagishi, M., Fujito, Y., Lukowiak, K., and Ito, E. (2014) An increase in insulin is important for the acquisition conditioned taste aversion in *Lymnaea*. Neurobiol Learn Mem 116, 132-138.
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4. Matsuo, R., Kobayashi, S., Wakiya, K., Yamagishi, M., Fukuoka, M., and Ito, E. (2014) The cholinergic system in the olfactory center of the terrestrial slug *Limax*. J Comp Neurol 522, 2951-2966.
5. Watabe, S., Kodama, H., Kaneda, M., Morikawa, M., Nakaishi, K., Yoshimura, T., Iwai, S., Miura, T., and Ito, E. (2014) Ultrasensitive enzyme-linked immunosorbent assay (ELISA) of proteins by combination with the thio-NAD cycling method. BIOPHYSICS 10, 49-54.
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2013

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2. Elekes, K., Battonyai, I., Kobayashi, S., and Ito, E. (2013) Organization of the procerebrum in terrestrial pulmonates

(*Helix*, *Limax*) reconsidered: Cell mass layer synaptology and its serotonergic input system. Brain Struct Funct 218, 477-490.
3. Murakami, J., Okada, R., Fujito, Y., Sakakibara, M., Lukowiak, K., and Ito, E. (2013) Paired pulse ratio analysis of insulin-induced synaptic plasticity in the snail brain. J Exp Biol 216, 1771-1773.
4. Hatakeyama, D., Okuta, A., Otsuka, E., Lukowiak, K., and Ito, E. (2013) Consolidation of long-term memory by insulin in *Lymnaea* is not brought about by changing the number of insulin receptors. Commun Integr Biol 6, e23955.
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6. Iwai, A., Yoshimura, T., Wada, K., Watabe, S., Sakamoto, Y., Ito, E., and Miura, T. (2013) Spectrophotometric method for the assay of steroid 5α-reductase activity of rat liver and prostate microsomes. Anal Sci 29, 455-459.
7. Matsuo, R, Yamagishi, M., Wakiya, K., Tanaka, Y., and Ito, E. (2013) Target innervation is necessary for neuronal polyploidization in the terrestrial slug *Limax*. Dev Neurobiol 73, 609-620.
8. Otsuka, E., Matsunaga, M., Okada, R., Yamagishi, M., Okuta, A., Lukowiak, K., and Ito, E. (2013) Increase in cyclic AMP concentration in a cerebral giant interneuron mimics part of a memory trace for conditioned taste aversion of the pond snail. BIOPHYSICS 9, 161-166.
9. Tanimoto, E., Karasawa, S., Ueki, S., Nitta, N., Aoki, I., and Koga, N. (2013) Unexpectedly large water-proton relaxivity of TEMPO incorporated into micelle-oligonucleotides. RSC Adv 3, 3531-3534.

2012

1. Yamagishi, M., Ito, E., and Matsuo, R. (2012) Whole genome amplification in large neurons of the terrestrial slug *Limax*. J Neurochem 122, 727-737.
2. Ito, E., Otsuka, E., Hama, N., Aonuma, H., Okada, R., Hatakeyama, D., Fujito, Y., and Kobayashi, S. (2012) Memory trace in feeding neural circuitry underlying conditioned taste aversion in *Lymnaea*. PLoS ONE 7, e43151.
3. Okada, R., Akamatsu, T., Iwata, K., Ikeno, H., Kimura, T., Ohashi, M., Aonuma, H., and Ito, E. (2012) Waggle dance effect: dancing in autumn reduces the weight loss of a honeybee colony. J Exp Biol 215, 1633-1641.
4. Kobayashi, S., Matsuo, R., Sadamoto, H., Watanabe, S., and Ito, E. (2012) Excitatory effects of GABA on procerebrum neurons in a slug. J Neurophysiol 108, 989-998.
5. Okada, O., Odai, K., Sugimoto, T., and Ito, E. (2012) Molecular dynamics simulations for glutamate-binding and cleft-closing processes of the ligand-binding domain of GluR2. Biophys Chem 162, 35-44.
6. Ito, E., Okada, R., Sakamoto, Y., Otshuka, E., Mita, K., Okuta, A., Sunada, H., and Sakakibara, M. (2012) Insulin and memory in *Lymnaea*. Acta Biol Hung 63 (Suppl. 2), 320-327.
7. Matsuo, R., Yamagishi, M., and Ito, E. (2012) Analyses of DNA endoreplication in the brain neurons in the terrestrial slug *Limax valentianus*. Acta Biol Hung 63 (Suppl. 2), 297-304.
8. Kobayashi, S., and Ito, E. (2012) GABAergic effects on the slow oscillatory neural activities in the procerebrum of *Limax valentianus*. Acta Biol Hung 63 (Suppl.2), 217-221.
9. Okada, R., Ikeno, H., Kimura, T., Ohashi, M., Aonuma, H., and Ito, E. (2012) Mathematical analysis of the honeybee waggle dance. Acta Biol Hung 63 (Suppl. 2), 201-205.
10. Abe, J., Ueki, S., Arata, T., Nakazawa, S., Yamauchi, S., and Ohba, Y. (2012) Improved sensitivity by isotopic substitution in distance measurements based on double quantum coherence EPR. Appl Magn Reson 42, 473-485.

2011

1. Yamagishi, M., Ito, E. and Matsuo, R. (2011) DNA endoreplication in the brain neurons during body growth of an adult slug. J Neurosci 31, 5596-5604.
2. Matsuo, R., Kobayashi, S., Morishita, F. and Ito, E. (2011) Expression of Asn-d-Trp-Phe-NH_2 in the brain of the terrestrial slug *Limax valentianus*. Comp Biochem Physiol B 160, 89-93.
3. Watabe, S., Sakamoto, Y., Morikawa, M., Okada, R., Miura, T. and Ito, E. (2011) Highly sensitive determination of hydrogen peroxide and glucose by fluorescence correlation spectroscopy. PLoS ONE 6, e22955.
4. Sadamoto, H., Saito, K., Muto, H., Kinjo, M. and Ito, E. (2011) Direct observation of dimerization between different CREB1 isoforms in a living cell. PLoS ONE 6, e20285.
5. Kawai, R., Kobayashi, S., Fujito, Y. and Ito, E. (2011) Multiple subtypes of serotonin receptors in the feeding circuit of a pond snail. Zool Sci 28, 517-525.
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2010

1. Matsuo, R., Kobayashi, S., Murakami, J., and Ito, E. (2010) Spontaneous recovery of the injured higher olfactory center in the terrestrial slug *Limax*. PLoS ONE 5, e9054.
2. Hatakeyama, D., Mita, K., Kobayashi, S., Sadamoto, H., Fujito, Y., Hiripi, L., Elekes, K. and Ito, E. (2010) Glutamate transporters in the central nervous system of a pond snail. J Neurosci Res 88, 1374-1386.
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5. Matsuo, R., Kobayashi, S., Tanaka, Y., and Ito, E. (2010) Effects of tentacle amputation and regeneration on the morphology and activity of the olfactory center of the terrestrial slug *Limax valentianus*. J Exp Biol 213, 3144-3149.
6. Kobayashi, S., Hattori, M., Elekes, K., Ito, E., and Matsuo, R. (2010) FMRFamide regulates oscillatory activity of the olfactory center in the slug. Eur J Neurosci 32, 1180-1192.
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9. Aihara, T., Nakamura, M., Ueki, S., Hara, H., Miki, M., and Arata, T. (2010) Switch action of troponin on muscle thin filament as revealed by spin labeling and pulsed EPR. J Biol Chem 285, 10671-10677.

[Review articles]

1. Ito, E., Hsu, W.L., and Yoshioka, T. (2014) A role for proton signaling in the induction of somatic cells to pluripotent

embryonic stem cells. J Phys Chem Biophys 4, 2.

2. Ito, E., Kojima, S., Lukowiak, K., and Sakakibara, M. (2013) From likes to dislikes: conditioned taste aversion in the pond snail *Lymnaea stagnalis*. Can J Zool 91, 405-412.
3. Ito, E., Matsuo, R., and Okada, R. (2013) Involvement of nitric oxide in memory formation in microbrains. Neurosci Lett 541, 1-3.
4. Matsuo, R., and Ito, E. (2012) Robustness and adaptive flexibility of the pulmonate's brain, (Snails: Biology, Ecology and Conservation, E. M. Hämäläinen and S. Järvinen eds., Nova Science Publishers, NY) pp.151-162.
5. Matsuo, R., and Ito, E: (2011) Spontaneous regeneration of the central nervous system in gastropods. Biol Bull 221, 35-42.
6. Matsuo, R., Kobayashi, S., Yamagishi, M., and Ito, E. (2011) Two pairs of tentacles and a pair of procerebra in Pulmonata: optimized functions and redundant structures in the sensory and central organs involved in olfactory learning of terrestrial pulmonates. J Exp Biol 214, 879-886.

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Behavioral Neuroscience

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1972 Ph.D. in Physical Chemistry from The University of Tokyo
1972 JSPS Postdoctoral Fellow, The University of Tokyo
1973 Postdoctoral Fellow at Carnegie-Mellon University
1975 Assistant Professor and then Associate Professor at The University of Tokyo
1985 Professor at Kyushu University
1993-2005 Professor, School of Pharmaceutical Sciences, The University of Tokyo
2001-2003 Dean, School of Pharmaceutical Sciences, The University of Tokyo
2005 Executive Vice-President for Research, The University of Tokyo
2006 Professor and President, Tokushima Bunri University
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2006 Assistant Professor, Tokushima Bunri University
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2005 Ph.D. in Pharmacology from Kyushu University
2005 Assistant Professor, Tokushima Bunri University

Research

Molecular and neural mechanisms of eyeblink classical conditioning

Introduction: Associative learning is a fundamental form of cognition in humans and animals. Eyeblink classical conditioning (EBCC) is a form of associative learning that has been most extensively studied at the neurological and behavioral level. Its basic neural circuitry and neural mechanisms have been demonstrated to be similar in all mammals. Since the same paradigm is applicable to humans as well as non-human mammals, there is growing interest in EBCC for the study of human diseases of motor and memory impairment, in parallel with detailed studies of the molecular and neural mechanisms in animal models.

Typical EBCC experiments use a tone as the conditioned stimulus (CS), and a periorbital shock or corneal air puff as the unconditioned stimulus (US). By repeated presentations of the CS paired with the US, the CS comes to elicit an eyeblink, which is called the conditioned response (CR). Previous studies have indicated that the cerebellum and brainstem are sufficient for *delay* conditioning in which the US is delayed and co-terminates with the CS. On the other hand, *trace* conditioning, in which a stimulus-free trace interval intervenes between the CS and US, requires other brain regions, including the hippocampus and the medial prefrontal cortex (mPFC).

Basic Functions of the Cerebellum and Brainstem: To investigate the basic properties of the essential neural circuits in the cerebellum and brainstem, we have developed a decerebrate guinea pig preparation, in which a section is made between the thalamus and the superior colliculus and all of the brain tissue above the section is aspirated. Decerebrate animals readily acquire the CR in delay conditioning. When a longer tone CS is used, the learning becomes slower. These CRs are adaptive and appropriately timed relative to the US. Subsequent CS-alone trials cause extinction of the CR. These characteristics of eyeblink conditioning are similar to those reported previously in intact animals of various species, suggesting that the cerebellum and brainstem are sufficient for this type of learning.

We also study trace eyeblink conditioning in decerebrate guinea pigs. A 350-ms tone CS is paired with a 100-ms periorbital shock US with a trace interval of either 0, 100, 250, or 500 ms. Decerebrate animals readily acquire the CR with a trace interval of 0 or 100 ms. Even in the paradigm with a 500-ms trace interval, which is known to depend critically on the hippocampus in all animal species examined, the decerebrate guinea pigs acquire the CR, with the adaptive timing seen in the other paradigms with a shorter trace interval. However, it takes many more trials to learn when we employ the 500-ms trace paradigm rather than the shorter trace-interval paradigms, and the CR expression is unstable from trial to trial. When decerebrate animals are conditioned step by step with a trace interval of 100, 250, and 500 ms (in that order), they easily acquire the adaptive CR with the 500-ms trace interval. However, the CR% decreases after the trace interval is shifted from 250 ms to 500 ms, a decrease that is not observed with the shift from 100 ms to 250 ms. These results suggest that the cerebellum and brainstem can maintain

the "trace" of the CS and associate it with the US even in the 500-ms trace paradigm, but that the forebrain might be required to facilitate the association and stabilization of the memory.

Cerebellar Cortical Mechanism of EBCC: Long-term depression (LTD) at parallel fiber-Purkinje cell synapses in the cerebellar cortex has been proposed as the neural substrate for EBCC. Since the glutamate receptor subunit δ2 (GluRδ2) is selectively expressed at the dendritic spines of the Purkinje Cell (PC) and is essential for the induction of cerebellar LTD, GluRδ2-null mice (in which cerebellar LTD is specifically impaired) provide a useful means to test the cerebellar LTD hypothesis. Mutant mice lacking GluRδ2 show severe learning impairment in *delay* conditioning, but learn normally in *trace* conditioning. This surprising finding has now been confirmed in experiments with another line of mutant mice lacking phospholipase Cβ4 and with wild-type mice subjected to intracerebellar injection of the NO synthase inhibitor L-NAME, both of which lack cerebellar LTD. Therefore, there may be variations in the cerebellar neural substrates for eyeblink conditioning, depending on the CS-US temporal overlap.

We have recently found that the muscarinic acetylcholine receptor antagonist scopolamine and the NMDA receptor antagonist MK-801 impair learning in trace conditioning experiments with a zero trace-interval (trace 0 paradigm experiments) in GluRδ2$^{-/-}$ mice, and that the metabotropic glutamate receptor subtype 1 outside the cerebellum is essential for trace conditioning but not for delay conditioning. These findings suggest a contribution of the hippocampus to the LTD-independent learning mechanism. To examine this possibility further, we have looked at the effects of hippocampal lesions on learning in GluRδ2$^{-/-}$ mice. GluRδ2$^{-/-}$ mice whose dorsal hippocampi were aspirated exhibit severe learning impairment in the trace 0 paradigm experiments, while control GluRδ2$^{-/-}$ mice that received a lesion in the cortex overlying the hippocampus are able to learn promptly. Wild-type mice do not show such hippocampal dependency in the trace 0 paradigm. We therefore concluded that the hippocampus is essential for learning with a trace 0 paradigm when cerebellar LTD is disrupted. In contrast, GluRδ2$^{-/-}$ mice that receive post-training hippocampal lesions retain the memory as well as the control GluRδ2$^{-/-}$ mice do. The hippocampal lesion also does not affect memory retention in wild-type mice. These results suggest that the hippocampus is *not* essential for *retention* of motor memory with a trace 0 paradigm in LTD-deficient mice. Thus, the present studies clearly argue that the hippocampus is essential for memory formation in cerebellar motor learning when cerebellar LTD is disrupted. These studies suggest that the neural network which underlies learning and memory is both flexible and robust.

Reorganization of Brain Circuitry during Memory Consolidation: Previous studies, including those described above, have confirmed the time-limited involvement of the hippocampus in mnemonic processes and have suggested that there is reorganization of the responsible brain circuitry during memory consolidation. For temporal characterization of such reorganization, we carried out EBCC experiments with a trace interval of 500 ms in rats with ablation of one of three brain regions: the hippocampus, the mPFC, or the cerebellum. At various time intervals after establishing the trace conditioned response (CR), rats receive an aspiration of one of these regions. After recovery, the animals are tested for their retention of the CR. When ablated one day after the learning, both the hippocampal lesion and the cerebellar lesion groups exhibit severe impairment in their retention of the CR, whereas the mPFC lesion group show only a small decline. As we increase the interval between the lesion and the learning, the effect of the hippocampal lesion decreases and that of the mPFC lesion increases. When ablated 4 weeks after the learning, the hippocampal lesion group exhibits CRs that are as robust as those of the corresponding control group. In contrast, the mPFC lesion and cerebellar lesion groups fail to retain the CRs. These results indicate that the hippocampus and the cerebellum, but only marginally the mPFC, constitute a brain circuitry that mediates recently acquired memory. As time elapses, the circuitry is reorganized to use mainly the mPFC and the cerebellum, but not the hippocampus, for remotely acquired memory.

Application of EBCC to Studies of Human Memory Loss: In addition to its great advantage as a model system for learning and memory, EBCC is expected to have wide potential application to clinical studies, including motor impairment (e.g. Parkinson's disease), dementia (e.g. Alzheimer's disease), and other psychopathologies. Such studies using mouse models of human memory loss (experiments using aged animals, senescence- accelerated SAMP8 mice, obese mice and animals injected with β-amyloid peptide) are currently under way.

Publications (2010~2015)

2014

1. Ohtani Y, Miyata M, Hashimoto K, Tabata T, Kishimoto Y, Fukaya M, Kase D, Kassai H, Nakao K, Hirata T, Watanabe M, Kano M, Aiba A. (2014) The synaptic targeting of mGluR1 by its carboxyl-terminal domain is crucial for cerebellar function. J. Neurosci. **34**, 2702-2712.
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4. Iihara N, Nishio T, Goda T, Anzai H, Kagawa M, Houchi H, Kirino Y (2014) "Effect of enduarance for adverse drug reactions on the preference for aggressive treatments in cancer

patients," Support Care Cancer, DOI 10.1007/s00520-014-2439-1

2013

1. Kishimoto Y, Hirono M, Atarashi R, Sakaguchi S, Yoshioka T, Katamine S, Kirino Y (2013) Age-dependent impairment of eyeblink conditioning in prion protein-deficient mice. PLOS ONE 8:e60627
2. Kishimoto Y, Kirino Y (2013) Presenilin 2 mutation accelerates the onset of impairment in trace eyeblink conditioning in a mouse model of Alzheimer's disease overexpressing human mutant amyloid precursor protein. Neurosci. Lett. 538:15-19.
3. Hiasa M*, Isoda Y*, Kishimoto Y *, Saitoh K, Kimura Y, Kanai M, Shibasaki M, Hatakeyama D, Kirino Y, Kuzuhara T (2013) Inhibition of MAO-A and stimulation of behavioural activities in mice by the inactive prodrug form of the anti-influenza agent oseltamivir. Br. J. Pharmacol. 169:115-129. (*Equal contribution)
4. Kishimoto Y, Higashihara E, Fukuta A, Nagao A, Kirino Y (2013) Early impairment in a water-finding test in a longitudinal study of the Tg2576 mouse model of Alzheimer's disease. Brain Res. 1491:117-126
5. Ishihara Y, Itoh K, Mitsuda Y, Shimada T, Kubota T, Kato C, Song SY, Kobayashi Y, Mori-Yasumoto K, Sekita S, Kirino Y, Yamazaki T, Shimamoto N (2013) Involvement of brain oxidation in the cognitive impairment in a triple transgenic mouse model of Alzheimer's disease: noninvasive measurement of the brain redox state by magnetic resonance imaging. Free Radic Res. 47: 731-9

2012

1. Kishimoto Y, Oku I, Nishigawa A, Nishimoto A, Kirino Y (2012) Impaired long-trace eyeblink conditioning in a Tg2576 mouse model of Alzheimer's disease. Neurosci. Lett. 506:155-159
2. Kirino, Y. (2012) Regulatory science of medical products. Folia Pharmacol. Jpn. 139, 215-218

2011

1. Miyata M*, Kishimoto Y*, Tanaka M, Hashimoto, K, Hirashima, N, Murata, Y, Kano, M, Takagishi, Y. (2011) A role for myosin Va in cerebellar plasticity and motor learning: a possible mechanism underlying neurological disorder in myosin Va disease. J. Neurosci. 31: 6067-6078. (*Equal contribution)
2. Iihara N, Kirino Y, Yamagara D, Yokoi H, Hara K. (2011) Team care incorporating community pharmacies enhances patient's satisfaction –Based on a questionnaire survey to participants in a small trial of an electronic prescription network system. Jpn. J. Telemed. Telecare, 7: 35-38.

