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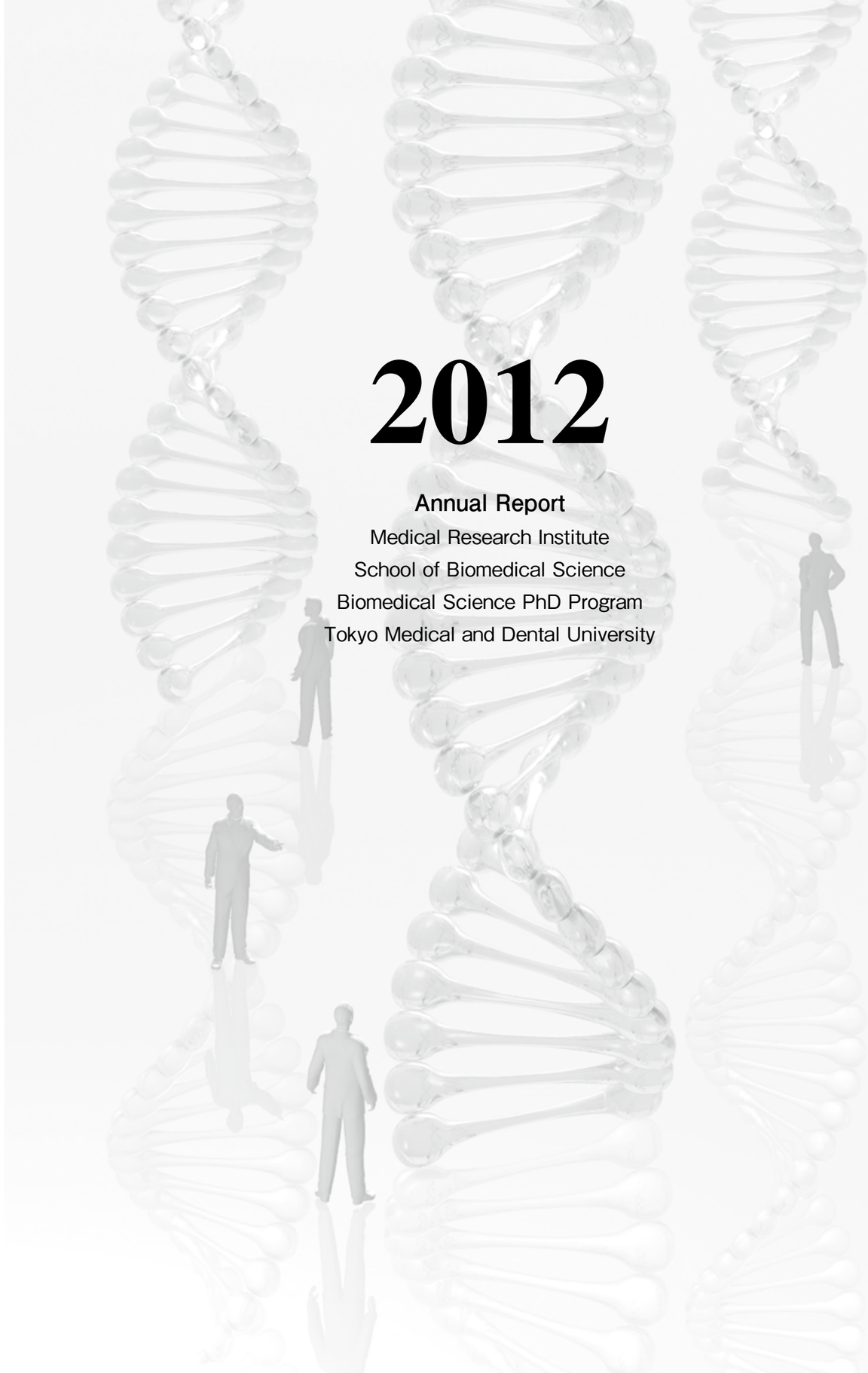
ANNUAL REPORT 2012