2010

1. Iihara N, Kirino Y, Hara K, Yokoi H, Ueno T, Harada A, Nakagawa M, Saito Y, Morioka K, Ogata Y. (2010) Development of an electronic prescription interactive network system enhancing collaboration of medical staffs between a hospital and community pharmacies. Iryo-Joho-Gaku, 30, 225-231
2. Kishimoto Y. (2010) "Experimental verification of classical conditioning models by inducible On/Off control of gene expression. Seibutubutsuri. 50: 195-198.

Kagawa School of Pharmaceutical Sciences
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Development and Application of Advanced Instrumentation in Analytical Chemistry

Staff

Professor: Kentaro Yamaguchi, Ph. D (Apr. 2004)
Educational History:
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Latest Work Record:
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Associate Professor: Masatoshi Kawahata, Ph. D
Educational History:
Graduated from Graduate School of Tokushima Bunri University in Mar. 2006

Assistant Professor: Kazuaki Ohara, D. Eng. (Apr. 2010)
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Graduated from Graduate School of Tokyo University in Mar. 1992

Assistant Professor: Shinsuke, Komagawa, Ph. D
Educational History:
Graduated from Graduate School of Tokyo Science University in Mar. 2009

Research

Laboratory of Analytical chemistry has designated that it contributes to pharmaceutical technology with analyzing molecular structures, physical properties, chemical reactivity, and functions. The connection between dynamic behavior of biomolecules and its reactivity-function relationship as well as the detection of unstable reaction intermediate in solution are our major subjects. We have been engaged in the study to produce new analysis method, which reveals the generating mechanism of the outstanding functionalities and the physical properties of the materials in solution based on microanalysis in the atomic resolution. We also develop the analytical and chemical technique in solution that is based on quantum mechanics and statistical mechanics. These results might be applied to pharmaceutical organic synthesis.

Development in the field of mass spectrometry:

In the laboratory, we have succeeded in the development of the 'Cold-Spray Ionization (CSI)' method. CSI is designed for mass spectrometric detection of labile organic species. It may be an appropriate method to analyze in solution the structures of biomolecular complexes, labile organic species including Grignard reagents, asymmetric catalysis, and supermolecules. The method, a variant of Electrospray ionization (ESI)-MS, operates at low temperature, allow simple and precise characterization of labile non-covalent complexes that are difficult or impossible to observe by conventional MS techniques, including fast atom bombarment (FAB), and matrix assisted laser desorption ionization (MALDI), as well as ESI.

Biopolymer analysis:

The behavior of important in vivo molecule such as protein, nucleic acid, and lipid, will be made clear by using our newly developed method CSI mass spectrometry. The structure of multistranded DNA of duplex, triplex and quadruplex DNA have been examined by electrospray ionization (ESI) MS. However, non-covalent complexes of multiply stranded DNA are difficult to observe by conventional methods, because of their low melting temperature (T*m*). The characterization of triple- and quadruple-stranded oligodeoxynucleotides was carried out by means of CSI-MS. In consequence, it is proved that DNA has been made clear to take hyper-stranded structures combining various multiply stranded helical components.

Analysis of organic reaction mechanism:

A new CSI mass spectrometric procedure RTS (reaction tracking system), which can trace the time-dependent simultaneous change of the raw materials, products and inter mediates in an organic reaction under CSI condition has been developed. It would be expected that behavior of each component becomes clear and that the appropriate design become possible to make the reaction more efficiently.

Observation of the giant molecules by means of mass spectrometry:

Mass Spectrometry (MS) has been developed and adopted to wide variety of analytical chemistry in recent years.
Although MS was basically developed for high molecular weight substances in the field of biochemistry, the measurement of huge molecules over 10k Da is still very difficult. This is caused by the ionizing problems, stability of the compounds and the existence of various impurities.
We develop some new techniques to overcome these problems by using newly equipped FT-ICR mass spectrometer.

Publications (2010~2015)

[Original papers]

2015

1. Danjo, H.; *Nakagawa, T.; Katagiri, K.; Kawahata, M.; Yoshigai, S.; Miyazawa, T.; Yamaguchi, K. (2015). Formation of Lanthanide(III)-Containing Metallosupramolecular Arrays Induced by Tris(spiroborate) Twin Bowl. Cryst. Growth. Des., 15, 384-389.

2014

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Design and Synthesis of Novel Nuclear Receptor Ligands

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Research

Design and synthesis of the novel vitamin D receptor ligands

We have been interested in functions of the nuclear receptors modulated by small molecules, which can be critical to certain disease states. In particular, novel analogues targeted to vitamin D receptor (VDR) were designed and synthesized to understand how the subtype-free, singular VDR can deliver the diverse biological activities vitamin D, as well as to allow the development of potential therapeutic agents with selective activity profiles for the treatment of cancer or osteoporosis. Syntheses of the analogues were carried out by a convergent method using a palladium catalyst. Separate preparation of the requisite A-ring enyne precursors has developed from the 3-buten-1-ol derivatives. Modification in the A-ring, as well as in the side chain of vitamin D, resulted in exceptionally potent compounds with unique activity profiles.

Publications (2010~2015)

[Original papers]

2014

1. Fujishima, T.; Suenaga, T.; Nozaki, T. (2014). Concise synthesis and characterization of novel *seco*-steroids bearing a spiro-oxetane instead of a metabolically labile C3-hydroxy group, *Tetrahedron Lett.*, **2014**, *55, 3805-3808.*
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6. Fujishima, T; Fujii, S.; Harayama, T. Synthesis and biological activity of fluorinated vitamin D. *Current Org. Chem.*, **2010**, *14*, 962.

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Spontaneous Resolution and Polymorphism

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Research

Spontaneous Resolution of Optical Activity

The spontaneous resolution of an achiral compound (total asymmetric transformation) has been of great interest in connection with the origin of life. This phenomenon also holds potential in that the spontaneously resolved chiral crystals could find application as chiral sources in asymmetric synthesis to produce compounds with fixed chirality. During the course of our study on the stereochemistry of aromatic amides or sulfonamides, we found that several groups of compounds with a common skeleton showed spontaneous resolution more frequently than other achiral compounds. We also have developed an effective screening method for spontaneous resolution of aromatic sulfonamides, which relies on parallel syntheses and solid-state CD measurements. We are now exploring a photoreaction in the chiral crystalline state of achiral compounds which will produce high enantioselectivities. Furthermore, conformational chirality, which is retained when chiral crystals are dissolved at low temperature, will be utilized in diastereoselective syntheses. Another approach is to utilize the spontaneously resolved chiral crystals as catalytic ligands to produce chiral compounds with fixed chirality.

Construction of Adamantane-Based Macrocycles and Cages

Synthetic macrocyclic compounds are considerably important receptor molecules, and they provide an opportunity to interact with various guest molecules by the binding sites of multiple functional groups and well-defined cavities within their frameworks. Adamantane derivatives are unique and specific compounds, because they are mechanically rigid and conformationally well-defined. However, the design and synthesis of adamantane-based host molecules and their applications in host-guest systems remain largely unexplored. We reported the construction and structural analysis of various types of nano-sized adamantane-based macrocycles and cages bearing rectangle, square, and spherical shapes. Their macrocycles encapsulate electron-poor guest molecule, 1,3,5-trinitrobenzene via charge-transfer interactions. Further, adamantane-based macrocycles showed the generation of unique molecular networks in the solid state. The adamantane-based macrocycles formed tubular structures with channels, which were assembled into three-dimensional networks through multiple intermolecular interactions.

Self-assembled Nano-scale Structures Using Macrocyclic Framework as Molecular Block

Recently, several functionalized macrocycles were utilized as building blocks for self-assembled nanostructures including vesicles, tubes, fibers, and others. We synthesized two types of adamantane-based oxacyclophanes, which are consisted of two disubstituted adamantane bearing benzene derivatives and two pyrazine moieties connected with oxygen atoms. By using the specific properties of spherical shape, bulky skeleton, and lipophilicity of the adamantane derivatives, adamantane-based oxacyclophanes were induced into hollow spherical aggregates with a multilayer membrane in organic and aqueous solution. The hollow spheres were induced into fibrous and network aggregates, which were eventually transformed into single crystals. These results afforded the evidences for a morphological change and phase transition occurring from the solution into a solid.

Publications (2010~2015)

[Original papers]

2015

1. Katagiri, K., Tohaya, T., Shirai, R., Kato, T., Masu, H., Tominaga, M., Azumaya, I. (2015). Folded-to-unfolded structural switching of a macrocyclic aromatic hexaamide based on conformation changes in the amide groups induced by *N*-alkylation and dealkylation reactions. J. Mol. Struct. 1082, 23–28.
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Search of the Functional Molecules from Natural Resources

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Research

We conduct chemical and biological research on the components of medicinal plants and crude drugs in order to advance the development of therapeutic pharmaceuticals. The lab focuses on determining the structure of potential compounds, the relationship between that structure and their biological activity, and the role of genes in the biosynthesis of compounds and their function in human physiology. In addition, new avenues in systems biology and metabolomics are being explored and there is ongoing research on the prevention of illegal drug circulation and use, and on the evaluation of drug quality.

I. *Research on the biological active chemical constituents of health foods*

We found that royal jelly acted at the early stages of the G_1 phase and the S phase of a cell cycle and controlled multiplication of human osteosarcoma cell line, MG-63 cell. Separation of the active water extract by a dialysis membrane and a solid phase extraction suggested that active substances were high polar low molecular compounds. Furthermore, the existence of nitrogen-containing compounds having acidic groups was suggested by LC/MS (ESI^-) analyses. Further isolation procedure identified that the main active component was AMP N_1-oxide. Continuous examination revealed also the existence of adenosine N_1-oxide, ADP N_1-oxide, ATP N_1-oxide, and NAD N_1-oxide as active ingredients. AMP N_1-oxide and adenosine N_1-oxide inhibited multiplication of MG-63 cell strongly, and their control of the G_1 to S phase comparing with AMP as 1/100 low concentration was found. From these facts, AMP N_1-oxide, adenosine N_1-oxide, and other N_1-oxide are considered to be the main ingredients that contribute at MG-63 cell-growth control of royal jelly.

II. *The search of the Alzheimer therapeutic drug from natural resources*

Donepezil hydrochroloride of the choline esterase inhibitor is used as Dementia and Alzheimer therapeutic drag. Galantamine, the metabolite of the Amaryllidaceae plant acts in similar mechanism, and approval in the country is examined. In addition, Kampo medicine nominates an effect for condition improvement. We perform construction pro-screening to search for the therapeutic drug from a crude drug and a medical plant.

III. *The study of anti-Leishmaniasis therapeutics*

Leishmaniasis is a parasitic disease caused by species of the genus Leishmania. Over 20 of which are known to be pathogenic to humans、and the disease is endemic in some tropical and subtropical regions of the world. *Leishmaniasis* is transmitted by small biting sandflies (*Phlebotomus* spp.), causing a disease which currently afflicts twelve million people in 88 countries. *Leishmania major*, the causative agent of cutaneous leishmaniasis, is a digenetic parasite that exists as an extracellular promastigote within the insect vector (sandfly), and as a nonmotile intracellular amastigote within the phagolysosome of macrophages and other cells of the reticuloendothelial system of the mammalian host. Treatment options for leishmaniases include pentavalent antimonials as first-line drugs, and amphotericin B and pentamidine as second-line drugs. However, these drugs are extremely toxic and usually too expensive for general use, and more economical and less toxic drugs are thus being sought. We have been searching for plant compounds that are active against *Leishmania major*, *L. panamensis, L. guyanensis, and L. peruviana*, exhibited significant activity against the pathogenic protozoan, and newly assay method. Recently, we isolated leishmanicidal naphthoquinones from *Tectona grandis*.

IV. *Research on the chemical components of illegal drugs of plant origin*

1. Khat is a fresh leaf of evergreen shrub *Catha edulis* (Celastaceae) that grows naturally or is grown in Ethiopia, East and Southern Africa, and Yemen, etc., and a lot of people in Africa and Arabia nations use this leaf traditionally as a stimulant biting, and, as a result, it is assumed to obtain the feeling of well-being at the same time as hungry and tiredness's softening. The stimulating component of Khat was believed to be *d*-norpseudoephedrine until cathinone was identified as a main active constituent at the end of 1970's. This cathinone is regulated as narcotics and psychotropic drug, and there is an action similar to (+)-amphetamine that is the synthetic central nervous system stimulation medicine and the strength is assumed to be this level. I synthesized cathinone and ephedrine as an authentic sample to use for the analysis of the drug.

2. *Salvia divinorum* which belongs to Labiatae family is used in traditional spiritual and curative practices by the indigenous Mazatec people of southern Mexico. Salvinorin A (Sal A), which is the neoclerodane ditrepene and is an extremely potent and highly selective kappa opioid receptor agonist, is the main active constituent isolated from the leaves of *S. divinorum.* The sale of *S. divinorum* has become prohibited due to its psychoactive effect in Japan in recent years. The main objectives of this research are to develop immunoassays using anti-Sal A monoclonal antibody (MAb). The icELISA, which has a measuring range from 0.156 μg/ml to 1.25 μg/ml for Sal A, was developed to distinguish *S. divinorum* among various Labiatae plants. In addition, we are preparing the immunochromatographic strip to realize much more rapid analysis. These immunoassays using anti-Sal A must be a convenient authentification method for *S. divinorum* samples.

V. ***Research on Development of Preparative Separation Method of Biologically Active Natural Products by Centrifugal Partition Chromatography : Preparative separation of lancemaside A from Codonopsis lanceolata by CPC***

The roots of *Codonopsis* sp. (Campanulaceae) have been used in folk medicine in China, Korea, and Japan for the treatment of bronchitis, cough, spasm, and inflammation. Recently, it was demonstrated that a hot water extract of *C. lanceolata* roots promoted spermatogenesis and improved sexual motion. Moreover, three phenylpropanoids were identified as the active compounds that promoted spermatogenesis, while several saponins, including lancemaside A, were isolated, and lancemaside A was identified as the active compound that improves sexual motion. Although it is assumed that *C. lanceolata* roots are highly safe since they have been used for a long time, general and specific toxicity tests for safety assurance of the active integrants are required. In general, a large amount of purified compounds is required to assess the effectiveness and to perform safety tests. Therefore we attempted to develop a simple and efficient method for the preparative isolation of lancemaside A from the hot water extract of *C. lanceolata* roots, and resulted in the successful preparative separation of lancemaside A along with two other saponins from the saponin fraction of *C. lanceolata* by CPC.

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Design and synthesis of novel nucleic acid analogs

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Research

Stimulator of angiogenesis

Stimulators of angiogenesis are sometimes desired for clinical treatment of diseases evoked by an impaired blood supply including ulcers associated with diabetes or burn wounds. However, availability of stimulators is few till date because of their size. Most of the stimulators known are endogenous large molecules like VEGF and FGF. Those are expensive proteins, hard to synthesize, and not so stable. We developed 2-Cl-C.OXT-A as a stable candidate compound. This compound strongly stimulates the tube formation of HUVEC. Its maximun potency at 100μM was stronger than VEGF (10ng/mL). 2-Cl-C.OXT-A will be applicable to medicine instead of endogenous growth factors such as VEGF and/or FGF.

Effect of uracil analog against HIV-1

Human immunodeficiency virus type 1 (HIV-1) contains an important enzyme, reverse transcriptase (RT), which catalyzes the conversion of the viral genome RNA into the double-stranding DNA. Since this process is essential for viral replication, many drugs targeting this enzyme have been developed. Within the class of the anti-HIV agents which inhibit reverse transcriptase, non-nucleoside reverse transcriptase inhibitors (NNRTIs) are rapidly increasing. It is interesting that some NNTRIs have an aromatic group at the 6 position of uracil. Under the background of these reports, we undertook a search for an anti-HIV agent by the SAR of the 1,3-disubstituted uracil. Two compounds showed excellent anti-HIV-1 activity with moderate cytotoxicity.

Publications (2010~2015)

[Original papers]

2015

1. Sakakibara, N., Baba, M., Okamoto, M., Toyama, M., Demizu, Y., Misawa, T., Kurihara, M., Irie, K., Kato, Y., Maruyama, T. (2015). Design, synthesis, and anti-HIV-1 activity of 1-aromatic methyl-substituted 3-(3,5-dimethylbenzyl)uracil and *N*-3,5-dimethylbenzyl-substituted urea derivatives. Antiviral Chemistry & Chemotherapy, 24, 3-18.

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2012

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Research on Molecular Biology

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Research

I. Gene expression analysis of mouse embryonic carcinoma P19 cells induced to form neural cells. (Rie Komori, Takanobu Kobayashi and Hiroshi Miyazawa)

Mouse embryonic carcinoma P19 cells are pluripotent cells that can be induced to differentiate into multiple cell types by cellular aggregation in the presence of differentiating agents. When aggregated in the presence of all-*trans* retinoic acid (ATRA), P19 cells differentiate into neural cells (including neurons and glia cells), whereas the same cells aggregated in the presence of DMSO differentiate into cardiomyocytes. These cells can simulate the molecular and morphological events occurring during early embryonic development, and have been used extensively as a model to study the molecular mechanisms controlling the process of differentiation into cardiomyocytes or neural cells.

To identify the genes associated with induction of neural differentiation, we carried out a transcriptome analysis of P19 cells induced to form neural cells by ATRA. We employed the DNA microarray method, which can produce an accurate and derailed profile of gene expression. Numerous genes were activated in P19 cells in response to ATRA treatment. We compared the expression profiles from control (undifferentiated) and ATRA-treated P19 cells, which provided an abundance of information about the gene products involved in neural differentiation. We confirmed the sequential expression patterns of some genes over the course of differentiation. We are investigating the relationship between ATRA treatment and expression patterns of these genes, interactions with other factors, and functions in neural differentiation. These findings will provide useful clues to a more comprehensive understanding of the complex processes involved in the induction of neural differentiation. We are undertaking additional investigations to better understand the role played by these genes during the induction of neural differentiation.

Recently, we identified a candidate gene associated with induction of neural differentiation. We found that the expression of Csn3 was induced by all-trans retinoic acid (ATRA) during neural differentiation in P19 cells from our study using DNA microarray. We describe the induction mechanism of Csn3 transcription activation in this process. In conclusion, the Csn3 expression is upregulated via ATRA-bound RARα and binding of this receptor to the RARE in the Csn3 promoter region. This will certainly serve as a first step forward unraveling the mysteries of induction of Csn3 in the process of neural differentiation.