Tokyo Medical and Dental University

Medical Research Institute / School of Biomedical Science / Biomedical Science PhD Program / Tokyo Medical and Dental University

Annual Report 2012



2012

Annual Report

Medical Research Institute
School of Biomedical Science
Biomedical Science PhD Program
Tokyo Medical and Dental University

Contents

Yushima Area

1-5-45 Yushima Bunkyo-ku Tokyo 113-8510 Japan

Medical Research Institute
Department of Molecular Pharmacology, Department of Molecular Neuroscience, Department of Pathological Cell Biology, Department of Developmental and Regenerative Biology, Department of Immunology, Department of Molecular Cytogenetics, Department of Molecular Genetics, Department of Biochemical Genetics, Department of Bioinformatics, Department of Molecular Medicine and Metabolism, Department of Molecular Cell Biology, Department of Bio-informational Pharmacology, Department of Molecular Pathogenesis, Department of Epigenetics, Department of Stem Cell Regulation, Department of Neuropathology, Department of Structual Biology, Department of Biodefense Research, Department of Stem Cell Medicine, Frontier Research Unit, Project Research Unit

Surugadai Area

2-3-10 Kanda-surugadai Chiyoda-ku Tokyo 101-0062 Japan

Medical Research Institute
Department of Molecular Epidemiology

School of Biomedical Science
Laboratory of Chemical Bioscience, Laboratory of Organic and Medicinal Chemistry

Medical Research Institute

Director
Shigetaka Kitajima
Faculty Meeting

Research Divisions

Division of Advanced Molecular Medicine	Department of Molecular Medicine and Metabolism	Professor	Yoshihiro Ogawa
	Department of Molecular Pharmacology	Professor	Masaki Noda
	Department of Molecular Cell Biology	Professor	Hiroshi Shibuya
	Department of Molecular Neuroscience	(SBS, Laboratory of Molecular Neuroscience)	
	Department of Biodefense Research	Professor	Toshiaki Ohteki
	Department of Bio-informational Pharmacology	Professor	Tetsushi Furukawa
	Department of Stem Cell Regulation	Professor	Tetsuya Taga
	Frontier Research Unit Laboratory of Oxygen Biology		
	Project Reserch Unit		
Division of Pathophysiology	Department of Neuropathology	Professor	Hitoshi Okazawa
	Department of Pathological Biochemistry	(under consideration)	
	Department of Pathological Cell Biology	Professor	Shigeomi Shimizu
	Department of Developmental and Regenerative Biology	Professor	Hiroshi Nishina
	Department of Stem Cell Medicine	Professor	Emi K. Nishimura
	Department of Immunology	(SBS, Laboratory of Immunology)	
	Department of Molecular Pathogenesis	Professoror	Akinori Kimura
	(SBS, Laboratory of Genome Diversity)		
	Frontier Research Unit Virus Research Unit		
	Project Research Unit		
Division of Medical Genomics	Department of Molecular Cytogenetics	Professor	Johji Inazawa
	Department of Molecular Genetics	Professor	Yoshio Miki
	Department of Molecular Epidemiology	Professor	Masaaki Muramatsu
	Department of Biochemical Genetics	Professor	Shigetaka Kitajima
	(SBS, Laboratory of Genome Structure and Regulation)		
	Department of Function Genomics	(SBS, Laboratory of Gene Expression)	
	Department of Epigenetics	Professor	Fumitoshi Ishino
	Department of Bioinfomatics	(SBS, Laboratory of Computational Biology)	
	Frontier Research Unit		
	Redox Response Cell Biology Laboratory		
	Project Reserch Unit		
Division of Integrative Research	System Biology for Intractable Diseases	Visiting Professor	Satoru Miyano
Division of Donation	Department of Organ Network and Metabolism	Tokunin Professor	Yasutomi Kamei
Advanced Technology Laboratory	Genome Laboratory		
	Cellular and Proteome Research Laboratory		
	Laboratory of Recombinant Animals		
	Laboratory of Anatomy and Cell Function		
	Bioresource Laboratory		
	Animal Research Laboratory		
	Laboratory for Structure Analysis		
Chemical Biology Screening Center			
Administrative Office	General Affairs Section		