II. DNA Oxidation, Point Mutation and DNA repair. (Katsuhito Kino)

The genome is constantly assaulted by oxidation reactions that are likely to be associated with oxygen metabolism, and oxidative lesions are generated by many of these oxidants. Such genotoxin-induced alterations in the genomic message have been implicated in aging and in several pathophysiological processes, particularly those associated with cancer.

1. *Guanine Oxidation*

Photosensitized oxidation of guanine provides various oxidation products, including 8-oxoguanine (8-oxoG) and imidazolone. Riboflavin (vitamin B2) is known to be an effective photosensitizer for the oxidation of guanine. We have demonstrated: the user-friendly synthesis and photoreaction of a flavin-linked oligonucleotide; the practical synthesis of hydroxyethyl-flavin from commercially available riboflavin; and the preparation of a flavin-linked oligonucleotide using a phosphoramidite of hydroxyethyl-flavin. To demonstrate the usefulness of this method, the flavin-linked oligomer was synthesized. The flavin-linked oligomer and its complementary oligomer containing 8-oxoG were then irradiated under UV light (366 nm) at neutral pH. Enzymatic digestion of the irradiated mixture indicated that the 8-oxoG residue was oxidized to

imidazolone. These results demonstrated that 8-oxoG is effectively oxidized to imidazolone by photosensitization of the terminal flavin via a hole-transfer mechanism, and imidazolone is formed by one-electron oxidation of 8-oxoG at neutral pH.

In addition, 8-OxoG was specifically oxidized by iodine with aqueous KI. Under acidic conditions, the major product was dehydro-guanidinohydantoin. Under basic conditions, two diastereoisomers of spirohydantoin were chiefly obtained. In addition, unstable diimine was detected for the first time.

2. *Point Mutation by Guanine Oxidation.*

The guanine base (G) in genomic DNA is highly susceptible to oxidative stress because it has the lowest oxidation potential. Therefore, G-C-->T-A and G-C-->C-G transversion mutations frequently occur under oxidative conditions. One typical lesion of G is 8-oxoguanine (8-oxoG), which can pair with A, and this pairing may cause G-C-->T-A transversion mutations. Although the number of G-C-->C-G transversions is rather high under specific oxidation conditions such as riboflavin photosensitization, the molecular basis of G-C-->C-G transversions is not known.

We have shown that Iz is a key oxidation product of G when 8-oxoG in DNA photosensitized with riboflavin or anthraquinone. Primer extension experiments have demonstrated that Iz can specifically pair with G in vitro. Thus, specific Iz-G base pair formation can explain the G-C-->C-G transversion mutations that appear under oxidative conditions.

Moreover, we found that guanine is preferentially incorporated opposite 2,2,4-triamino-5(2H)-oxazolone (Oz) by eukaryotic DNA polymerases alpha, beta and epsilon, and we first propose the chemical structure of an Oz:G base pair having hydrogen bonds. Especially, since DNA polymerases alpha and epsilon play an important role in eukaryotic DNA replication, our results indicate that Oz is the premutagenic lesion that causes G:C-C:G transversions. Our results first clarify the mechanism of G:C-C:G transversions in eukaryote, and we mention the chemical consideration in guanine insertion opposite Oz. Thus we believe that our present study has novel insights into the molecular mechanism of point mutations underlying the first trigger which causes several diseases.

In addition to Oz, guanidinohydantoin (Gh)/iminoallantoin (Ia) and spiro-imino-dihydantoin (Sp) are known products of oxidative guanine damage. These damaged bases can base pair with guanine and cause G:C-C:G transversions. In this study, the stabilization energies of these bases paired with guanine were calculated in vacuo and in water. The calculated stabilization energies of the Ia:G base pairs were similar to that of the native C:G base pair, and both bases pairs have three hydrogen bonds. By contrast, the calculated stabilization energies of Gh:G, which form two hydrogen bonds, were lower than the Ia:G base pairs, suggesting that the stabilization energy depends on the number of hydrogen bonds. In addition, the Sp:G base pairs were less stable than the Ia:G base pairs. Furthermore, calculations showed that the Oz:G base pairs were less stable than the Ia:G, Gh:G and Sp:G base pairs, even though experimental results showed that incorporation of guanine opposite Oz is more efficient than that opposite Gh/Ia and Sp.

Next, we demonstrated that hNEIL1 and hNTH1 cleave Oz sites as efficiently as 5-hydroxyuracil sites. Thus, hNEIL1 and hNTH1 can repair Oz lesions. Furthermore, the nicking activities of these enzymes are largely independent of nucleobases opposite Oz; this finding indicates that removing Oz from Oz:G and Oz:A base pairs might cause an increase in the rate of point mutations in human cells.

3. *Product analysis of photooxidation in isolated quadruplex DNA*

(1) The formation of quadruplex structure changed the site reactivity and the kinds of guanine photooxidation products of d(TGGGGT). In quadruplex DNA, 8-oxo-7,8-dihydroguanine (8oxoG) and dehydroguanidinohydantoin (Ghox) were mainly formed, although 2,5-diamino-4H-imidazol-4-one (Iz) was mainly formed in single-stranded DNA. In addition, 3'-guanine was specifically oxidized in quadruplex DNA compared with single-stranded DNA, which depended on the localization of the HOMO.

(2) Guanine is the most easily oxidized among the four DNA bases, and some guanine-rich sequences can form quadruplex structures. In a previous study using 6-mer DNA d(TGGGGT), which is the shortest oligomer capable of forming quadruplex structures, we demonstrated that guanine oxidation products of quadruplex DNA differ from those of single-stranded DNA. Therefore, the photooxidation products of double-stranded DNA (dsDNA) may also differ from that of quadruplex or single-stranded DNA, with the difference likely explaining the influence of DNA structures on guanine oxidation pathways. In this study, the guanine oxidation products of the dsDNA d(TGGGGT)/d(ACCCCA) were analyzed using HPLC and electrospray ionization-mass spectrometry (ESI-MS). As a result, the oxidation products in this dsDNA were identified as 2,5-diamino-4H-imidazol-4-one (Iz), 8-oxo-7,8-dihydroguanine (8oxoG), dehydroguanidinohydantoin (Ghox), and guanidinohydantoin (Gh). The major oxidation products in dsDNA were consistent with a combination of each major oxidation product observed in single-stranded and quadruplex DNA. We previously reported that the kinds of the oxidation products in single-stranded or quadruplex DNA depend on the ease of deprotonation of the guanine radical cation (G•+) at the N1 proton. Similarly, this mechanism was also involved in dsDNA. Deprotonation in dsDNA is easier than in quadruplex DNA and more difficult in single-stranded DNA, which can explain the formation of the four oxidation products in dsDNA.

4. *Chemistry of flavins*

Photoirradiation in the presence of riboflavin led to guanine oxidation and the formation of imidazolone. Meanwhile, riboflavin itself was degraded by ultraviolet light A (UV-A) and visible light (VIS) radiation, and the end product was lumichrome. VIS radiation in the presence of riboflavin oxidized guanine similarly to UV-A radiation. Although UV-A radiation with lumichrome oxidized guanine, VIS radiation with lumichrome did not. Thus, UV-A radiation with riboflavin can oxidize guanine even if riboflavin is degraded to lumichrome. In contrast, following VIS radiation degradation of riboflavin to lumichrome, VIS radiation with riboflavin is hardly capable of oxidizing guanine. The consequences of riboflavin degradation and guanine photooxidation can be extended to flavin mononucleotide and flavin adenine dinucleotide. In addition, we report advanced synthesis; carboxymethylflavin was obtained by oxidation of formylmethylflavin with chlorite and hydrogen peroxide; lumichrome was obtained by heating of formylmethylflavin in 50% AcOH; lumiflavin was obtained by incubation of formylmethylflavin in 2 M NaOH, followed by isolation by step-by-step concentration.

III. Identification of novel low molecular compounds that inhibit binding of NF-□B to DNA (Takanobu Kobayashi)

The nuclear transcription factor-κB (NF-κB) is one of the central regulators of an organism's response to various stress signals. In response to an extracellular signal, NF-κB translocates into the nucleus, binds to DNA, and activates the transcription of specific genes. NF-κB regulates the transcription of a number of genes involved in immune and inflammatory pathways and in apoptosis. Dysregulation of NF-κB contributes to a variety of pathological conditions. Therefore, the down-modulators of NF-κB could have important therapeutic implications. One of the strategies for the down-regulation of NF-κB transcriptional activity is the specific inhibition of the DNA binding of NF-κB.

We have screened a virtual library using our structure-based computational screening method, thus enabling us to identify several compounds that inhibit DNA-NF-κB interactions. In our most recent studies, the inhibitory effects of the hit compounds selected from the virtual library were measured using fluorescence correlation spectroscopy (Olympus MF20) and an Electrophoresis Mobility Shift Assay. Using these methods, we found some compounds that inhibit the DNA-NF-κB interaction. We expect that these compounds may down-modulate the transcriptional function of NF-κB.

IV. Regulation of DNA replication machinery (Hiroshi Miyazawa)

DNA contains the genetic information which can be viewed as the organism's vital plan. Maintenance and replication of DNA, and the expression of the genetic information in DNA are the bases for life. In addition, information units of more than 10^9 are packaged and condensed in the nucleus of living cells. The condensation/decondensation of DNA molecules is dynamically repeated in growing cells during development and differentiation, necessitating strict control of the expression of genetic information.

Our purpose is to elucidate the functions of DNA replication factors and the proteins interacting with replication factors, and to ask how these factors act in various reactions occurring in DNA, such as DNA repair or transcription. We are investigating the behavior of these factors in nuclear structure, and studying how the DNA replication machinery is regulated during the cell cycle and cell differentiation.

So far, we have found that the second largest subunit of DNA polymerase ε (DPE2) interacts with SAP18, a polypeptide associated with the co-repressor protein Sin3. DNA polymerase ε is involved in chromosomal DNA replication, DNA repair and cell-cycle checkpoint control in eukaryotic cells. The Sin3 complex consists of several peptides containing the histone deacetylases, HDAC1 and HDAC2. By deacetylating histones in the chromosome, HDAC condenses chromatin structure, resulting in the repression of gene expression. The interaction of HDAC activity with replication factors predicts that DNA polymerase ε is involved in the maintenance of chromatin structure and transcriptional silencing during DNA replication. Thus DNA polymerase □ appears to be involved in epigenetic regulation. We are investigating how the interaction of DNA polymerase □ and the replication complex with proteins involved in epigenetic regulation (i.e. DNA methyltransferases, histone acetylases and deacetylases, and so on) change in the process of DNA replication and cell differentiation.

Publications (2010~2015)

[Original papers]

2015

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2. Komori R., Kobayashi T., Matsuo H., Kino K., Miyazawa H.* (2013) "Csn3 Gene Is Regulated by All-Trans Retinoic Acid during Neural Differentiation in Mouse P19 Cells." PLOS ONE, 8(4), e61938.
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2011

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3. Kino, K.*, Kobayashi T., Komori, R., Miyazawa, H. (2011) "Chapter 7: Science education through research." Sci. Edu. Rapidly Changing World, pp.125-136. (Nova Science Pub.) & Sci. Edu. through Res. (as Online Book; https://www.novapublishers.com/catalog/product_info.php?cPath=23_54&products_id=17694)
4. Morikawa, M., Kino, K., Miyazawa, H. (2010) "The chemical point of generation and biological effects of DNA damages." Hoshasen seibutsu kenkyu [Radiat. Biol. Res. Common], 45, 268-285. (Japanese)

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Diversity of Epigenetics Regulation and Malignancies

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Research

Functional regulation of proteins by post-translational modifications (Ohshima)

Post-translational modifications such as ubiquitination, phosphorylation, and acetylation regulate the function of many proteins. Recently, a number of ubiquitin-like proteins (Ubl) have been identified that are covalently linked to lysine residues in target proteins. One Ubl, SUMO-1, also known as PIC1, UBL1, sentrin, GMP1, and SMT3, is an 11-kDa protein that is structurally homologous to ubiquitin. SUMO-1 modification plays an important role in altering the function of modified proteins, including transcriptional activation, nuclear localization, and decreased turnover. SUMO-1 is conjugated to proteins through a series of enzymatic steps. Initially, the ATP-dependent formation of a thioester bond between SUMO-1 and the E1 enzyme complex (SAE1/Uba2) is formed, and SUMO-1 is then transferred to the E2-conjugating enzyme Ubc9. Finally, SUMO-1 is conjugated from Ubc9 directly to a lysine residue of target proteins. The E3 ligase that conjugates SUMO-1 to target molecules in vitro and in vivo has only recently been identified. One group of such E3 ligases, protein inhibitor of activated STAT (PIAS) family proteins homologous to the yeast Siz family protein, has a conserved RING-finger domain, which regulates transactivation of many transcription factors by conjugating SUMO-1. In order to understand the molecular mechanisms by which transcriptional regulation through SUMO-1 modification, we focus the transcription (co)factors involving in cell growth, differentiation, immortalization and attempt to define the biological significance of sumoylation in carcinogenesis.

Furthermore, we have been also analyzing the molecular mechanisms by which human T-cell leukemia virus type-1 (HTLV-1) infection is the cause of morbidity and mortality in adult T-cell leukemia (ATL).

Publications (2010-2015)

[Original papers]

2015

1. Mukai, R., Ohshima, T. (2013) HTLV-1 bZIP factor suppresses the centrosome protein B (CENP-B)-mediated trimethylation of histone H3K9 through the abrogation of DNA binding ability of CENP-B. *J. Gen. Virol.* 96, 159-164.

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2010

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Regulation of Immune functions through nuclear receptors

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Research

Research Themes:

The mechanisms of immune cell trafficking and the regulation of immune responses are our main themes to clarify. Especially, we study roles of nuclear receptor ligands including vitamin A & D and various hormones in regulating immune functions especially in mucosal systems including the gut. By pursuing these biologically fundamental questions, we set a goal to establish a solid basis of new remedies and drug discovery for various diseases.

Recent Study:

For efficient immune responses, immune cells with proper functions need to migrate into right sites in the body. T cells, known as the control tower of the immune system, patrol the whole body along with the blood vessels and lymphatic vessels. However, they cannot migrate into non-lymphoid tissues before they are activated with antigen in the secondary lymphoid organs. Once they are activated and become effector or memory T cells, however, they can migrate into non-lymphoid tissues. They tend to migrate into the tissue that is associated with the secondary lymphoid organ where they are activated. This type of migration is called “homing”. For example, T cells that are activated with antigen in the small intestine-relate secondary lymphoid organs, Peyer’s patches (PP) and mesenteric lymph nodes (MLN), tend to migrate into small intestinal tissues including the lamina propria. In 2004, we found that vitamin A-derived retinoic acid is the physiological factor that imprints gut-homing specificity on T cells. We also found that subpopulations of dendritic cells (DC) in PP and MLN express the key enzyme of retinoic acid synthesis, RALDH (retinaldehyde dehydrogenase), and are capable of producing retinoic acid from vitamin A (retinol). They imprint T cells with the gut-homing specificity by delivering retinoic acid to T cells during antigen presentation. In 2006, we also found that a similar mechanism is involved in the imprinting of B cells with gut-homing specificity by a collaboration mainly with Dr. von Andrian’ s group and Dr. Adams’ group.

In 2009, we established a method for estimating the enzyme activity of RALDH in each DC, and identified the retinoic acid-producing subpopulation in MLN-DC and PP-DC. The RALDH2 isoform was mostly responsible for the activity. Depending on these results, we searched for the physiological factors that induce RALDH2 expression in DC in the gut or in MLN. We found that GM-CSF (granulocyte-macrophage colony-stimulating factor) plays a major role in the induction, and that retinoic acid itself plays a role as an essential cofactor. IL-4 and IL-13 exhibited effects similar to those of GM-CSF on RALDH2 expression, but are found to be dispensable by the analysis of their receptor-deficient mice. The stimulation through Toll-like receptors enhanced the RALDH2 expression in DC as well as DC maturation.

In 2007, several groups reported that the retinoic acid-producing DC also enhance the differentiation of $Foxp3^+$ inducible regulatory T cells (iTreg) and suppress that of pro-inflammatory Th17 cells. Accordingly, we found that GM-CSF-treated DC that expressed RALDH2 could enhance the differentiation of $Foxp3^+$ iTreg and suppress that of Th17. These results suggest that retinoic acid contributes to oral tolerance and regulation of immune responses to specific antigens. We have recently found that vitamin A deficiency affects not only the nature of T cells but also that of DC, and that MLN-DC in vitamin A deficient mice can induce oral antigen-specific $CD4^+$ T cells that produce high levels of IL-13 and tumor necrosis factor-α (TNF-α). Under vitamin A-deficient conditions, although it has been generally known that antibody responses are reduced, we found that markedly high levels of IgG1 antibody responses and IgE antibody responses against oral antigens can be induced. It is likely that these antibody responses involve the above-mentioned new IL-13-producing inflammatory helper T (Th) cells. Currently, we are investigating the molecular mechanism of differentiation of these Th cells and their role in allergic and inflammatory diseases.

We have been also analyzing some other aspects of retinoic acid effects on immune functions and regulation, including 1) The

molecular mechanism of retinoic acid effects on the expression of gut-homing receptors on immune cells, 2) The role of a retinoic acid-metabolizing system in the regulation of T cell functions, 3) Amplification and disruption of retinoic acid signals by RXR ligands and environmental chemicals, 4) The molecular mechanism of RALDH isoform 2 (RALDH2, encoded by *Aldh1a2*) expression in DC and the roles of a retinoic acid-bound RAR/RXR heterodimer and a retinoic acid response element (RARE) half-site at the proximal promoter of the *Aldh1a2* gene. The RARE half-site in this gene promoter was commonly found in many animal species.

Publications

＊ 2010-2014

2014

1.Ohoka, Y, Yokota-Nakatsuma, A, Maeda, N, Takeuchi, H, and Iwata, M.: Retinoic acid and GM-CSF coordinately induce retinal dehydrogenase 2 (RALDH2) expression through cooperation between the RAR/RXR complex and Sp1 in dendritic cells. ***PLoS One***. 9(5): e96512 (2014).

2. Yokota-Nakatsuma, A., Takeuchi, H., Ohoka, Y., Kato, C., Song, S.-Y., Hoshino, T., Yagita, H., Ohteki, T., and Iwata, M.: Retinoic acid prevents mesenteric lymph node dendritic cells from inducing IL-13-producing inflammatory Th2 cells. ***Mucosal Immunol.*** 7(4):786-801 (2014). Epub 2013 Nov 13.

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Kagawa School of Pharmaceutical Sciences
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Regulatory Mechanism of Inflammatory Immune Diseases

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Research

Research project: Analysis of the molecular mechanism by which lysophospholipids regulate inflammatory responses in rheumatoid arthritis synovial cells.(Nochi)

In the previous studies, we assessed the role of lysophosphatidic acid (LPA) and synovial fluid of rheumatoid arthritis (RA) patients in COX-2 induction in fibroblast-like RA synovial cells. We found that synovial fluid from RA patients stimulated COX-2 induction, which was associated with prostaglandin E_2 production, in RA synovial cells. The synovial fluid-induced responses were inhibited by pertussis toxin, $G_{i/0}$ protein inhibitor, and by Ki16425, antagonist for LPA receptors (LPA_1 and LPA_3). Indeed, LPA effectively induced COX-2 expression and prostaglandin E_2 production. The LPA-induced actions were markedly inhibited by pertussis toxin, Ki16425, and small interfering RNA targeted for the LPA_1.

The local acidification of extracellular pH causes augmentation of cell proliferation as observed in the cancer tissue and the inflammatory site. In RA, the proliferation of synovial cell is abnormally augmented and the pH of synovial fluid from RA patient is lower than that of normal synovial fluid. Therefore, we examined the possibility that the local acidification in intraarticular cavity may affect the inflammatory responses and contributes to exacerbation of pathological condition in RA. We found that extracellular acidic pH induced COX-2 and ADAMTS-4 expression through Gq-coupled proton-sensing receptor (OGR1) in RA synovial cells. Furthermore extracellular acidic pH synergistically enhanced LPA-induced COX-2 and ADAMTS-4 expression through LPA_2. The LPA-induced COX-2 expression under acidic condition lasted for several hours, though the LPA-induced COX-2 expression via LPA_1 under physiological condition was transient. To examine details of the intracellular signaling mechanism by which COX-2 and ADAMTS-4 induction are regulated under acidic circumstances, further studies are now under way.

Research project: characterization of retinoid X receptor signaling on T cell differentiation. (Takeuchi)

Retinoic acid (RA) is an immune-modulating molecule, and its signaling is known to affect T cell differentiation. It can enhance differentiation toward regulatory T cell (Treg), and suppress that toward Th17. RA receptor is consist of two components, RAR and RXR. Although RXR has a ligand-binding domain, it does not bind RA at physiological condition. It is not well-studied whether RXR-specific signaling can affect T cell differentiation.

We found that RXR signaling actually played some roles on T cell differentiation. RXR signaling dramatically enhanced RA-mediated Treg differentiation. On the other hand, it suppressed that of Th17 in cooperation with some nuclear receptor signaling. The effect of RXR signal was observed in vivo as well as in vitro. Thus, this finding can apply to develop new methods to regulate immune-response and inflammatory diseases.

Research project: Analysis of the molecular mechanism of autophagy and mitophagy. (Kiriyama, Nochi)

In the course of our investigation of the effects of weakly basic agents on leukocytes, we found that these agents induced giant autolysosomes. We found that only GABARAPL1 mRNA among LC3 family members and mitochondria protein Nix mRNA are upregulated by weakly basic agents or mitochondrial membrane potential disrupter, CCCP. In addition, GABARAPL1 and mitochondria colocalized in autolysosomes. Moreover, we found that GABARAPL1 mRNA induction is independent of mTOR pathway, which is the main pathway of autophagy. These results suggest that GABARAPL1 may play an important role in mitophagy, an autophagy-related pathway specific for mitochondria. Further studies on the mechanism of autophagy are under way.

Publications (2010~2015)

[Original papers]

2014

1. Ohoka, Y, Yokota-Nakatsuma, A, Maeda, N, Takeuchi, H, and

Iwata, M.: Retinoic acid and GM-CSF coordinately induce retinal dehydrogenase 2 (RALDH2) expression through cooperation between the RAR/RXR complex and Sp1 in dendritic cells. ***PLoS One***. 9(5): e96512 (2014).

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1. Takeuchi, H., Yokota-Nakatsuma, Y., Ohoka, Y., Kagechika, H., Kato, C., Song, S.-Y., and Iwata, M.: Retinoid X receptor agonists modulate Foxp3^{+} regulatory T cell and Th17 cell differentiation with differential dependence on retinoic acid receptor activation. ***J Immunol*** 191(7): 3725-3733 (2013).

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Pathological analysis of neural disease using animal model

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Research

It's clear that the future direction of the Pharmaceutical science is "the development of therapeutic method of human diseases based on the understanding of pathophysiology at molecular level". This direction is also one of the most important bases of Pharmaceutical education. Thus we set final goal of our research on the integrative understanding of the pathogenesis of human diseases from molecular to individual level. To pursue this task, animal models for human diseases are beneficial experimental tools. From the analyses of these animals, we have a chance to combine analyses of molecular and cellular level with clinical changes at individual level. Further, these animals are valuable, because we can obtain most early changes in the pathogenesis of diseases, which are hardly examined in human patients. Thus one of the main methods of our division is histopathological analysis of these animals. These analyses require systemic approaches from macroscopic anatomy, conventional histological methods, immuno histochemical and *in situ* hybridization histochemical methods using light microscopy and electron microscopy. Now we are trying to establish a system for analyses integrating these lesions identified by histological techniques with biochemical and molecular biological analyses. We also pursue cooperative research projects with medical institutions outside our university for analyses using human materials that fulfill ethical criteria, depending on the progress in each research project. These trials using good animal models for human disease are expected to contribute to the better understanding of the pathophysiology of human disease. Following are the detailed information of each research project.

Following are the detailed information of each research project.

1. Functional analysis of lanosterol 14 alpha-demethylase (LDM, CYP51) in the processes of myelination and remyelination using oligodendrocyte-specific LDM transgenic mouse.

Lanosterol 14 alpha-demethylase (LDM, CYP51) is the only cytochrome P450 enzyme that is involved in cholesterol biosynthesis in eukaryotes. It is expressed abundantly in the liver and moderately in the brain. Cholesterol is not only a major component of plasma or endoplasmic reticulum membrane but also an essential component of myelin sheath in the central and peripheral nervous system. Cholesterol required for myelination in the brain is newly synthesized in oligodendrocytes, which form myelin sheaths, because cholesterol synthetized in the liver can't cross the blood brain barrier. Previously, our immunohistochemical and biochemical analyses indicated that LDM was predominantly expressed in oligodendrocyte and Schwann cell in the central and peripheral nervous system, respectively, and that its expression increased in the process of myelination during postnatal development and remyelination in the experimental demyelination-remyelination, which was induced by feeding ICR mice with a diet including cuprizone, then with a normal diet. These results suggest that augmentation of LDM expression in oligodendrocyte may have therapeutic significance in demyelinating disease, such as multiple sclerosis. In order to reveal whether LDM is critical enzyme for remyelination, we generated LDM transgenic mouse (LDM-Tg) driven by myelin proteolipid protein (PLP) promoter, which is oligodendrocyte-specific and PLP shows a similar expression pattern of LDM in the brain during myelination and remyelination. The oligodendrocyte-specific LDM expression cassette was constructed from PLP promoter with a length of 10 kbp, which was cloned from the first intron of mouse genomic DNA, LDM cDNA, which was cloned from total RNA derived from mouse brain, and polyadenylation signal sequence derived from SV40 late polyadenylation signal. The cell specificity of this expression cassette was confirmed by comparing the effect in culture cells, positive expression in Oli-neu cells derived from mouse oligodendrocyte and negative expression in HEK293T. This construct DNA was injected into pronuclei of fertilized egg and then LDM-Tg was obtained, which shows oligodendrocyte-specific overexpression of LDM with genetic background of C57BL/6. Western blot analyses of isolated proteins from various organs of LDM-Tg and control mouse revealed increased expression of LDM in the brain and spinal cord, while no such increase was observed in the liver, a major peripheral organ of cholesterol synthesis. Immunohistochemcal analyses also stronger LDM-immunoreactivity in the myelin sheath of the brain and spinal cord of LDM-Tg compared with those of control mouse. Now we are comparing changes of the LDM-expression and myelination during the postnatal development and

remyelination following experimental demyelination induced by the application of MOG peptide or cuprizone between LDM-Tg and control mouse to evaluate the effects of high expression of LDM on myelination and remyelination.

2. Expression changes of Stearoyl-CoA desaturase isoforms in neuronal and glial cells during the processes of demyelination, neurodegeneration and ischemia.

Stearoyl-CoA desaturase (SCD) is an enzyme involved in the synthesis of monounsaturated fatty acid such as oleic acid from saturated fatty acid such as stearic acid. Two isoforms (*SCD1* and *SCD5*) and four isoforms (*SCD1 – 4*) were identified in human and mice, respectively. It has been reported that oleic acid synthesized by SCD constitutes myelin sheath and induces the expression of MAP2 in neurons and its transition from soma to dendrites, and that oleic acid works as a kind of neurotrophic factor in the recovery processes from neuronal injury. Recently it has been reported that the expression of SCD is increased in patients with Alzheimer's disease. These data suggest that monounsaturated fatty acid metabolism is involved in differentiation and repair responses of the brain. However, cell identity to express SCD and its subtype during these processes are not fully understood. RT-PCR revealed that *SCD1* and *SCD2* were mainly expressed in mice brain and *in situ* hybridization using specific probes for *SCD1* and *SCD2* detected mainly *SCD2* in neurons and oligodendroglia. Expression analyses at cell level using quantitative RT-PCR and laser capture microdissection (LCM) revealed that neurons express SCD1 as well as SCD2, but the expression of SCD1 mRNA was quite low compared with that of SCD2 mRNA in the examined areas such as the cerebral cortex, pyramidal cell layer and stratum radiatum of the hippocampus. These data suggests that we failed to detect SCD1 mRNA expression by *in situ* hybridization due to its detection limit.

Immunohistochemical analyses using an antibody for SCD1 and SCD2 indicated that increased SCD-immunoreactivity in astrocytes and neurons at a peak of demyelination induced by feeding ICR mice with cuprizone containing diet for 6 weeks. Similar increased SCD-immunoreactivity in neurons was observed in a central part of stratum radiatum of hippocampal CA1 region of 3 x Tg Alzheimer model mice at postnatal day 200 as compared with control mice. Immunohistochemical analyses using antibodies specific for SCD1 and SCD2 and expression analyses using quantitative RT-PCR and LCM revealed that the expression of SCD2 mRNA remarkably increased in the white matter. SCD2-immunoreactivity decreased in the pyramidal cell layer of the hippocampus of 3 x Tg Alzheimer model mice compared with control mice, while SCD1-immunoreactivity showed no apparent change. These data suggest SCD2 mainly changes its expression during the processes of remyelination and neurodegeneration. It awaits further analyses to clear functional significance of SCD2 in those processes.

Publications (2010-2015)

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1. Yokota-Nakatsuma A, Takeuchi H, Ohoka Y, Kato C, Song S-Y, HoshinoT, Ohteki T and Iwata M. Retinoic acid prevents mesenteric lymph node dendritic cells from inducing IL-13-producing inflammatory Th2 cells. Mucosal Immunol 7:786-801 (2014).

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3. Yokota-Nakatsuma A, Takeuchi H, Ohoka Y, Kato C, Song S-Y, HoshinoT, Ohteki T and Iwata M. Retinoic acid prevents mesenteric lymph node dendritic cells from inducing IL-13-producing inflammatory Th2 cells. Mucosal Immunol Epub 2013 Nov 13.

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3. Satake S., Song S.-Y., Konishi S. and Imoto K. Glutamate transporter EAAT4 in Purkinje cells controls intersynaptic diffusion of climbing fiber transmitter- mediating inhibition of GABA release from interneurons. European Journal of Neuroscience, 32: 1843-53 (2010).

Kagawa School of Pharmaceutical Sciences
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Neuroscience Study on Regulation and Plasticity at Inhibitory Synapses

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Manami Hanafusa (Second year of master's course)

Research

Since the expansion of the Institute of Neuroscience, Tokushima Bunri University April, 2007, all the staffs have been appointed joint members of the institute and have been involved in a collaborative research project aiming at etiology and early diagnosis of Alzheimer disease based on our expertise described below. This project has been supported by the MEXT.

Our research in the Neurophysiology lab has focused on three topics in neuroscience: (1) the cellular and molecular mechanisms underlying plasticity at inhibitory GABAergic synapses in the central nervous system (CNS); (2) the molecular mechanisms for GABA receptor trafficking into inhibitory synaptic sites; and (3) the search for lead compounds that have therapeutic potential in the mental disorders associated with GABAergic synapses.

Using molecular imaging technique and dissociated neuron culture system, Kuriu and Konishi published in 2012 a paper entitled "Activity-dependent coordinated mobility of hippocampal inhibitory synapses visualized with presynaptic and postsynaptic tagged-molecular markers" in *Mol. Cell. Neurosci.* Also, Shuntaro Kohnomi has joined the lab from April, 2012, and he has started an electrophysiological project on the brain reward system using brain slice-patch clamp techniques.

Numerous brain functions depend on the balance between excitatory and inhibitory synaptic activity in the CNS. Any changes in synaptic transmission therefore seriously affect brain function, often leading to neurological and mental disorders. In particular, GABAergic inhibitory synapses play a pivotal role in a number of mental disorders, which is reflected in the fact that one group of GABA receptor enhancers, benzodiazepines, includes the most frequently prescribed CNS drugs worldwide. Thus, elucidation of the mechanisms underlying regulation of the strength of inhibitory neurotransmission at GABAergic synapses provides not only basic information about brain mechanisms, but also may suggest critical strategies in the search for drug targets in mental disorders such as severe anxiety, depression and cognitive dysfunction.

Recently, we have been interested in the mechanisms underlying control of inhibitory neurotransmission at GABAergic synapses and have found three novel and distinct modes of synaptic modulation in the cerebellar cortex. First, the monoamines noradrenaline and serotonin, released from afferent inputs originating from the brainstem, elicit short-term and long-term enhancement of GABA release at inhibitory synapses between cerebellar interneurons and Purkinje cells, the sole output neuron from the cerebellar cortex. In short-term enhancement, activation of $ß_2$-adrenergic receptors in the nerve terminal of interneurons by noradrenaline leads to an acceleration of hyperpolarization- and cyclic nucleotide-gated cation (HCN) channels and depolarizes interneurons, which in turn causes repetitive action potentials and an increase in the frequency of spontaneous GABA release from the nerve terminals of interneurons. In long-term enhancement, the $ß_2$-adrenergic receptor activation couples to stimulation of cyclic AMP-dependent protein phosphorylation and thereby enhances action potential-induced GABA release via protein kinase A-dependent increases in Ca^{2+} sensitivity of the release machinery, as well as the size of the readily releasable pool of GABA in the interneuron nerve terminal (Saitow et al., 2000, 2005). In addition to this presynaptic regulation of GABAergic inhibitory neurotransmission, ATP has been shown to enhance GABAergic transmission through a postsynaptic mechanism in which activation of P2Y-type purinergic receptors by ATP and its metabolites increases the sensitivity of $GABA_A$ receptors in Purkinje cells (Saitow et al., 2004; Ono et al., 2006).

Second, the cerebellar GABAergic synapse between interneurons and Purkinje cells is under the control of presynaptic

inhibition induced by the excitatory neurotransmitter, possibly glutamate, released from the climbing fiber input (Satake et al., 2000). The climbing fiber transmitter not only excites Purkinje cells but also acts on AMAP-type glutamate receptors in the presynaptic nerve terminal of interneurons to inhibit GABA release (Satake et al., 2004, 2006, 2010). The climbing fiber transmitter-mediated inhibition of GABA release is caused by inhibition of voltage-gated Ca^{2+} channels in the presynaptic terminal following activation of AMPA receptors (Rusakov et al., 2005). Therefore, the climbing fiber transmitter glutamate spills out of the synaptic cleft and reaches the presynaptic terminal of interneurons, thereby inhibiting GABA release through activation of AMPA receptors coupling to inhibition of Ca^{2+} channels in the nerve terminals.

A third form of novel synaptic mechanism that we found around cerebellar GABAergic synapses is cross-talk between $GABA_B$ receptors and Group I type metabotropic glutamate receptors (mGluR1); GABA released from interneurons acts on $GABA_B$ receptors expressed in the periphery of nearby excitatory synapses (peri-synaptic regions) between parallel fibers and Purkinje cells and enhances mGluR1-mediated slow synaptic excitation in Purkinje cells (Hirono et al., 2001, 2011). Therefore, under certain circumstances, GABA appears to elicit an excitatory action following cross-talk between its own receptors and metabotropic glutamate receptors. Furthermore, because mGluR1 is critically involved in synaptic plasticity, it is highly likely that a combination of $GABA_B$ receptors and its selective ligands is a promising therapeutic target for cognitive dysfunction.

As exemplified above, the strength of inhibitory neurotransmission at GABAergic synapses in the CNS is modulated by multiple regulatory mechanisms, which will likely yield clues for developing therapeutics for the treatment of CNS diseases. Based on these findings and considerations, our aims include: (1) to further elucidate the cellular and molecular mechanisms that underlie synaptic modulation of inhibitory GABAergic transmission, and (2) to devise a drug screening system to search for potential lead compounds that fit the profile of GABA synapse enhancers. We are utilizing three approaches: (1) thin brain slices from rats mice combined with electrophysiological techniques using patch-clamp recordings, allow us to study the properties of GABAergic inhibitory synapses, both physiologically and pharmacologically; (2) optical recording of neuronal activity in the brain using VSDs (voltage-sensitive dyes), provides not only spatial information about brain activity but also a powerful means for screening neuron/synapse-acting compounds; and (3) primary cultures of neurons dissociated from the brain combined with confocal imaging and electrophysiology, which allow us to study the molecular mechanisms of $GABA_A$ receptor delivery into inhibitory synaptic sites.

Combining these three different experimental approaches, we are now characterizing the synaptic mechanisms associated with the control at GABAergic inhibitory synapses as well as the neural mechanisms of learning and memory formation in the brain. We are pursuing the four research projects described above in order to gain further understanding of the brain mechanisms, which may lead to drug therapies for neurological and mental disorders caused by dysfunction of central GABAergic inhibitory synapses.

Publications (2010～2015)

[Original papers]

2014

1. Okabe A, Shimizu-Okabe C, Arata A, Konishi S, Fukuda A & Takayama C. (2015) KCC2-mediated regulation of respiration-related rhythmic activity during postnatal development in mouse medulla oblonga. *Brain Res.* 1601: 31-39.
2. Isshiki M., Tanaka S., Kuriu T., Tabuchi K., Takumi T., Okabe S. (2014) Enhanced synapse remodelling as a common phenotype in mouse models of autism. *Nature Commun.* 5: 4742: 1-15.

2013

1. Watanabe T., Sadamoto H. and Aonuma H. (2013) Molecular basis of the dopaminergic system in the cricket Gryllus bimaculatus. *Invert Neurosci* 13, 107-23.
2. Sadamoto H. and Muto H. (2013) Fluorescence Cross-correlation Spectroscopy (FCCS) to Observe Dimerization of Transcription Factors in Living Cells. *Methods Mol Biol.* 977, 229-41.
3. Murakami J., Okada R., Sadamoto H., Kobayashi S., Mita K., Sakamoto Y., Yamagishi M., Hatakeyama D., Otsuka E., Okuta A., Sunada H., Takigami S., Sakakibara M., Fujito Y., Awaji M., Moriyama S., Lukowiak K. and Ito E. (2013) Involvement of insulin-like peptide in long-term synaptic plasticity and long-term memory of the pond snail Lymnaea stagnalis. *J Neurosci.* 33, 371-83.
4. Shin E., Kashiwagi Y., Kuriu T., Iwasaki H., Tanaka T., Koizumi H., Gleeson JG., Okabe S*. (2013) Doublecortin-like kinase enhances dendritic remodeling and negatively regulates synapse maturation. *Nature Commun.* 4: 1440: 1-14.