School of Biomedical Science

Dean of School of Biomedical Science — Research Divisions
Hiroyuki Kagechika
Faculty Meeting

Dean of Biomedical Science PhD Program
Hiroyuki Kagechika

Department of Medical Bioinformatics	Laboratory of Genome Diversity	(MRI, Department of Molecular Pathogenesis)	
	Laboratory of Systems Biology	Professor	Hiroshi Tanaka
	Laboratory of Genome Structure and Regulation	(MRI, Department of Department of Biochemical Genetics)	
Department of Applied Structural Biology	Laboratory of Structural Biology	Professor	Nobutoshi Ito
	Laboratory of Organic and Medicinal Chemistry	Professor	Hiroyuki Kagechika
	Laboratory of Chemical Bioscience	Professor	Takamitsu Hosoya
Department of Functional Biology	Laboratory of Gene Expression		
	Laboratory of Molecular Neuroscience	Professor	Kohichi Tanaka
	Laboratory of Immunology	Professor	Takeshi Tsubata
	Laboratory of Biosystem Modeling		

Medical Research Institute

Director
- Shigetaka Kitajima
  - Faculty Meeting
  - Research Divisions
    - Division of Advanced Molecular Medicine
      - Department of Molecular Medicine and Metabolism — (under consideration)
      - Department of Molecular Pharmacology — Professor — Masaki Noda
      - Department of Molecular Cell Biology — Professor — Hiroshi Shibuya
      - Department of Molecular Neuroscience — Professor — Kohichi Tanaka
      - Department of Biodefense Research — Professor — Toshiaki Ohteki
      - Department of Bio-informational Pharmacology — Professor — Tetsushi Furukawa
      - Department of Stem Cell Regulation — Professor — Tetsuya Taga
      - Department of Structual Biology — Professor — Nobutoshi Ito
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    - Division of Pathophysiology
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      - Department of Stem Cell Medicine — Professor — Emi K. Nishimura
      - Department of Immunology — Professor — Takeshi Tsubata
      - Department of Molecular Pathogenesis — Professoror — Akinori Kimura
      - Frontier Research Unit
        Virus Research Unit
      - Project Research Unit
    - Division of Medical Genomics
      - Department of Molecular Cytogenetics — Professor — Johji Inazawa
      - Department of Molecular Genetics — Professor — Yoshio Miki
      - Department of Molecular Epidemiology — Professor — Masaaki Muramatsu
      - Department of Biochemical Genetics — Professor — Shigetaka Kitajima
      - Department of Function Genomics — (under consideration)
      - Department of Epigenetics — Professor — Fumitoshi Ishino
      - Department of Bioinfomatics — Professor — Hiroshi Tanaka
      - Frontier Research Unit
        Redox Response Cell Biology Laboratory
      - Project Reserch Unit
    - Division of Integrative Research
      - System Biology for Intractable Diseases — Visiting Professor — Satoru Miyano
    - Advanced Technology Laboratory
      - Genome Laboratory
      - Cellular and Proteome Research Laboratory
      - Laboratory of Recombinant Animals
      - Laboratory of Anatomy and Cell Function
      - Bioresource Laboratory
      - Animal Research Laboratory
      - Laboratory for Structure Analysis
    - Administrative Office
      - General Affairs Section

Highlight

The 6th International Symposium of Institute Network/ The 10th Surugadai Symposium/ The 2nd MRI Joint Usage/Research Symposium

Since 2006, National University (Tokyo University, Kyoto University, Kyushu University, Osaka University, Tohoku University, Kanazawa University and our University)-affiliated Research Institutes have held the annual international symposium that allows top scientists invited from abroad and Japanese scientists to discuss advanced research in the fields of medical and biological science.