2012

1. Hirono M*, Saitow F, Kudo M, Suzuki H, Yanagawa Y, Yamada M, Nagao S, Konishi S*, Obata K. (2012) Cerebellar Globular Cells Receive Monoaminergic excitation and monosynaptic inhibition from Purkinje cells. *PLoS One* 7: e29663.
2. Kohnomi S, Koshikawa N, Kobayashi M*. (2012) D_2-like dopamine receptors differentially regulate unitary IPSCs depending on presynaptic GABAergic neuron subtypes in rat nucleus accumbens shell. *J. Neurophysiol.* 107: 692-703.
3. Kuriu T, Yanagawa Y, Konishi S.* (2012) Activity-dependent coordinated mobility of hippocampal inhibitory synapses visualized with presynaptic and postsynaptic

tagged-molecular markers. *Mol. Cell. Neurosci.* 49: 184-195.

2011

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2. Sadamoto, H., Saito, K., Muto, H., Kinjo, M. and Ito, E. (2011) Direct observation of dimerization between different CREB1 isoforms in a living cell. *PLoS ONE* 6, e20285.
3. Watanabe, T., Sadamoto, H., and Aonuma, H. (2011) Identification and expression analysis of the genes involved in serotonin biosynthesis and transduction in the field cricket Gryllus bimaculatus. *Insect Mol Biol* 20, 619-35.

2010

1. Satake, S., Song, S.-Y., Konishi, S.*, and Imoto, K. (2010). Glutamate transporter EAAT4 in Purkinje cells controls intersynaptic diffusion of climbing fiber transmitter mediating inhibition of GABA release from interneurons. Eur *J Neurosci.* 32 (11): 1843-53. PMID: 21070388
2. Saito K, Kakizaki T, Hayashi R, Nishimaru H, Furukawa T, Nakazato Y, Takamori S, Ebihara S, Uematsu M, Mishina M, Miyazaki JI, Yokoyama M, Konishi S, Inoue K, Fukuda A, Fukumoto M, Nakamura K, Obata K, Yanagawa Y. (2010) The physiological roles of vesicular GABA transporter during embryonic development: a study using knockout mice. *Mol Brain.* 3 (1): 40. PMID: 21190592; PMC3023674.
3. Masuoka, T., Mikami, A., Kamei, C. (2010) Ameliorative effect of a hippocampal metabotropic glutamate- receptor agonist on histamine H1 receptor antagonist-induced memory deficit in rats. *J Pharmacol Sci* 113(1): 41-47.
4. Kohnomi, S., Suemaru, K., Goda, M., Choshi, T., Hibino, S, Kawasaki, H., Araki, H. Ameliorating effects of tropisetron on dopaminerigc distuption of prepulse inhibition via the α_7 nicotinic acetylcholine receptor in Wistar rats. *Brain Res*. 1353: 152-158. PMID: 20673759.
5. Hatakeyama, D., Mita, K., Kobayashi, S., Sadamoto, H., Fujito, Y., Hiripi, L., Elekes, K. and Ito, E. (2010) Glutamate transporters in the central nervous system of a pond snail. *J Neurosci Res* 88, 1374-1386.
6. Sadamoto, H., Kitahashi, T., Fujito, Y., and Ito, E. (2010) Learning-dependent gene expression of CREB1 isoforms in the molluscan brain. *Front Behav Neurosci* 4, 25.

[Review articles]

1. Konishi S. and Satake S. (2013) Physiological interactions between neurons and glia: roles of transporters in the control of intersynaptic crosstalk. Chapter 9, In Glial Cells: Embryonic Development, Types, Functions and Role in Disease, edit by K. Charanjit and Eng-Ang Ling, pp.177-191, Nova Science Publishers, New York.

[Books]

1. Konishi S., Kirino Y., Ito E., and Song S-.Y. (2013) "Mechanism of Memory" Vol. 1 & 2: translated from "Memory From Mind to Molecules" by E.R. Kandel & L.R. Squire. pp.1-298, pp.1-300, Blubacks B-1842 & B-1843, Kodansha, Tokyo

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Research on Pharmacotherapy and Experimental Neurology

Staff

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Research

【Research aims】

Our research goal is the novel molecular target for new drugs. To achieve this goal, we are working on molecular mechanism for the epileptogenesis of partial and generalize epilepsy through the several approaches such as pharmacological, behavioral, cell biological, biochemical and *in vivo* imaging techniques.

【Research Scopes】

1. Prevention of status epilepticus-induced brain edema and neuronal cell loss by new antiepileptic drugs.

Status epilepticus (SE) refers to neurologic emergencies that may lead to death or permanent neurologic injury. To avoid life threating injury, patients must be properly and quickly treated. Furthermore, SE causes 3~5% of symptomatic epilepsy (~35% of epileptic syndromes), thus SE patients are at a high risk of developing acquired epilepsy (Hesdorffer, 1998; Temkin, 2003; Jacobs et al., 2009). The management of SE is important to prevent mortality and the development of post-SE symptomatic epilepsy. Seizures must be treated as soon as possible and benzodiazepines (lorazepam or diazepam) are typically administered as first-line antiepileptic drugs (AEDs). However, when these drugs fail, second-line AEDs (phenytoin; PHT, fosphenytoin; fosPHT, valproate; VPA, and midazolam) are administered in refractory SE prior to giving phenobarbital; PB (Manno, 2011). Various clinical trials have indicated that conventional AEDs (e.g., DZP, PB, VPA, or PHT) suppressed acute seizures, but thus far there has been no success at preventing the development of post-SE acquired epilepsy under various conditions (Temkin, 2001; 2003; 2009). Although the mechanisms underlying the development of acquired epilepsy as part of the epileptogenic process are not well understood, the lack of efficacy of the AEDs suggests that the biological mechanisms of the acquired epilepsy process may be quite different from that of the established epileptic brain (Pitkanen et al., 2009).

Levetiracetam ([(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide]) with broad-spectrum antiepileptic effects is an established second-generation AED that is widely used in patients with either generalized or partial epilepsy (Lyseng-Williamson, 2011). In addition, levetiracetam is one of currently available candidates as second-line AED for SE (Manno, 2011) and as an anti-epileptogenic drug (Pearl et al., 2013; Klein, et al., 2012). Animal studies have shown that levetiracetam possesses anticonvulsant activity and results in neuroprotective effects (Mazarati et al., 2004; Zheng et al., 2010). In addition, levetiracetam has been considered for the treatment of pilocarpine (PILO)-SE due to its anti-epileptogenic effects in basic and clinical studies. Two phase II clinical trials for levetiracetam indicated the possibility that it may decrease the risk of acquired epilepsy or prevent the development of acquired epilepsy (Pearl et al., 2013; Klein, et al., 2012). However, the previous evidence in SE animal models has been conflicting and whether levetiracetam can prevent or modify epileptogenesis remains controversial (Löscher et al., 1998; Glien 2002; Klitgaard, Pitkanen, 2003; Stratton et al., 2003; Gibbs et al., 2006; Brandt, et al., 2007).

Temporal lobe epilepsy (TLE) is the most frequent type (75%) of symptomatic partial epilepsies that originate from the limbic regions (e.g., hippocampus and amygdala) after an initial brain insult, such as SE, stroke, and traumatic brain injury (TBI). Additionally, it is also one of the most refractory forms of epilepsy with approximately 30% of patients being unresponsive to AEDs (Engel, 1996; Kwan and Brodie, 2000). In this present study, we used a PILO-induced SE mice as a model of TLE to determine the effects of repeated administration of high-dose levetiracetam after the termination of SE by DZP. We observed that repeated high-dose levetiracetam prevented the development of brain edema in the limbic regions at the initial period of post-SE, and the incidence of spontaneous recurrent seizures.

2. Study on the relationship between blood brain barrier (BBB) disruption and generalized epilepsy.

In recent years, it have been well recognized that the therapies for epilepsy by the AEDs, which is represented by valproate, have been definitely effective. On the other hand, no less than 30% of epileptic patients were intractable, so there are difficulties in achievement of high level of QOL for them. In order to dissolve this problem, the development of new AEDs with novel mechanisms is an important for drug-resistant patients. We aim to find out the novel molecular target for new drugs. Recently, we have focused the relationship between BBB disruption and generalized epilepsy. Although conventional evaluation methods of BBB disruption are to measure the diffusion of low molecular weight dye (ex. Evans blue) to brain parenchyma, they are not available in animals alive. In our laboratory, the spatial and sequential changes of the BBB disruption in PTZ-induced convulsive alive mice were elucidated by the technique of gadolinium-enhanced magnetic resonance imaging using the MRI for rodent (MRminiSR, 1.5T). In addition, we have investigated the involvement of NO in the BBB disruption in generalized epilepsy.

3. Beneficial effects of supplementation of the rare sugar "D-allulose" against hepatic steatosis and obesity

The rise in obesity is a major public health concern worldwide. Obesity is a common nutritional disorder, defined as an excessive overweight status presenting with a high body fat, often associated with numerous health problems. The prevalence of obesity in the Organization for Economic Co-operation and Development (OECD) countries is more than half of the adult population (53%) based on latest surveys (OECD Health Statistics, 2012), and being overweight is often associated with type 2 diabetes as a result of insulin resistance (Saltiel, 2001; Wang et al., 2005). The rate of obesity is the lowest (4.1% in 2011) and highest (36.5% in 2010) in Japan and the USA, respectively, based on the WHO criteria, among OECD member countries (Factbook Country Statistical Profiles in OECD, 2014). However, the prevalence of obesity has also been rising in Japan due to the increased adoption of a Westernized meal style and decreased physical activity.

Although sugar has been a major component of the human diet since ancient times, a high intake of sugar may be associated with an increased risk of health conditions, such as obesity, cardiovascular disease, diabetes, gout, fatty liver and dental caries (Burt and Pai, 2001; Bristol et al., 1985; Johnson et al., 2007; Milich et al., 1986; van Baak and Astrup, 2009). In particular, the rising intake of sugar-sweetened beverages, sweets and desserts high in glucose and fructose has recently been identified to be a major contributor to the obesity epidemic (Ludwig et al., 2001; Mozaffarian et al., 2011; Te Morenga et al., 2012). Therefore, decreasing the intake of sugar is necessary to achieve weight loss. However, it is difficult to strictly control the intake of sugar and/or sugar-containing foods and beverages.

D-allulose (also called D-psicose), an epimer of D-fructose isomerized at C-3 position, is a rare ketohexose found in wheat, Itea plants, processed cane and beet molasses (Matsuo et al., 2001; Oshima et al., 2006; Baek et al., 2010) and is also present in the commercial complexes of D-glucose and D-fructose in small quantities (Cree and Perlin, 1968). Due to its rarity, the biological functions of this sugar have not yet been explored, although innovating and unique methods of production using Izumoring (Granstrom et al., 2004; Takeshita et al., 2000) have enabled a number of investigations, and this compound is expected to be approved for commercial use as a substitute for sugar in foodstuffs aiming to maintain the physiological levels of blood sugar and prevent excess fat deposition, thus controlling obesity. D-allulose has attracted much attention for its promising antihyperglycemic and anti-lipidemic effects, shown experimentally in normal rats and clinically on maltodextrin-stimulated glucose tolerance tests in healthy humans (Iida et al., 2008; Matsuo et al., 2006). Most recently, we demonstrated that D-allulose serves as a unique metabolic regulator in growing type 2 diabetes OLETF rats via the maintenance of blood glucose and prevention of abdominal fat deposition (Hossain et al., 2011, 2012). These results suggest that D-allulose may be a suitable candidate as an antidiabetic agent, even as an ingredient in food.

The inherited deficiency of leptin, an appetite-suppressing hormone, causes obesity and obesity-related syndromes (Bray and York, 1979; Herberg and Coleman, 1977; Ingalls et al., 1950; Mayer et al., 1951; Zhang, et al., 1994). Leptin-deficient Lepob/Lepob (ob/ob) mice develop obesity-related glucose intolerance, insulin resistance and fatty liver (excess fat deposition in the liver), and increased lipogenesis has been reported in both the liver and adipose tissue of these mice (Bray and York, 1979; Herberg and Coleman, 1977). Therefore, these animals provide a good model of obesity and related syndromes, including glucose intolerance and fatty liver disease (Montague et al., 1997).

In the present study, we examined the effects of the subchronic ingestion of D-allulose on obesity and hepatic steatosis in ob/ob mice. Hepatic steatosis is characterized by the abnormal and excessive accumulation of lipids within hepatocytes. In addition, we performed in vivo evaluations with the goal of characterizing the morphological aspects of adipose tissues and other visceral organs using magnetic resonance imaging (MRI). This study is the first to examine the dietary supplemental benefits of D-allulose in ob/ob mice, which particularly influenced the rate of obesity and development of obesity-related hepatic steatosis.

Publications (2010~2015)

[Original papers]

2015

1. Ishihara Y., **Itoh K.**, Ishida A., Yamazaki T. (2015) Selective estrogen-receptor modulators suppress microglial activation and neuronal cell death via an estrogen receptor-dependent pathway. ***J. Steroid Biochem. Mol. Biol***. 145:85-93
2. **Itoh, K**., Inamine, M., Oshima, W., Kotani, M., Chib, Y., Ueno, M., Ishihara, Y., (2015) Prevention of status epilepticus-induced brain edema and neuronal cell loss by repeated treatment with high-dose levetiracetam. ***Brain Res***., accepted

2014

1. Kobayashi T., **Komori R**., Ishida K., Kino K., Tanuma S., Miyazawa H., (2014) Tal2 expression is induced by all-trans retinoic acid in P19 cells prior to acquisition of neural fate. ***Sci. Rep.,*** 4, 4935

2013

1. Danjo, S., Ishihara, Y., **Watanabe, M**., Nakamura, Y. and **Itoh, K.** (2013) Pentylentetrazole-induced loss of blood-brain barrier integrity involves excess nitric oxide generation by neuronal nitric oxide synthase. ***Brain Res***. 1530, 44-53.
2. **Watanabe, M**., Miyai, A., Danjo, S., Nakamura, Y. and **Itoh, K**. (2013) The threshold of pentylenetetrazole-induced convulsive seizures, but not that of nonconvulsive seizures, is controlled by the nitric oxide levels in murine brains. ***Exp. Neurol.*** 247, 645-652.
3. Fujii H. G., Sato-Akaba H., Emoto M. C., **Itoh K.**, Ishihara Y., Hirata H., (2013) Noninvasive mapping of the redox status in septic mouse by in vivo electron paramagnetic resonance imaging. ***Magn. Reson. Imag***., 31:130-138
4. Ishihara Y, **Itoh K**, Mitsuda Y, Shimada T, Kubota T, Kato C, Song Si-Y, Kobayashi Y, Mori-Yasumoto K, Sekita S, Kirino Y, Yamazaki T, Shimamoto N. (2013) Involvement of brain oxidation in the cognitive impairment in 3xTg-AD mice: Non-invasive measurement of the brain redox state by magnetic resonance imaging. ***Free Radical Res.*** 47: 731-739

2012

1. Hama, S., Ishihara, Y., **Watanabe, M**., Danjo, S., Nakamura, Y. and **Itoh, K**. (2012) Effects of Sulfaphenazole after Collagenase-induced Experimental Intracerebral Hemorrhage in Rats. ***Biol. Pharm. Bull.*** 35, 1849-1853.
2. Kotani, M., **Itoh, K.**, Ito, T., Yamashita, T. and Imada, M. (2012) Generation and characterization of a monoclonal antibody, Namu mAb, which reacts to the subependymal zone and the neurospheres in mouse brain, ***Neuroreport.*** 23, 830-834.
3. Myllykoski, M., **Itoh, K**., Kangas, SM., Heape, AM., Kang, SU., Lubec, G., Kursula, I. and Kursula, P. (2012) The N-terminal domain of the myelin enzyme 2´,3´-cyclic nucleotide 3´-phosphodiesterase: Direct molecular interaction with the calcium sensor calmodulin. ***J. Neurochem.***123, 515-524.

2011

1. Ichikawa, Y., and **Itoh, K**. (2011) Blood-arachnoid barrier disruption in experimental rat meningitis detected using gadolinium-enhancement ratio imaging. ***Brain Res.*** 1390, 142-149.
2. **Watanabe, M**., and **Itoh, K**. (2011) Characterization of a novel posttranslational modification in neuronal nitric oxide synthase by small ubiquitin-related modifier-1. ***Biochem. Biophys. Acta*** 1814, 900-907.
3. **Itoh, K.**, Fujisaki K., and Watanabe, M. (2011) Human L1CAM carrying the missense mutations of the fibronectin-like type III domains is localized in the endoplasmic reticulum and degraded by poly-ubiquitylation. ***J. Neurosci. Res.*** 89, 1637-1645.
4. Ishihara, Y., Katayama, K., Sakabe, M., Kitamura, M., Aizawa, M., Takara, M., and **Itoh, K**. (2011) Antioxidant properties of rare sugar D-allose: Effects on mitochondrial reactive oxygen species production in Neuro2A cells. ***J. Biosci. Bioeng.*** 112, 638-642.

2010

1. Suzuki, A., Arikawa, C., Kuwahara, Y., **Itoh, K**., **Watanabe, M.**, Watanabe, H., Suzuki, T., Funakoshi, Y., Hasegawa, H., and Kanaho, Y. (2010) The scaffold protein JIP3 functions as a downstream effector of the small GTPase ARF6 to regulate neurite morphogenesis of cortical neurons. ***FEBS Lett*** 584, 2801-2806.

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Laboratory for Neural Circuit Systems

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Yoko Tominaga
Research Assistant since 2006

Research

Since the expansion of the Institute of Neuroscience, Tokushima
Research areas of the laboratory

I. Study of neural circuit mechanisms of learning and memory with optical recording methods

The primary interest of the laboratory is the neural circuit mechanisms of higher cognitive functions, such as learning and memory, in the brain. A measurement method that makes the laboratory unique in the field is an optical recording method that uses voltage-sensitive dye (VSD) with electrophysiology. As one of the leading laboratories in the use of this technique, we have been continuously developing the method since the 90s and have provided established tools to colleges throughout the world.

II. Analysis of the electrophysiological control of excitable membranes in connection with ciliary structures.

By focusing on the role of information integration in the membrane potentials of cells, we have used the model organism, paramecium, which is the simplest single-celled animal, to study the mechanisms of the membrane potential control of cilia.

Specific research aims

Area I

1. Neural circuitry mechanisms of the limbic system: Optical study.

The limbic system is a brain structure that is critical for emotion and declarative memory. The limbic system consists of many areas, including the hippocampus, amygdala, and associated cortical systems, such as the entorhinal and piriform cortices. We are analyzing the function of these circuits by visualizing neural activity with the VSD optical recording methods.

We have revealed reverberation circuits and information integration mechanisms in the deep layers of the entorhinal and piriform cortices in association with the hippocampal neural circuit (Science, 1996; Neurosci. Res., 2008) with Professor Toshio Iijima's group at Tohoku University. In addition, we have found that neuronal signals from layer III of the medial entorhinal cortex are critical for temporal association memory formation (Science, 2011) with Professor Susumu Tonegawa's laboratory at the Picower center for learning and memory at MIT. We have also revealed information integration processes in the entorhinal cortex (Eur. J. Neurosci., 2007) with Dr. Riichi Kajiwara and Dr. Ichiro Takashima's group at AIST Japan.

In the 2012 fiscal year, we showed that the D-current plays an important role in the integration of neural activity in the entorhinal cortex in collaboration with Dr. Riichi Kajiwara (Japan Society for Neuroscience, 2012; Society for Neuroscience, 2012; supported by KAKENHI).

2. Development of an optical measurement microscope: stimulation pattern with a confocal microscope system and a new optical measurement.

The optical recording method with VSD requires high-speed and low-noise imaging. This requires new special optics. We have been developing special optics that meet these requirements (J. Neurosci. Methods, 2000; now commercially available as THT-microscope, BrainVision).

We have also developed special new ultra-high-speed and low-noise confocal optics (submitted; supported by JST tansaku, A-STEP).

In addition, we have developed a microscope that allows us to conduct light stimulus patterns to the neural networks (SFN abstr., 2011).

Recently, we have started a project to develop a special chamber that is suitable for these experiments (Supported by JST A-STEP, 2012-2013).

3. Mechanisms of late-onset brain dysfunctions caused by early transient exposure to chemicals and drugs.

There are several lines of evidence that indicate that the early transient exposure to certain chemicals during brain development results in the malfunctioning of cognitive function in adulthood. The neural mechanisms of these effects are largely unknown. We are evaluating these neural mechanisms with our optical recording methods as part of the research team that is supported by the Ministry of Health, Labour and Welfare (2008-).

We have shown that the administration of valproic acid, which is the first-line drug used in the treatment of epilepsy, during pregnancy causes a collapse of the balance of excitation and inhibition in children born to these mothers (Japanese Society of Toxicology, 2012). This study is joint research that is being conducted with Kentaro Tanemura sensei of Tohoku University, Dr. Yoshikazu Nakajima of Nara Institute of Science and Technology, and the teacher Katsuhide Igarashi of the Japan Institute of Health Sciences.

In addition, we will organize a symposium at the Japan

Neuroscience Society in Kyoto in June 2013.

4. Study of the Application of optical measurement methods to test ES cell function.

This study was initiated in 2012 and is intended to use the optical recording method with VSD to characterize cells that are differentiated from human ES cells. This is joint research that is being conducted with Prof. Katsunori Sasaki, Shinshu University [supported by KAKENHI (A)].

5. Visualization of cell-specific membrane potential responses by the introduction of voltage sensitive fluorescent protein (VSFP), which is a new membrane potential-sensitive protein.

In collaboration with Dr. Thomas Knopfel at RIKEN BSI, we have succeeded in detecting optical signal-specific hippocampal pyramidal cells by introducing a new VSFP from 2012. The detection of cell-specific signals are made possible in specimens in vivo by the further development of this technique.

6. Detection and use of the optical signals from neural excitation with a polarized light microscope.

This is joint research that is being conducted with Dr. Tomomi Tani and Dr. Oldenburg of Woods Hole MBL. In this study, we aim to detect changes in nerve optical properties, such as polarization, that are caused by nerve excitation. In March of 2013, we will visit the MBL for this purpose.

7. Studies of the mechanisms of regulation by a variety of factors and the neural responses of hippocampal neural synapses.

We are collaborating with various laboratories to apply our method in order to examine the neural pathologies of diseases, such as Alzheimer's disease, and other factors (J. Neurosci., 1996; Neurosci. Letters, 1997; J. Neurosci, 2002; PNAS, 2004; Neuropharmacol., 2005).

8. Regulation mechanisms of neural activity by inhibitory synapses in the hippocampus.

The unique feature of VSD imaging compared to other biological imaging methods is that it can measure hyperpolarization and, thus, inhibitory neural responses. From this point of view, we found depolarizing GABA responses in area CA1 in response to tetanic stimulation (J. Neurophysiol., 2002; Pfluger's Arch., 2010). In addition, we found perisomatic inhibitory actions with feedforward inhibition (Neurosci. Res., 2009).

Area II

1. Physiological studies of osmoregulatory mechanisms and contractile vacuoles of Paramecium.

For the first time, we have applied electrophysiological methods to the study of the Paramecium organelles, the contractile vacuoles, and have revealed the membrane dynamics that are involved in this periodic activity (J. Exp. Biol., 1997a, b; 1998a, b; J. Cell Sci., 1999; J. Exp. Biol., 2005).

2. Physiological studies of membrane proteins and cilia of paramecium response mechanisms.

The use of recent techniques of RNA interference knockdown in combination with the whole genome project of the Paramecium has enabled us to knock down particular proteins that are associated with cilia disease (so-called ciliopathy). We have found that the absence of some molecules that have been thought to be structural proteins induces behavioral changes. By applying electrophysiological methods to this mutant, we have examined the relationship of that behavior and the membrane responses and found that some of these "structural proteins" are actually involved in membrane potential-mediated signal transduction (e.g., Eukary. Cell, 2012). This is joint research that is being conducted with Prof. Hori of Yamaguchi University.

Publications (2010~2015)

[Original papers]

*Corresponding author

2014-

2013

1. Takashi Tominaga[CA], Riichi Kajiwara, and Yoko Tominaga (2013) VSD imaging method of ex vivo brain preparation *Journal of Neuroscience and Neuroengineering* 2, 211-219 (2013) *[Featured Article に採用]*
2. Tominaga T[CA] and Tominaga Y. (2013) A new non-scanning confocal microscopy module for functional voltage- sensitive dye and Ca2+ imaging of neuronal circuit activity Journal of Neurophysiology J Neurophysiol 110, 553-561; published ahead of print April 24, [Also featured as Key Scientific Articles on Global Medical Discovery]

2012

1. Kutomi, O., Hori, M., Ishida, M., Tominaga, T., Kamachi, H., Koll, F., Cohen, J., Yamada N and Noguchi M. (2012). Outer Dynein Arm Light Chain 1 Is Essential for Controlling the Ciliary Response to Cyclic AMP in Paramecium tetraurelia. Eukaryotic cell, 11(5), 645–653. doi:10.1128/EC.0527

2011

1. Suh, J., Rivest, A.J., Nakashiba, T., Tominaga, T., and Tonegawa, S. (2011). Entorhinal cortex layer III input to the hippocampus is crucial for temporal association memory. *Science 334*, 1415-1420.

2010

1.Tominaga T, and Tominaga Y. (2010) GABA(A) receptor-mediated modulation of neuronal activity propagation upon tetanic stimulation in rat hippocampal slices. Pflugers Arch 460: 875-889.

[Review articles]

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Design, Manufacturing and Evaluation of Novel Pharmaceutical Preparations for Patients

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Research

We have the research philosophy for Laboratory of Pharmaceutics, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University. Research projects were chosen based on the philosophy. The selected projects and those results were as follows:

(1) Development of a novel dosage form containing fluticasone propionate for inflammatory bowel diseases

Crohn's disease and ulcerative colitis are the two primary types of inflammatory bowel diseases (IBD). Glucocorticosteroids were the standard treatment for IBD, but due to adverse events, their use was limited. However, new formulations of glucocorticosteroids have been developed to reduce systemic action. Fluticasone propionate (FLT) is an inhaled corticosteroid with high anti-inflammatory potency, used for the topical treatment of asthma. The purpose of this project was the design and preparation of a new FLT dosage form for topical treatment of IBD. The physicochemical property of FLT was very important for the dosage form design. The information for FLT was not enough, so we started the determination of the property. As a recent result, chemical structures for degradation products of FLT in an alkaline solution were found by the support of Laboratory of Pharmacognosy and Natural Products Chemistry. The physicochemical property of FLT and the improvement of its solubility were presented as papers in 2014.

(2) Effect of the adsorption of drugs to insoluble additives in the disintegration process on the dissolution behavior and taste of orally disintegration tablets

It was known that the taste of an orally disintegration tablet containing amlodipine besilate changed with changing the concentration of corn starch in the tablet. This phenomenon was considered to be effect of adsorption of additives to drugs. This project started the idea. For amlodipine besilate and other drugs developed orally disintegration tablets, the effect of the adsorption of drugs to insoluble additives in the disintegration process on the dissolution behavior and taste of orally disintegration have been studied. Now we are trying the evaluation of the adsorption in initial dissolution process using a new apparatus.

(3) Effect of the simple suspension method on the dissolution behavior

The simple suspension method is usually used. However, there was no report regarding the change of the dissolution property of the drug. The purpose of this project was a comparison of the dissolution profile between original and generic drugs after applied the simple suspension method. We are trying the pharmaceutical preparations with amlodipine besilate and with enalapril malate.

(4) Cleaning validation for machines used in the dispensary of pharmacy

When machines used in the dispensary, for an example, a dividing and packing machine was applied for a granule or a powder, it was easily considered that the little amount of the granule or powder was left in the machine. Therefore, cleaning the machine was required. This cleaning will be performed according the procedure which is decided by each pharmacy. In the case of a pharmaceutical plant, cleaning validation was required for machines for manufacturing pharmaceutical preparations by GMP. The purpose of this project is to introduce the concept of cleaning validation to pharmacies. We evaluated the residual amount of theophylline in a dividing and packing machine using a theophylline preparation. Further, a cleaning effect of lactose and NaHCO3 on the residual amount of theophylline was determined.

(5) Degradation rate of ebastine in an acidic solution and the effect of cyclodextrins (CDs) on the its degradation rate

Degradation rate of ebastine in an acidic solution and the effect of cyclodextrins on its degradation rat were examined. In addition, the degradation rates of CDs were determined.

(6) Development of novel pharmaceutical technology for poorly water-soluble drugs

To enhance oral bioavailabilitiy of poorly water-soluble drugs such as curcumin etc, we tried to prepare powders or water-based suspensions contain novel nano-particles by build-up or break-down methods, and estimated its particle characteriscics.

Publications (2010~2015)

[Original papers]

2014

1. Tokumura, T., Isaka, H., Kanou, M., Miyazaki, E., Kaneko, N., Kurita, T., (2015). An inclusion complex of fluticasone propionate with γ-cyclodextrin in aqueous solution and in a solid state. J. Drug Del. Sci. Tech. 26, 24-27.
2. Tokumura, T., Kanou, M., Miyazaki, E., Kaneko, N., Isaka, H. (2014). Degradation rate of fluticasone propionate in an alkaline solution of 0.1N NaOH : methanol = 1 : 1. Int. Res. J. Pharm. App. Sci., 4(5), 1-3.
3. Tokumura, T., Miyazaki, E., Isaka, H., Kaneko, N., Kanou, M., (2014). Solubility of fluticasone propionate in aqueous solutions measured by a method avoiding its adsorption to experimental tools. Int. Res. J. App. Sci., 4(4), 19-24.

2013

1. Kurita, T., Makino, Y. (2013). Novel curcumin oral delivery systems. Anticancer Res 33, 2807-2821.

2012

1. Kubodera, M., Tokumura, T., and Machida, Y., (2012). Determination of metronidazole in a rat stomach by HPLC for obtaining basic data of eradication therapy of *Helicobacter pylori*. J Pharmaceutical Analysis 2, 378-381.
2. Tokumura, T., Nagaoka, M., and Machida, Y., (2012). Effect of doses and dosage forms on the bioavailability of amoxicillin in non-fasted rats. J Drug Del Sci Tech 22, 568-570.

2011

4. Tokumura, T., Nagaoka, M., and Machida, Y., (2011). Effect of doses and dosage forms on the gastro-intestinal absorption of amoxicillin in rats. J Drug Del Sci Tech 21, 237-239.

2010

5. Hidaka, S., Tokumura, T., Tomono, K., Suzuki, T., Ueda, H., Nagai, T., Nagaoka, M., Nakane, R., and Machida, Y., (2010). Effect of β-cyclodextrin on the degradation rate of amoxicillin in acidic solution. Yakugaku Zasshi 130, 889-893.
6. Kamiya, S., Kurita, T., Miyagishima, A., Itai, S. and Arakawa, M. (2010). Physical properties of griseofulvin-lipid nanoparticles in suspension and their novel interaction mechanism with saccharide during freeze-drying. Eur. J. Pharm. Biopharm. 74(3), 461-466.
7. Kubodera, M. Tokumura, T. and Machida, Y. (2010). Are the optimum pharmaceutical preparations used for the second-line eradication therapy for Helicobacter pylori infection in Japan? –A discussion from a simulation for the amount of antibiotics in stomach based on the data of dissolution studies-. J Basic Clinical Pharmacy 001, 231-237.

Kagawa School of Pharmaceutical Sciences
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Integrated study on pharmacokinetics and pharmacodynamics

Staff

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Research

1. Integrated study on pharmacokinetics and pharmacodynamics for efficient drug discovery and on the optimum drug therapy
2. Basic and clinical analysis of drug-metabolizing enzyme and transporters influencing pharmacokinetics and pharmaco-dynamics
3. Basic research on the toxicity mechanism of drugs, xenobiotics and their active metabolites
4. Basic research for synthesis of COA-Cl (2-Cl-C.OXT-A) analogs, and their tube formation activities of human umbilical vein endothelial cells (HUVEC)
5. Curcumin and doxorubicin co-encapsulation liposome effects on cell growth, apoptosis, and angiogenesis

The elucidation of relationship between pharmacokinetics (PK) and pharmacodynamics (PD) of drugs is critical not only for the discovery of novel drugs but also for their optimum uses in clinical settings. The effects of clinically used drugs are influenced by drug-metabolizing enzymes and drug-transporters. Therefore, one of our major research projects is to push an integrated analysis of PK and PD for investigational drugs by characterizing *in vivo* drug-metabolizing enzymes and drug-transporters under physiological and pathological conditions.

We are also conducting the research of mechanism(s) for the decrease in levels of serum thyroid hormones by xenobiotics and their active metabolites, and the species difference and extrapolation to human in these compounds-induced alteration of the hormone levels.

In addition to these projects, we have also focused on simultaneous determination of polybrominated diphenyl ethers (PBDEs), hydroxylated (OH-) and methoxylated (MeO-) PBDEs found in marine sponge by atmosphere pressure chemical ionization tandem mass spectrometry (APCI-LC/MS/MS), and immunotargeting of liposome to tumor and endothelial cell expressed membrane type-1 matrix metalloproteinase (MT1-MMP).

Publications (2010-2015)

[Original papers]

2015

1. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., Kimura, O., and Koga, N. (2015). Distribution and excretion of 2,2',3,4',5,5',6-heptachlorobiphenyl (CB187) and its metabolites in rats and guinea pigs. Chemosphere, 118, 5-11.
2. Sakakibara, N., Baba, M., Okamoto, M., Toyama, M., Demizu, Y., Misawa, T., Kurihara, M., Irie, K., Kato, Y., Maruyama, T. (2015). Design, synthesis, and anti-HIV-1 activity of 1-aromatic methyl-substituted 3-(3,5-dimethylbenzyl)uracil and *N*-3,5-dimethylbenzyl-substituted urea derivatives. Antiviral Chemistry & Chemotherapy, 24, 3-18.
3. Endo, T., Kimura, O., Ogasawara, H., Ohta, C., Koga, N., Kato, Y., and Haraguchi, K. (2015). Mercury, cadmium, zinc and copper concentrations and stable isotope ratios of carbon and nitrogen in tiger sharks (*Galeocerdo cuvier*) culled off Ishigaki Island, Japan. Ecol. Indic., 55, 86-93.

2014

1. Kato, Y., Haraguchi, K., Onishi, M., Ikushiro, S., Endo, T., Ohta, C., Koga, N., Yamada, S., and Degawa, M. (2014). 3,3',4,4'-Tetrachlorobiphenyl-mediated decrease of serum thyroxine level in C57BL/6 and DBA/2 mice occurs mainly through enhanced accumulation of thyroxine in the liver. Biol. Pharm. Bull., 37, 504-509.
2. Kimura, O., Ohta, C., Koga, N., Haraguchi, K., Kato, Y., and Endo, T. (2014). Carrier-mediated uptake of nobiletin, a citrus polymethoxyflavonoid, in human intestinal Caco-2 cells. Food Chemistry, 154, 145-150.
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5. Kimura, O., Haraguchi, K., Ohta, C., Koga, N., Kato, Y., and Endo, T. (2014). Uptake of aristolochic acid I into Caco-2 cells by the monocarboxylic acid transporters. Biol. Pharm. Bull., 37, 1475-1479.
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Pharmacology Research & Perspectives, 2, e00068.

2013

1. Kato, Y., Onishi, M., Haraguchi, K., Ikushiro, S., Ohta, C., Koga, N., Endo, T., Yamada, S., and Degawa, M. (2013). A possible mechanism for 2,3',4,4',5'-pentachlorobiphenyl-mediated decrease in serum thyroxine level in mice. Biol. Pharm. Bull., 36, 1594-1601. (Highlighted paper selected by Editor-in-Chief)
2. Hidaka, N., Suemaru, K., Kato, Y., and Araki, H. (2013). Involvement of α4β2 nicotinic acetylcholine receptors in working memory impairment induced by repeated electroconvulsive seizures in rats. Epilepsy Research, 104, 181-185.
3. Endo, T., Hisamichi, Y., Kimura, O., Ogasawara, H., Ohta, C., Koga, N., Kato, Y., and Haraguchi, K. (2013). Levels of mercury in muscle and liver of star-spotted dogfish (*Mustelus manazo*) from the northern region of Japan: A comparison with spiny dogfish (*Squalus acanthias*). Arch. Environ. Contam. Toxicol., 64, 467-474.
4. Sakakibara, N., Hamasaki, T., Baba, M., Demizu, Y., Kurihara, M., Irie, K., Iwai, M., Asada, E., Kato, Y., and Maruyama, T. (2013). Synthesis and evaluation of novel 3-(3,5-dimethylbenzyl)uracil analogs as potential anti-HIV-1 agents. Bioorg. Med. Chem., 21, 5900-5906.
5. Sakakibara, N., Tsukamoto, I., Isono, Y., Takata, M., Konishi, R., Kato, Y., and Maruyama, T. (2013). A new method for synthesis and angiogenic evaluation of leteprinim potassium and its novel analogs. Heterocycles, 87, 2369-2384.
6. Umezawa, T., Ragamustari, S.K., Nakatsubo, T., Wada, S., Li, L., Yamamura, M., Sakakibara, N., Hattori, T., Suzuki, S., and Chiang, V.L. (2013). A lignan *O*-methyltransferase catalyzing the regioselective methylation of matairesinol in *Carthamus tinctorius.* Plant Biotechnology, 30, 97-109.
7. Okabe, N., Nakamura, E., Himi, N., Narita, K., Tsukamoto, I., Maruyama, T., Sakakibara, N., Nakamura, T., Itano, T., and Miyamoto, O. (2013). Delayed administration of the nucleic acid analog 2Cl-C.OXT-A attenuates brain damage and enhances functional recovery after ischemic stroke. Brain Research, 1506, 115-131.
8. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., and Koga, N. (2013). Species difference in the metabolism of 2,2',3,4',5,5'-hexachlorobiphenyl (CB146) by animal and human liver microsomes. Fukuoka Acta Medica 104: 161-169.