In the year 2011, the 6th International Symposium of Institute Network was organized by the Medical Research Institute, Tokyo Medical and Dental University with the main theme of "Research Breakthroughs in Intractable Diseases". This symposium was held at M&D tower Akio Suzuki Memorial Hall with joint sponsorship from the 10th Surugadai Symposium and the 2nd MRI Joint Usage/Research Symposium.

Surugadai Symposium is annually held by Medical Research Institute and Graduate School of Biomedical Science. The purpose of this symposium is to have eminent speakers discuss their great achievement and to help young researchers and senior scientists exchange knowledge and promote research. The 10th Surugadai Symposium was organized by the Division of Pathophysiology.

The talk given by the top scientists were very interactive and interesting. Participants were able to learn a lot. Active discussion could be seen during the symposium.

Program

The 6th International Symposium of Institute Network

The 10th Surugadai Symposium

The 2nd MRI Joint Usage/Research Symposium

"Research Breakthroughs in Intractable Diseases"

Date: June 9-10, 2011

【Invited Speakers】

Kozo Tanaka (Institute of Development, Aging and Cancer, Tohoku University)

Kouhei Tsumoto (Institute of Medical Science, University of Tokyo)

Chiaki Takahashi (Cancer Research Institute, Kanazawa University)

Masahiko Sugita (Institute for Virus Research, Kyoto University)

Jun Takahashi (Institute for Frontier Medical Sciences, Kyoto University)

Yasuhiko Horiguchi (Research Institute for Microbial Diseases, Osaka University)

Akira Shinohara (Institute for Protein Research, Osaka University)

Hiroyuki Sasaki (Medical Institute of Bioregulation, Kyushu University)

Toshiaki Ohteki (Medical Research Institute, Tokyo Medical and Dental University)

Mayumi Ito (New York University School of Medicine, USA)

Richard J. Youle (NINDS, NIH, USA)

Kirsten C. Sadler (Mount Sinai School of Medicine, USA)

Shingo Kajimura (University of California, San Francisco, USA)

Division of Advanced Molecular Medicine

【Aim and Scope】

The mission of Division of Advanced Molecular Medicine is to conduct basic and applied research on the cause, prevention and treatment of intractable diseases including life-style related diseases, bone diseases, immune diseases, neurological diseases, cardiovascular diseases and cancer. For this purpose, we are actively undertaking a broad spectrum of medical research with an emphasis on cross-disciplinary approaches. Topics of research projects in each Department are as follows:

【Molecular Medicine and Metabolism】

- Role of central leptin signaling in the starvation-induced alteration of B cell development has been elucidated.
- We have demonstrated that melanocortin-4 receptor-deficient mice serve as a novel mouse model of non-alcoholic steatohepatitis.
- Increased systemic glucose tolerance with increased muscle glucose uptake in transgenic mice overexpressing RXR γ in skeletal muscle has been demonstrated.

【Molecular Pharmacology】

- Elucidation of novel role of Osteopontin in sympathetic tone-induced bone loss via modulation of ß2-Adrenergic receptor.
- Identification of Dok-1/Dok-2 as negative regulators of bone resorption by modulating M-CSF activity.
- Identification of MuRF1 gene as a positive regulator of unloading-induced bone loss.

【Molecular Cell Biology】

- WNK signaling pathway is involved in neural development.
- IQGAP regulates the nuclear localization of β-catenin in Wnt signaling.

【Molecular Neuroscience】

- BDNF signalling in glia has important roles in neural protection and regeneration.
- Deficits in glutamate transporter function compound the effects of familial AD AβPP/PS1 mutant transgenes in younger animals.

【Biodefense Research】

- Identification of a clonogenic progenitor with prominent plasmacytoid DC differentiation potential.
- Identification of new tolerance induction machinery by DC; DC performs hemophagocytosis to fine-tune excessive immune responses.

【Bio-informational Pharmacology】

- Genome-wide association study (GWAS) identified 12 atrial fibrillation-associated genetic risks, including 2 Japanese-specific genetic risks.
- Gene mutations and variants confer familiar and common cardiac arrhythmias.
- Human iPS-derived cardiomyocytes (hiPS-CM)-based drug screening system and diseased hiPS-CM models were established.