2012

1. Kato, Y., Tamaki, S., Haraguchi, K., Ikushiro, S., Sekimoto, M., Ohta, C., Endo, T., Koga, N., Yamada, S., and Degawa, M. (2012). Comparative study on 2,2',4,5,5'-pentachloro-biphenyl-mediated decrease in serum thyroxine level between C57BL/6 and its transthyretin-deficient mice. Toxicol. Appl. Pharmacol., 263, 323-329.
2. Kato, Y., Okada, S., Atobe, K., Endo, T., and Haraguchi, K. (2012). Selective determination of mono- and dihydroxylated analogs of polybrominated diphenyl ethers in marine sponges by liquid-chromatography tandem mass spectrometry. Anal. Bioanal. Chem., 404, 197-206.
3. Sakakibara, N., Tsukamoto, I., Tsurura, T., Takata, M., Konishi, R., and Maruyama, T. (2012). Novel synthesis of carbocyclic oxetanocin analogs (2-alkoxy-C.OXT-A) and their tube formation activities of human umbilical vein endothelial cells (HUVEC). Heterocycles, 85, 1105-1116.
4. Ordonez, P., Hamasaki, T., Isono, Y., Sakakibara, N., Ikejiri, M., Maruyama, T., and Baba, M. (2012). Anti-human immunodeficiency virus type 1 activity of novel 6-substituted 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives. Antimicrob Agents Chemother, 56, 2581-2589.
5. Sakakibara, N., Kakoh, A., and Maruyama, T. (2012). First synthesis of [6-^{15}N]-cladribine using ribonucleoside as a starting material. Heterocycles, 85, 171-182.

2011

1. Kato, Y., Onishi, M., Haraguchi, K., Ikushiro, S., Ohta, C., Koga, N., Endo, T., Yamada, S., and Degawa, M. (2011). A possible mechanism for 2,2',4,4',5,5'-hexachlorobiphenyl-mediated decrease in serum thyroxine level in mice. Toxicol Appl Pharmacol 254, 48-55.
2. Misaka, S., Kurosawa, S., Uchida, S., Yoshida, A., Kato, Y., Kagawa, Y., and Yamada, S. (2011). Evaluation of the pharmacokinetic interaction of midazolam with ursodeoxycholic acid, ketoconazole and dexamethasone by brain benzodiazepine receptor occupancy. J Pharm Pharmacol 63, 58-64.
3. Koga, N., Ohta, C., Kato, Y., Haraguchi, K., Endo, T., Ogawa, K., Ohta, H., and Yano, M. (2011). *In vitro* metabolism of nobiletin, a polymethoxy-flavonoid, by human liver microsomes and cytochrome P450. Xenobiotica 41, 927-933.
4. Haraguchi, K., Kato, Y., Ohta, C., Koga, N., and Endo, T. (2011). Marine sponge: a potential source for methoxylated polybrominated diphenyl ethers in the Asia-Pacific food web. J Agric Food Chem 59, 13102-13109.
5. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., and Koga, N. (2011). *In vivo* metabolism of 2,2',3,4,4',5'-hexachlorobiphenyl (CB138) in guinea pigs. Fukuoka Acta Medica 102: 167-174.
6. Sakakibara, N., Komatsu, M., and Maruyama, T. (2011). One-pot synthesis of 2-nitrooxyalkoxylated inosine analogs using cyclic ether and isoamyl nitrite. Heterocycles, 83, 2865-2872.
7. Sakakibara, N., Tsuruta, T., Komatsu, M., Iwai, M., and Maruyama, T. (2011). A new method for synthesis of 2-alkoxyadenosine analogs. Heterocycles, 83, 2299-2311.
8. Isono, Y., Sakakibara, N., Ordonez, P., Hamasaki, T., Baba, M., Ikejiri, M., and Maruyama, T. (2011). Synthesis of 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives with potential anti-HIV activity. Antiviral Chemistry & Chemotherapy, 22, 57-65.
9. Yamamura, M., Wada S., Sakakibara, N., Nakatsubo, T., Suzuki, S., Hattori, T., Takeda, M., Sakurai, N., Suzuki, H., Shibata, D., and Umezawa, T. (2011). Occurrence of guaiacyl/*p*-hydroxyphenyl lignin in *Arabidopsis thaliana* T87 cells. Plant Biotechnology, 28, 1-8.

2010

1. Kato, Y., Haraguchi, K., Kubota, M., Seto, Y., Okura, T., Ikushiro, S., Koga, N., Yamada, S., and Degawa, M. (2010). A possible mechanism for the decrease in serum thyroxine level by a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-like polychlorinated biphenyl congener, 3,3',4,4',5-pentachloro-biphenyl in mice. Drug Metab Dispos 38, 150-156.
2. Kato, Y., Haraguchi, K., Ito, Y., Fujii, A., Yamazaki, T., Endo, T., Koga, N., Yamada, S., and Degawa, M. (2010). Polychlorinated biphenyl-mediated decrease in serum thyroxine level in rodents. Drug Metab Dispos 38, 697-704.
3. Kato, Y., Suzuki, H., Haraguchi, K., Ikushiro, S., Ito, Y., Uchida, S., Yamada S., and Degawa, M. (2010). A possible mechanism for the decrease in serum thyroxine level by phenobarbital in rodents. Toxicol Appl Pharmacol 249, 238-246.
4. Ito, Y., Harada, T., Fushimi, K., Kagawa, Y., Oka, H., Nakazawa, H., Homma, R., Kato, Y., and Yamada, S. (2010). Pharmacokinetic and pharmacodynamic analysis of acetylcholinesterase inhibition by distigmine bromide in rats. Drug Metab Pharmacokinet 25, 254-261.
5. Tsukamoto, I., Sakakibara, N., Maruyama, T., Igarashi, J., Kosaka, H., Kubota, Y., Tokuda, M., Ashino, H., Hattori, K., Tanaka, S., Kawata, M. and Konishi, R. (2010). A novel nucleic acid analogue shows strong angiogenic activity. Biochemical and Biophysical Research Communications 399, 699-704.
6. Suzuki, S., Sakakibara, N., Li, L., Umezawa, T. and Chiang, V.,

L. (2010). Profiling of phenylpropanoid monomers in developing xylem tissue of transgenic aspen (Populus tremuloides). Journal of Wood Science, 56, 71-76.

[Others]

1. Sakakibara, N., Maruyama, T., and Kato, Y. (2013). Design and synthesis of novel nucleic acid analogs as potential angiogenesis or anti-HIV-1 agents. Journal of Kagawa Pharmaceutical Association Kagayaku 150, 68-70.
2. Ohta, C., Kato, Y., Haraguchi, K., Endo, T., and Koga, N. (2013). *In Vitro* metabolism of nobiletin in the small intestine and kidney of rats and guinea pigs. Bulletin of Nakamura Gakuen Univ and Nakamura Gakuen Univ Junior Coll 45, 141-149.
3. Ohta, C., Haraguchi, K., Endo, T., Kato, Y., Matsubara, F., and Koga, N. (2012). *In Vitro* metabolism of 2,4,6-tribromo-anisole found in marine biota by animal liver microsomes and anti-oxidative activity of its related compounds. Bulletin of Nakamura Gakuen Univ and Nakamura Gakuen Univ Junior Coll 44, 215-223.
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5. Kato, Y. (2010). Integrated study on pharmacokinetics, pharmacodynamics and the toxicity mechanism of xenobiotics. J Pharm Sci Technol 70, 303-308.
6. Ohta, C., Haraguchi, K. Kato, Y., Endo, T., and Koga, N. (2010). Synthesis of 2,3',4,5,5'-pentachlorobiphenyl (CB120) and the postulated hydroxylated metabolite. Bulletin of Nakamura Gakuen Univ and Nakamura Gakuen Univ Junior Coll 42, 333-338.

[Proceedings]

2014

1. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., and Koga, N. (2014). The participation of rat CYP3A enzymes in the metabolism of 2,2',4,5,5'-pentachlorobiphenyl (CB101). Organohalogen Compds 76, 466-469.

2013

1. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., Kimura, O., and Koga, N. (2013). *In vitro* metabolism of 2,2',4,4',5-pentachlorobiphenyl (CB99) by rat and guinea pig liver microsomes. Organohalogen Compds 75, 587-590.

2012

1. Kato, Y., Tamaki, S., Haraguchi, K., Ikushiro, S., Sekimoto, M., Ohta, C., Endo, T., Koga, N., Yamada, S., and Degawa, M. (2012). 2,2',4,5,5'-Pentachlorobiphenyl-mediated inhibition of a serum T_4-transthyretin complex formation is one of causes for the PCB-induced changes in the serum and hepatic T_4 levels in mice. Organohalogen Compds 74, 1377-1380.
2. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., and Koga, N. (2012). Involvement of rat CYP3A enzymes in the metabolism of 2,2',3,4',5',6-hexachlorobiphenyl (CB149). Organohalogen Compds 74, 1475-1478.

2011

1. Kato, Y., Haraguchi, K., Onishi, M., Ikushiro, S., Ohta, C., Koga, N., Endo, T., Yamada, S., and Degawa, M. (2011). 2,2',4,4',5,5'-Hexachlorobiphenyl-mediated decrease in serum thyroxine level in mice. Organohalogen Compds 73, 726-729.
2. Haraguchi, K., Kato, Y., Ohta, C., Koga, N., and Endo, T. (2011). A Potential source for hydroxylated and methoxylated analogs of brominated diphenyl ethers in the Asia-Pacific food web. Organohalogen Compds 73, 182-185.
3. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., and Koga, N. (2011). Metabolism of 2,3',4,5,5'-pentachlorobiphenyl (CB120) by liver microsomes of rats and guinea pigs. Organohalogen Compds 73, 2251-2254.

2010

1. Ohta, C., Haraguchi, K., Kato, Y., Endo, T., Matsubara F., and Koga, N. (2010). Species difference in the *in vitro* metabolism of 2,2',3,4,4',5,5'-heptachlorobiphenyl (CB180). Organohalogen Compds 72, 1796-1799.

Kagawa School of Pharmaceutical Sciences
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Pharmaceutical care and drug efficacy, toxicity correlation

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Research

1. Modulation of multi-drug resistance related protein transport by interaction with dietary supplements
(Nakatsuma A.)

An interaction is taken to be the situation in which administration of a drug or substance induces changes in the pharmacokinetics of another simultaneously administered drug – by increasing either the plasma or intracellular concentration of the latter, and thus giving rise to the possibility of an adverse reaction.

The ABC-transporter superfamily, which functions as a drug efflux pump, is known to limit the absorption of a variety of drugs. We investigated the effects of food extracts on anticancer drug transport by the multi-drug resistance related proteins (MRPs). MRPs are efflux transporters expressed in human glioblastoma cell line T98G. The effects on MRP mediated transport were also evaluated using calcein, which is the substrate of MRP. Acute exposure to kaempferol caused a concentration-dependent decrease in the extracellular efflux of calcein compared with the control As for the simultaneous exposure to kaempferol and cisplatin, the cytotoxicity of cisplatin was expected to be potent because MRP and glutathione *S*-transferases (GST) are both inhibited by kaempferol. However, the cytotoxicity of cisplatin decreased

Western blot analysis and reverse transcription–polymerase chain reaction (RT–PCR) showed that treatment with 10 and 20 μM kaempferol for up to 72 hr was able to significantly lower MRP2 expression, whereas increased expression in a concentration-dependent on GST mRNA and protein levels. Furhermore, GST was strongly activated in T98G cell treated with kaempferol.

The results of the study also point to possible kaempferol-drug interaction, especially when the cytotoxicity of anticancer drugs are dependent on glutathione *S*-transferases and MRP-mediated transport processes. Hereafter, these possible efficacies need to be examined under in vivo conditions in detail.

2. Infection treatment caused by multiple-drug-resistant pseudomonas aeruginosa in a patient underwent allogeneic hematopoietic stem cell transplantation (Ninomiya M.)

Infections caused by multiple-drug-resistant Pseudomonas aeruginosa (MDRP) are a clinically significant problem. We reported here the effective use of combination therapy in a patient with infection caused by MDRP according to an interventional treatment strategy suggested by a pharmacist. The patient was a 70-year-old male who underwent allogeneic hematopoietic stem cell transplantation. On day 45 after transplant, MDRP was newly isolated from urine, but the diagnosis at that time was colonization. On day 61, the patient developed a fever(≧38．0℃). In addition, laboratory data showed that C-reactive protein (CRP) was also increased. At the medical team conference, the pharmacist proposed the following treatment strategy for this infection. Aztreonam and amikacin were intravenously administered at doses of 2g/day and 800mg/day, respectively. The subsequent clinical course was well controlled, but the infection recurred and was aggravated. Aztreonam and ciprofloxacin were then intravenously administered at doses of 4g/day and 600mg/day, respectively, resulting in the alleviation of fever in the patient as well as a decrease in CRP and disappearance of MDRP isolates from urine on day 67 ; that is, MDRP infection was consequently well controlled. In conclusion, the combination therapy between aztreonam and amikacin, or ciprofloxacin may be clinically useful for severe infections of MDRP in compromised hosts.

Publications (2010-2015)

[Original papers]

2014

1. Iihara N, Yoshida T, Okada T, Nakatsuma A, Kirino Y : Survey of usage of medication with driving with prohibition or caution by the national health insurance claims database in Japan. Jpn. J. Pharmaceu. Health Care Sci. 40(2), 67-77

2010

1. Nakatsuma A, Fukami T, Suzuki T, Furuishi T, Tomono K, Hidaka S (2010): Effects of kaempferol on the mechanisms of drug resistance in human glioblastoma cell line T98G. PHARMAZIE 65, 379-383

Kagawa School of Pharmaceutical Sciences
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Evaluation of Medicines and Pharmacotherapy Based on Informatics

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Research

(1) Patient–Healthcare Professional Relationship

Patients accept or refuse their medication therapy based on their personal beliefs. We analyze relationship between patient's perceptions of medication therapy and their behavior such as medication adherence.

We developed Medication Acceptance, Preference and Adherence Scale (MAPAS), which assessed each patient's beliefs, values and ideas concerning their acceptance and preference for medications and treatments. We found that patients' dissatisfaction consistent determinants of intentional non-adherence to medication, but not unintentional non-adherence. In addition, we found that cancer patients prefer aggressive therapies, even when self-estimations of ADR endurance are not very high, especially if they have been receiving chemotherapy for a short period of time.

(2) Pharmacoepidemiology Study Encouraging Proper Use of Medication

Post-marketing surveillance is important because it can evaluate medication use in the real world.

We surveyed use of medication with driving with prohibitions or cautions in outpatient settings for patients aged 25 years and older using the National Health Insurance Claims Database. Of outpatients aged 25 years and older who were administered medications, 73% outpatients were given the medications with cautions or prohibitions on driving. For the elderly, prescriptions were found with dosages that often exceeded the recommended limits.

(3) Bio- and Chem-Informatics of Traditional Medication Systems and Medicinal Resources

We demonstrate the computational sciences and informatics based on the evidences related to medical and pharmaceutical issues. This research is performed by biological and chemical experiments, database construction, and factor analysis by multivariate statistical analysis. In recent studies, we have focused on the theoretical analysis of the traditional and empirical medication system in Kampo (traditional Japanese medicine), and the transcriptome and metabolome analyses of medicinal bioresources and a model organism.

Publications (2010-2015)

[Original papers]

2015

1. Iihara, N., Nishio, T., Goda, M., Anzai, H., Kagawa, M., Houchi, H., and Kirino, Y. (2015). Effect of endurance for adverse drug reactions on the preference for aggressive treatments in cancer patients. Support Care Cancer 23(4), doi: 10.1007/s00520-014-2439-1.

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2. Iihara, N., Kirino, Y., Kazuhiro Hara, Hideto Yokoi, Tetsuo Ueno, Akinori Harada, Masahiko Nakagawa, Yukio Saito, Kei

Morioka, Yuhko Ogata (2010) Development of an Electronic Prescription Interactive Network System Enhancing Collaboration of Medical Staffs Between a Hospital and Community Pharmacies. Japan Journal of Medical Informatics 30(4), 225-231.

[Review articles]

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2. Tsuchiya, F., Iihara, N. (2014) “Healthcare IT and medication: How does healthcare IT-ization affect medication development and medication safety assurance?” YAKUGAKU ZASSHI. 134(5), 583-584.
3. Iihara, N., Kirino, Y. (2014) “A community electronic prescription system connecting physicians, pharmacists, and patients, and utilization of clinical information” YAKUGAKU ZASSHI. 134(5), 589-593.
4. Okada, T., Afendi, F.M., Altaf-Ul-Amin, M., Takahashi, H., Nakamura, K., and Kanaya, S. (2010). Metabolomics of medicinal plants: the importance of multivariate analysis of analytical chemistry data. Curr Comput Aided Drug Des 6(3), 179-196.

[Books]

1. Okada, T., and Noji, M. (2014). Metabolome analysis of medicinal plants and crude drugs –a study case of *Ephedra* plants by comprehensive metabolite analysis using a mass spectrometer and multivariate statistical analysis of its data–. (Chapter 1 in Part III) In: Kawahara, N. (Sv.) Recent progress of medicinal plants and crude drugs –cultivation and quality evaluation of medicinal plants and development of Kampo products– (in Japanese). CMC Publishing, Tokyo, pp. 122–131.
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Kagawa School of Pharmaceutical Sciences
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Drug information and side effects

Staff

Hitomi Yokota

Professor since 2011

School of Pharmaceutical Sciences, Osaka university, 1970

Research

I have worked as Hospital pharmacist for 40years. My major interests are in the utility of physical assessment by pharmacist to evaluate the drug side effect.

Two key elements of this work are the improve the clinical skill of pharmacist for early detection of drug side effect and analyzing the strong and weak aspect of physical assessment. Last year we held 8 study session of physical assessment to assess the resent status.

I also have an interest in the communication method. Increasing the proportion of elderly population weights the importance of home care. This trend would change the role of pharmacist. More communication between patients for early detection of side effect would be expected.

Publications (2010-2015)

[Original papers]

2012

1. Naomi Iihara, Takayuki Nishio, Hitomi Yokota, Takayo Yoshioka, Akihiko Iwamoto, Nobushige Obika, Shinji Kosaka, Yaeko Sogo, Hideaki Anzai(2012) Pharmacist Barriers to Handling Patients with Adverse Drug Events at Community Pharmacies Jpn. J. Drug Inform. 13(4):194-198.

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Kagawa School of Pharmaceutical Sciences
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Molecular Mechanisms in the regulation of Synaptic Transmission

Staff

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D.Sc. Kyushu University, Pharmaceutical Sciences, 1989

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Assistant Professor, Sapporo Medical University
Ph. D. in Hokkaido University, Biological Sciences, 2000

Research

Theme 1. The molecular mechanism of complexin (Hiroshi Tokumaru, Hisayo Sadamoto)

Action potential-evoked neurotransmitter release is triggered by Ca^{2+} influx through voltage-gated calcium channels located next to the active zone. The increase in Ca^{2+} concentration initiates rapid signaling cascades that lead to the exocytosis of synaptic vesicles containing high concentrations of neurotransmitter. Two soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins from the presynaptic membrane, syntaxin 1 and SNAP-25, and one SNARE protein from the synaptic vesicle membrane, synaptobrevin-2 (also known as VAMP-2), form a four-helix bundle (called the *trans*-SNARE complex or SNAREpin) that catalyzes membrane fusion. The synaptic vesicle protein synaptotagmin 1 (Syt1) serves as a major Ca^{2+} sensor for fast action potential-evoked synaptic vesicle exocytosis. The rapid interactions between Syt1, the SNARE complex and membrane phospholipids induced by Ca^{2+} are critical for membrane fusion.

The precise control of evoked neurotransmitter release requires several cytosolic proteins including complexin (also known as synaphin). Complexin and its binding to the SNARE complex are critical for fast neurotransmitter release, as demonstrated by studies in knockout mice and in *Drosophila* mutants. In addition, intra-presynaptic terminal injection of a SNARE-binding domain peptide that blocks complexin binding to the SNARE complex also inhibits rapid neurotransmitter release. Despite this evidence, complexin's function remains controversial. Furthermore, recent studies suggest that complexin contains several functional domains that either stimulate or inhibit neurotransmitter release. Thus, the function of complexin is likely more complex than expected from its small size (134 aa for mammalian complexin 1 and 2).

Like Syt1-deficient mice, complexin 1/2 double-knockouts exhibit an impairment of action potential-evoked synchronous (but not asynchronous) neurotransmitter release. However, an important difference exists between the two types of knockout mice: elevated external Ca^{2+} can rescue synchronous release in complexin 1/2 double-knockouts but not in Syt1 knockouts. Thus, the functions of complexin and Syt1 in action potential-evoked fast neurotransmitter release are intimately related to each other yet distinct.

Biochemically investigating the relationship between the two proteins, we previously demonstrated that complexin directly binds to Syt1 even in the absence of Ca^{2+}. Because Syt1 binds to SNARE complexes weakly in the absence of Ca^{2+}, we proposed that complexin recruits Syt1 to the SNARE complex prior to Ca^{2+} influx. Recently, we examined the interaction between complexin, Syt1 and the SNARE complex using recombinant proteins. Our results indicate that Syt1 recruitment to the SNARE-driven fusion machinery by complexin is essential for vesicle exocytosis.

Syt1 bound weakly to complexin alone, but the addition of three SNARE proteins (syntaxin 1, SNAP-25 and VAMP/synaptobrevin-2) in combination, but not individually, markedly enhanced binding. Unlike full-length complexin (amino acid [aa] 1–134) and an NH2 (N)-terminally truncated complexin (aa 46–134), carboxy (C)-terminally truncated complexin s (aa 1–104 and 1–124) could not support Syt1 binding even in the presence of the SNARE complex. These results indicate that the binding of Syt1 to the C-terminal region of complexin promotes its recruitment to the SNARE-driven fusion machinery, and that this process is crucial for Ca^{2+}-dependent vesicle exocytosis.

Theme 2. Differential localizations of GKAP/SAPAP1 isoforms in developing hippocampal neurons (Hisayo Sadamoto)

Guanylate kinase-associated protein (GKAP) and SAP90/PSD-95-associated protein 1 (SAPAP1) proteins form complexes with PSD-95 and Shank at excitatory postsynaptic sites, and are implicated in synapse formation and synaptic plasticity. GKAP/SAPAP1 proteins, which displayed different N termini, have appeared as multiple alternatively spliced isoforms. However, specific functional roles of individual isoforms still remain unclear. To understand particular functions of GKAP/SAPAP1 isoforms in formation and maintenance of synaptic connections, we here investigated expression and

subcellular distributions of these isoforms in hippocampal neurons during synaptic development. First, we identified two isoforms of SAPAP1 (named as SAPAP1b and 1c) in mice hippocampus, which exhibited an alternative usage of two exons in the middle part of SAPAP1 transcript. Using primary culture of mouse hippocampal neurons and confocal microscope, we sought to examine localizations of each EGFP-tagged GKAP/SAPAP1 isoform (GKAP, SAPAP1, SAPAP1b or SAPAP1c). During synaptic maturation, GKAP/SAPAP1 isoforms were found to display differences in cluster formation at the dendritic spines. EGFP-SAPAP1 formed clusters at dendritic shafts on an early stage of synapse formation and did not change the rate of accumulation (clustering index) in mature dendritic spines at later stages. Clusters of EGFP-SAPAP1b and EGFP-SAPAP1c were also found to occur at an early stage, but tended to disappear during synaptic maturation. In contrast, GKAP clearly accumulated in dendritic spines at a later stage of synaptic maturation. These results suggest the possibility that each spliced isoform of GKAP/SAPAP1 has a specific function in synapse formation.

Theme 3. Synaptic modulation and oscillatory network modulation in odor information processing (Suguru Kobayashi)

Synchronous oscillatory activity is common in the olfactory behavior of both vertebrates and invertebrates. In the olfactory center of terrestrial animals, changes in the oscillatory frequency of the local field potential (LFP) are thought to be involved in olfaction-based behavior and olfactory memory. We study GABAergic and FMRFamidergic neuromodulation of oscillatory activity in odor information processing of the procerebrum (PC) in the land slug *Limax valentianus*. We found that GABA and FMRFamide are present in the PC and these modulatory roles are involved in the oscillatory neural network of the PC. A part of results for excitatory GABAergic and FMRFamidaergic neuromodulation are published in J. Neurophysiol. (2012) and Eur. J. Neurosci. (2010). Furthermore, recent study shows the presence of cholinergic excitatory modulation for PC neurons via nicotinic ACh receptors activation and feedforward inhibition in the cholinergic afferents from the tentacles to the PC. We use electrophysiology and optical recording methods to understand the role of oscillatory dynamics in odor recognition and memory storage.

Grant Support: Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research.

Collaborations: With other universities.

Publications (2010-2015)

[Original papers]

2014

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3. Sadamoto H. and Muto H. (2013) Fluorescence Cross-correlation Spectroscopy (FCCS) to Observe Dimerization of Transcription Factors in Living Cells. Methods Mol Biol. 977, 229-41.
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6. Kobayashi, S., Matsuo, R., Sadamoto, H., Watanabe, S., and Ito, E. (2012) Excitatory effects of GABA on procerebrum neurons in a slug. J Neurophysiol 108, 989-998.
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8. Elekes K., Battonyai I., Kobayashi S., Ito E. (2012) Organization of the procerebrum in terrestrial pulmonates (*Helix*, *Limax*) reconsidered: cell mass layer synaptology and its serotonergic input system. Brain Structure and Function 218, 477-490.
9. Kobayashi S., Ito E. (2012) GABAergic effects on the slow oscillatory neural activities in the procerebrum of *Limax valentianus*. Acta Biologica Hungarica 63 (Suppl. 2), pp. 217–221.

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13. Matsuo R., Kobayashi S., Yamagishi M., Ito E. (2011) Two pairs of tentacles and a pair of procerebra: optimized functions and redundant structures in the sensory and central organs involved in olfactory learning of terrestrial pulmonates. J Exp Biol 214, 879-886.
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16. Matsuo R., Kobayashi S., Murakami J., Ito E. (2010) Spontaneous Recovery of the Injured Higher Olfactory Center in the Terrestrial Slug *Limax*. PLoS One 5, e9054.
17. Sadamoto, H., Kitahashi, T., Fujito, Y., and Ito, E. (2010) Learning-dependent gene expression of CREB1 isoforms in the molluscan brain. Front Behav Neurosci 4, 25.
18. Matsuo R., Kobayashi S., Tanaka Y., Ito E. (2010) Effects of tentacle amputation and regeneration on the morphology and activity of the olfactory center of the terrestrial slug *Limax valentianus*. J Exp Biol 213:3144-3149.
19. Kobayashi S., Fujito Y., Matsuyama K., Aoki M. (2010) Spontaneous respiratory rhythm generation in in vitro upper cervical slice preparations of neonatal mice. J Physiol Sci 60, 303–307.
20. Kobayashi S., Fujito Y., Matsuyama K., Aoki M. (2010) Raphe modulation of the pre-Bötzinger complex respiratory bursts in in vitro medullary half-slice preparations of neonatal mice. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 196, 519–528.
21. Kobayashi1 S., Hattori1 M., Elekes K., Ito E., Matsuo R. (2010) FMRFamide regulates oscillatory activity of the olfactory center in the slug. Eur J Neurosci 32, 1180-1192.

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22. Matsuo, R., Kobayashi, S., Watanabe, S., Namiki, S., Iinuma, S., Sakamoto, H., Hirose, K., and Ito, E. (2009) Glutamatergic neurotransmission in the procerebrum (olfactory center) of a terrestrial mollusk. J Neurosci Res 87, 3011-3023.

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Kasai H, Takahashi N, Tokumaru H. (2012) “distinct Initial SNARE Configurations Underlying the Diversity of Exocytosis” Physiol Rev. 92(4), 1915-1964

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Mochida S., Tokumaru H. et al., (2015) “Presynaptic Terminals” Springer ISBN978-4-431-55165-2

Kagawa School of Pharmaceutical Sciences
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Human Cell Immortalization and Tissue Regeneration

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Assistant Professor since 2009
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Visiting Fellow since 2012

Research

We have been mainly investigating the molecular mechanisms of immortalization of human normal cells, cancerization of human immortal cells, and differentiation of germ cells. The present research areas of interest, are as follows,

1. Mechanisms of immortalization of human normal cells

1-1 Control of life span in human fibroblasts and endothelial cells

It is widely accepted that telomerase, which compensates for telomere shortening, govern cellular life span. Telomerase is activated in most of human malignant neoplasms. And ectopic expression of telomerase may endow some kinds of human somatic cells with indefinite proliferation capacity, i.e., immortality. On the basis of this strategy, we have transfected hTERT gene into human normal skin fibroblasts and vascular endothelial cells, and finally established several immortal cell lines.

To clarify the changes in gene expression required for immortality, we investigated intrinsic responses required in acquiring immortality. Thus, we compared by real-time RT-PCR the changes in the expression levels of the cell cycle and apoptosis-related genes in human normal fibroblasts and endothelial cells versus hTERT-transfected cell lines. We found that immortal fibroblast cell lines upregulated cell-cycle promoting genes and down-regulated apoptosis-inducing genes at early phase after transfection, whereas the endothelial cell did not. In addition, the microarray analysis of the fibroblast cell lines revealed that the dysregulated genes during cellular immortalization were different from those found in endothelial cells, which probably have acquired telomere maintenance ability by expressing exogenous hTERT. These findings indicate that cell-type specific gene expression after telomerase expression may be important to acquire telomere-maintenance capacity and immortality in some non-cancerous human cells. A future investigation of the cell-type specific molecules investigated in these process may elucidate the differences in the capacity of acquiring immortality in cancer and normal somatic cells.

1-2 EBV-transformed B-lymphoblastoid cells

It is well known that Epstein-Barr virus (EBV) -infected B lymphoid cells are maintained in culture for long period, however only few of which becomes immortal during cell culture passages. We studied phenotypic characteristics of pre-, post-immortal and tumorigenic human B-lymphoblastoid cell lines (LCLs) established from normal B cells with the same genetic background. Pre-immortal LCLs showed low telomerase activity and a normal diploid karyotype, while post-immortal LCLs showed much higher telomerase activity and maintained a clonal aneuploidic state. Among five post-immortal LCLs tested, LCLs N0005 and N6803 formed colonies in agar medium and showed a marked aneuploidy. And N6803 was transplantable into nude mice indicating that it gained a complete malignant property, but all pre-immortal LCLs and the remaining three post-immortal LCLs lacked these characteristics. The products of tumor suppresser genes, p16(INK4A) and pRb were downregulated in N0005 and N6803 LCLs, and the p53 gene was mutated in N0005 LCL. These results indicated that some pre-immortal EBV-transformed LCLs can become immortal and then, tumorigenic *in vitro* culture, and that these LCLs will provide an *in vitro* model of tumorigenesis induced by EBV.

For further screening of genes involved in immortality and malignancy in EBV-transformed LCLs, we performed microarray analysis between pre- and post-immortal LCL and obtained a list of up- or down-regulated genes during cell immortalization. Cloning of each genes and elucidation of their role in immortalization is now under investigation.

1-3 Aim for application to clinical disorders (cell therapy)

The immortal endothelial cell lines obtained in our lab can be applied for clinical use of aging-related disorders, such as atherosclerosis lesions. One of these immortal endothelial cell (EC) lines, IMEC-1, retained a normal morphological feature of young endothelial cells and proliferated in response to specific angiogenic factors, such as bFGF. Thereafter, we assessed the carotid balloon catheter injury model, in which neointima formation have developed by denudation of the left common carotid artery, and examined the effect of implantation of immortal ECs into the denuded area of mouse carotid artery. Two weeks after the implantation of IMEC-1, neointima formation was significantly thiner, compared with that in control carotid injected with saline. These results suggest that implantation of immortal ECs may be of potential therapeutic value in vascular injury, and a possible

treatment strategy for the prevention of the progression of atherosclerosis and restenosis after angioplasty. In this way, our long-term goal is to improve age-related disorders by replacing aged, disfunctional cells to immortal, functional cells (that is cell therapy).

1-4 Establishment of human iPS cell lines from normal fetal lung fibriblast, TIG-1

TIG-1 is a normal human fibroblast strain which has been used extensively on studies of cellular senescence and numerous data on it have been accumulated at molecular level. Recently, a method for generating induced pluripotent stem cells (iPSCs) was developed. We introduced four reprogramming genes to TIG-1 and succeeded to isolate colonies which had embryonic stem cells (ESC)-like morphologies. They expressed ESC marker, OCT4, SOX2, SSEA4 and TRA-1-81 proteins and OCT4 and NANOG transcripts, showing establishment of iPSC lines from TIG-1. The iPSC clones could differentiate to all three germ layers as shown by mRNA expressions for a-fetoprotein (endoderm), MSX1 (mesoderm) and microtubule associated protein 2 (ectoderm). Immunostaining also detected a-fetoprotein, a-smooth muscle actin and b-tubulin. The iPSCs formed tetatoma containing all three germ layers in SCID mice. Thus, by comparing aging of parental TIG-1 cells and differentiation to myofibroblasts from iPSCs, we will be able to reveal and exact differentiation in process between senescence and terminal differentiation.

2. Differentiation of germ cells.

1) tesmin: We have cloned a novel cDNA encoding a testis-specific metallothionein-like protein, tesmin, by randomized RT-PCR on RNA from mouse tissues. Two tesmin-related transcripts (2.2 and 1.8 kb) in mouse and one (2.1 kb) in human were detected and sequenced. These encode a cysteine-rich 60 kDa protein (475 amino acid residues) that contained a metallothionein-like motif. A search of databases indicated that tesmin is a member of the CXC-hinge-CXC family, which is highly conserved through plants, tetrahimena, C. elegans, mouse and human.

In situ hybridization analysis in adult mouse testis showed that tesmin is specifically expressed in spermatocytes. Quantitative RT-PCR at different stages of mouse postnatal development (days 4, 8, 12, 18, and 42) revealed that tesmin is expressed as early as day 8 and coincides with the entry of germ cells into meiosis. Furthermore, adult W/Wv sterile mice that harbor the c-kit mutation was found to lack tesmin expression. The gene is assigned to mouse chromosome 19B, which translocated (11;19) in male sterile mice.

An immunohistochemical study indicated that tesmin exhibits dynamic changes in subcellular localization during spermatogenesis. Before meiosis, it was localized in the cytoplasm of early to late spermatocytes and then translocated into the nucleus just before meiotic division. After meiosis, it appeared in spermatids, starting from the acrosomal vesicles, moving to the nuclear membrane and then to the caudal end as the spermatids elongated, and finally relocating into the cytoplasm. Oxidative stress by cobalt chloride, as well as by diethylmaleate, induced premature translocation of tesmin from the cytoplasm to the nucleus and apoptotic morphologyin spermatocytes. A study on the mechanism of shuttling of tesmin between nucleus and cytoplasm is now intensively under way in this lab.

2) LIN54: The mammalian LIN complex (LINC) plays important roles in regulation of cell cycle genes. LIN54 is an essential core subunit of the LINC and has a DNA binding region (CHC domain), which consists of two cysteine-rich (CXC) domains separated by a short spacer. We generated various LIN54 mutants, such as CHC deletion mutant, and investigated their subcellular localizations and effects on cell cycle. Wild-type LIN54 was predominantly localized in the nucleus. We identified two nuclear localization signals (NLSs), both of which were required for nuclear localization of LIN54. Interestingly, deletion of one CXC domain resulted in an increased cytoplasmic localization. The cytoplasmic LIN54 mutant accumulated in the nucleus after leptomycin B treatment, suggesting CRM1-mediated nuclear export of LIN54. Point mutations (C525Y and C611Y) in conserved cysteine residues of CXC domain that abolish DNA binding activity also increased cytoplasmic localization. These data suggest that DNA binding activity of LIN54 is required for its nuclear retention. We also found that LIN54 (C525Y) and LIN54 (C611Y) inhibited cell cycle progression and led to abnormal nuclear morphology. Other CXC mutants also induced similar abnormalities in cell cycle progression. LIN54 (C525Y) led to a decreased expression of some G2/M genes, whose expressions are regulated by LINC. This cell cycle inhibition was partially restored by overexpression of wild-type LIN54. These results suggest that abnormal cellular localization of LIN54 may have effects on LINC activity

Publications (2010-2015)

[Original papers]

2014

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2013

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2012

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2011

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2. Nakamura K, Ikeda S, Matsuo T, Hirata A, Takehara M, Hiyama T, Kawamura F, Kusaka I, Tsuchiya T, Kuroda T, Yabe I. Patch clamp analysis of the respiratory chain in *Bacillus subtilis*. Biochim Biophys Acta. (2011) 1808(4): 1103-1107

Kagawa School of Pharmaceutical Sciences
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Analysis of Nonlinear Phenomena of Mathematical Models of Neurons

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Research

Individual neurons are capable of producing action potentials or spikes in response to external stimulation through interactions of various ionic channels expressed on the plasma membrane of neurons. Based on data derived from the electrophysiological experiments, various mathematical models which reproduce the electrical excitability of individual neurons are developed (e.g., nonlinear ordinary differential equations). By analyzing the mathematical models in detail one can understand the dynamics of individual neurons. Modeling studies are suggested to be useful for studying drugs which are used for therapy of channelopathy. In addition, based on the theory proposed by US mathematician and physicist, modeling studies are also expected to be useful for developing medical devices. Taking the above into account, this laboratory is investigating ordinary differential equations which describe the electrical excitability of individual neurons.

1. Research about ghostbursting phenomenon of electrosensory pyramidal neurons in weakly electric fish
2. Research about pacemaking of RPa1 neurons in snail Helix pomatia
3. Research about the spiking activity of neocortical pyramidal neuron

Publications (2010~2015)

[Original papers]

2015

1. Shirahata, T. (2015). Evaluation of kinetic properties of dendritic potassium current in ghostbursting model of electrosensory neurons. *Applied Mathematics*, 6(1), 128-135.

2014

2. Shirahata, T. (2014). Effect of sodium conductance variations on electrical behavior of a neocortical neuron model. *Acta Biologica Hungarica*, 65(4), 379-384.

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