【Stem Cell Regulation】

- We have shown that neural stem cells form neurospheres more efficiently under the hypoxic condition through VEGF production.
- Sox17 has been demonstrated to play an important role in the development and maintenance of hematopoietic stem cells in the AGM region.
- We have elucidated that glioma stem cells have a potential to differentiate into endothelial cells with increased chemo-uptake and chemoresistance.

Division of Advanced Molecular Medicine
Department of Molecular Medicine and Metabolism

Professor	Yoshihiro Ogawa, M.D., Ph.D.
Associate Professor	Takayoshi Suganami M.D., Ph.D.
GCOE Associate Professor	Naoki Sawada M.D., Ph.D.
Tokunin Assistant Professor	Misa Kim-Saijo M.D., Ph.D., Xunmei Yuan Ph.D., Mayumi Takahasi Ph.D., Ibuki Shirakawa Ph.D.,
JSPS Research Fellow	Rumi Hachiya M.D., Ph.D.

The concept of metabolic syndrome has been highlighted because it is a precursory state of atherosclerotic diseases. It has been defined as a constellation of abdominal obesity, insulin resistance, hyperlipidemia, and hypertension, and is a multi-factorial pathologic condition that arises from complex interactions between genetic and environmental factors. In our laboratory, all the staff and students have been provided with unique opportunities to investigate the pathophysiologic and therapeutic implications of adipocytokines, nuclear hormone receptors, and transcriptional co-activators/co-repressors toward better understanding of the molecular mechanism of metabolic syndrome.

Research Subjects

1. Molecular mechanism underlying adipose tissue inflammation

Obesity is associated with a state of chronic, low-grade inflammation, suggesting that inflammation may be a potential mechanism whereby obesity leads to insulin resistance. Using cDNA microarray analysis of a coculture of 3T3-L1 adipocytes and RAW264 macrophages, we have recently found that macrophage-inducible C-type lectin (Mincle, also called Clec4e and Clecsf9), a type II transmembrane C-type lectin, is induced selectively in macrophages during the interaction between adipocytes and macrophages. Mincle was induced by saturated fatty acid palmitate via Toll-like receptor 4 (TLR4) in cultured macrophages. Mincle mRNA expression was also markedly increased in adipose tissue from obese humans and mice. Our data suggest that Mincle may be a novel mechanism underlying obesity-induced adipose tissue inflammation. (**Diabetes** 60: 819-826, 2011.)

Non-alcoholic steatohepatitis (NASH) is considered a hepatic phenotype of metabolic syndrome and is a high risk for the progression to cirrhosis and hepatocellular carcinoma. Although the "two-hit" hypothesis points to the involvement of excessive hepatic lipid accumulation and chronic inflammation, the molecular mechanisms underlying the development of NASH are still unclear, partly because of no appropriate animal models. Recently, we demonstrated that melanocortin-4 receptor-deficient (MC4R-KO) mice developed steatohepatitis on a high-fat diet, which is associated with obesity, insulin resistance, and dyslipidemia. Notably, all the MC4R-KO mice examined developed well-differentiated hepatocellular carcinoma after 1-year high-fat diet feeding. They also showed enhanced adipose tissue inflammation (*i.e.* increased macrophage infiltration and fibrotic changes), which may contribute to excessive lipid accumulation and enhanced fibrosis in the liver. This study suggests that MC4R-KO mice provide a novel mouse model of NASH with which to investigate the sequence of events that comprise diet-induced hepatic steatosis, liver fibrosis, and hepatocellular carcinoma, and thus help to understand the pathogenesis of NASH, pursue its specific biomarkers, and evaluate its potential therapeutic strategies. (**Am. J. Pathol.** 179: 2454-2463, 2011).

2. Role of central leptin signaling in regulation of peripheral inflammation

Nutritional deprivation or malnutrition suppresses immune function in humans and animals, thereby conferring higher susceptibility to infectious diseases. Leptin, a major adipocytokine, is exclusively produced in the adipose tissue in response to the nutritional status and acts on the hypothalamus, thereby regulating energy homeostasis. Although it has been reported that leptin plays a critical role in starvation-induced T cell-mediated immunosuppression, little is known about its role in B cell homeostasis under starvation conditions. In this study, we observed the alteration of B cell development in the bone marrow of fasted mice, characterized by decreased pro-B, pre-B, and immature B cells and increased mature B cells. Interestingly, intracerebroventricular leptin injection was sufficient to prevent the alteration of B cell development of fasted mice. Our data also suggest that serum corticosterone concentrations and the neuropeptide Y pathway in the hypothalamus play a role in the leptin-mediated regulation of B cell development in the bone marrow. This study provides the first in vivo evidence for the role of central leptin signaling in the starvation-induced alteration of B cell development. The data from this study suggest that the central nervous system, which is inherent in the integration of information from throughout the organism, is able to control immune function. (**J. Neurosci.** 31: 8373-8380, 2011)

3. The skeletal muscle as a target organ in metabolic syndrome: Metabolic analysis of transgenic mice overexpressing RXR γ in skeletal muscle

The skeletal muscle plays roles in energy expenditure, glucose uptake and exercise. Retinoid X receptor (RXR) γ is a nuclear receptor-type transcription factor expressed mostly in the skeletal muscle, and is regulated by nutritional conditions, although its role in systemic glucose and lipid metabolism is unclear. We previously established transgenic mice overexpressing RXR γ in the skeletal muscle (RXR γ mice), showing lower blood glucose than in control mice. In this study, we investigated the glucose metabolism of RXR γ mice with induced obesity and impaired glucose metabolism. Glucose tolerance and the disposal rate were higher in lean RXR γ mice than in the controls. The skeletal muscle from lean RXR γ mice showed increased Glut1 expression, with increased glucose uptake, in an insulin-independent manner. In the obese condition, by crossing with genetically obese KKAy mice, systemic insulin resistance, glucose tolerance and obesity-induced fatty liver were markedly improved. This shows that increased glucose uptake in the skeletal muscle improved systemic glucose metabolism, and increasing RXR γ expression may be a novel therapeutic strategy against impaired glucose metabolism caused by obesity (**PLoS ONE** 6: e20467, 2011.).

4. Epigenetic regulation of metabolic diseases: Regulation of hepatic lipogenesis gene expression via DNA methylation

Epidemiological and animal studies have suggested that metabolic diseases in adulthood may be acquired during fetal and neonatal events. The metabolic phenotypes may be determined by epigenetic mechanisms, which are related to environmental factors, including the nutritional status, in early life with high plasticity, during pregnancy, and/or after birth. DNA methylation is a key epigenetic contributor to the maintenance of gene silencing. During the suckling period, when fat intake is high, the rate of hepatic *de novo* lipogenesis is very low, but it increases with the onset of weaning and diminished intake of milk. We have recently found that expression of *glycerol-3-phosphate acyltransferase 1* (*GPAT1*), which encodes a rate-limiting enzyme for *de novo* lipogenesis in the liver, is markedly increased in response to the physiologic demand of lipogenesis during the weaning period. This may be related to decreased DNA methylation of the *GPAT1* promoter, but not other lipogenic enzyme genes, such as fatty acid synthase and stearol CoA desaturase. We showed that DNA methyltransferases are recruited to the *GPAT1* promoter in the liver of neonates. Interestingly, feeding dams with a high-calorie diet before and during pregnancy and suckling, which is known to increase fatty liver in pups, decreased DNA methylation of the *GPAT1* promoter, increased *GPAT1* expression, and increased triglyceride levels in the liver of newborn pups. Our data suggest that environmental factors such as the nutritional status, affect DNA methylation and metabolic diseases.

Publications

[Original Articles]

1. M. Ichioka, T. Suganami, N. Tsuda, I. Shirakawa, Y. Hirata, N. Satoh-Asahara, Y. Shimoda, M. Tanaka, M. Kim-Saijo, Y. Miyamoto, Y. Kamei, M. Sata, Y. Ogawa. Increased expression of macrophage-inducible C-type lectin in adipose tissue of obese mice and humans. **Diabetes** 60: 819–826, 2011.
2. N. Satoh-Asahara, T. Sugamami, T. Majima, K. Kotani, Y. Kato, R. Araki, K. Koyama, T. Okajima, M. Tanabe, M. Oishi, A. Himeno, S. Kono, A. Sugawara, M. Hattori, Y. Ogawa, A. Shimatsu; The Japan Obesity Metabolic Syndrome Study (JOMS) Group. Urinary cystatin C as a potential risk marker for cardiovascular disease and chronic kidney disease in patients with obesity and metabolic syndrome. **Clin. J. Am. Soc. Nephrol.** 6: 265-273, 2011.
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4. S. Sugita, Y. Kamei, F. Akaike, T. Suganami, S. Kanai, M. Hattori, Y. Manabe, N. Fujii, T. Takai-Igarashi, J. Oka, H. Aburatani, T. Yamada, H. Katagiri, S. Kakehi, Y. Tamura, S. Takasuga, T. Sasaki, H. Kubo, K. Nishida, S. Miura, O. Ezaki, Y. Ogawa. Metabolic analysis of transgenic mice overexpressing RXR γ in skeletal muscle: increased glucose tolerance and suppression of obesity-induced fatty liver. **PLoS ONE** 6: e20467, 2011.
5. M. Itoh, T. Suganami, N. Nakagawa, M. Tanaka, Y. Yamamoto, Y. Kamei, S. Terai, I. Sakaida, Y. Ogawa. Melanocortin-4 receptor-deficient mice as a novel mouse model of non-alcoholic steatohepatitis. **Am. J. Pathol.** 179: 2454-2463, 2011.

[Review Articles]

1. M. Itoh, T. Suganami, R. Hachiya, and Y. Ogawa. Adipose tissue remodeling as homeostatic inflammation. **Int. J. Inflamm.** 2011: 720926, 2011.

Department of Molecular Pharmacology

Professor	Masaki Noda, M.D., Ph.D.
Associate Professor	Yoichi Ezura, M.D., Ph.D.
Assistant Professor	Tadayoshi Hayata, Ph.D.
GCOE Research Instructor	Takuya Notomi, Ph.D.
GCOE International Coordinator	Tetsuya Nakamoto, M.D., Ph.D.

Research Summary

In order to contribute to the establishment of therapy and prevention for osteoporosis and the other calcium-related disorders, we are elucidating molecular mechanisms underlying regulation of calcium metabolism with emphases on bone formation and resorption. Skeletal system is a largest storage site for calcium in a living body and its metabolism is conducted by a complex cell society consisting of bone-forming osteoblasts and bone-resorbing osteoclasts as well as stromal cells and chondrocytes. In our department, we take molecular and cellular biological approaches to study the mechanisms of regulation of the development, differentiation, and function of each group of these cells.

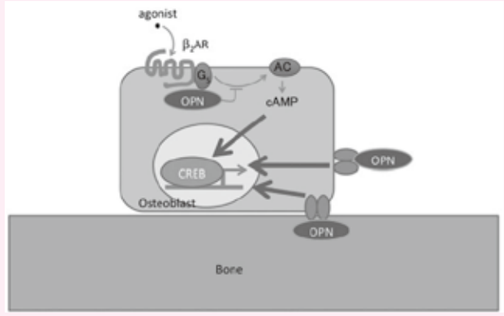
Research Projects

1. Dok-1 and Dok-2 deficiency induces osteopenia via activation of osteoclasts (Kawamata A, Hayata T, Ezura Y, Noda M).

Osteoporosis causes fractures that lead to reduction in the quality of life and it is one of the most prevalent diseases as it affects approximately 10% of the population. One of the important features of osteoporosis is osteopenia. However, its etiology is not fully elucidated. Dok-1 and Dok-2 are adaptor proteins acting downstream of protein tyrosine kinases that are mainly expressed in the cells of hematopoietic lineage. Although these proteins negatively regulate immune system, their roles in bone metabolism are not understood. Here, we analyzed the effects of Dok-1 and Dok-2 double-deficiency on bone. Dok-1/2 deficiency reduced the levels of trabecular and cortical bone mass compared to wildtype. In addition, Dok-1/2 deficiency increased periosteal perimeters and endosteal perimeters of the mid shaft of long bones. Histomorphometric analysis of the bone parameters indicated that Dok-1/2 deficiency did not significantly alter the levels of bone formation parameters including mineralizing surface/bone surface (MS/BS), mineral apposition rate (MAR) and bone formation rate (BFR). In contrast, Dok-1/2 deficiency enhanced the levels of bone resorption parameters including osteoclast number (N. Oc/BS) and osteoclast surface (Oc.S/BS). Analyses of individual osteoclastic activity indicated that Dok-1/2 deficiency enhanced pit formation. Systemically, Dok-1/2 deficiency increased the levels of urinary deoxypyridinoline (Dpyr). Search for the target point of the Dok-1/2 deficiency effects on osteoclasts identified that the mutation enhanced sensitivity of osteoclast precursors to macrophage colony-stimulating factor. These data revealed that Dok-1 and Dok-2 deficiency induces osteopenia by activation of osteoclasts (J Cell Physiol, 2011).

2. MURF1 deficiency suppresses unloading-induced effects on osteoblasts and osteoclasts to lead to bone loss. (Kondo H, Ezura Y, Hayata T, Noda M).

Loss of mechanical stress or unloading causes disuse osteoporosis that leads to fractures and deteriorates body function and affects mortality rate in aged population. This bone loss is due to reduction in osteoblastic bone formation and increase in osteoclastic bone resorption. MuRF1 is a muscle RING finger protein which is involved in muscle wasting and its expression is enhanced in the muscle of mice subjected to disuse condition such as hind limb unloading (HU). However, whether MuRF1 is involved in bone loss due to unloading is not known. We therefore examined the effects of MuRF1 deficiency on unloading-induced bone loss. We conducted hind limb unloading of MuRF1 KO mice and wild-type control mice. Unloading induced about 60% reduction in cancellous bone volume (BV/TV) in WT mice. In contrast, MuRF1 deficiency suppressed unloading-induced cancellous bone loss. The cortical bone mass was also reduced by unloading in WT mice. In contrast, MuRF1 deficiency suppressed this reduction in cortical bone mass. To understand whether the effects of MuRF1 deficiency suppress bone loss is on the side of bone formation or bone resorption, histomorphometry was conducted. Unloading reduced bone osteoblastic formation rate (BFR) in WT. In contrast, MuRF1 deficiency suppressed this reduction. Regarding bone resorption, unloading increased osteoclast number in WT. In contrast, MURF1 deficiency suppressed this osteoclast increase. These data indicated that the ring finger protein, MURF1 is involved in disuse-induced bone loss in both of the two major bone remodeling activities, osteoblastic bone formation and osteoclastic bone resorption (J Cell Biochem, 2011).



Model of regulation of β2AR signaling by OPN.
Our data suggest that intracellular OPN regulates β2AR signaling in osteoblasts by interacting with Gs to reduce the time course of cAMP production and CRE-dependent transcription activity to ultimately reduce bone mass. Extracellular OPN is also involved in regulation of bone cells by activation of CD44/integrin family of receptors.

Highlight

Sympathetic control of bone mass regulated by osteopontin (Nagao M, Ezura Y, Hayata T, Notomi T, Nakamoto T, Noda M).

The sympathetic nervous system suppresses bone mass by mechanisms that remain incompletely elucidated. Using cell-based and murine genetics approaches, we show that this activity of the sympathetic nervous system requires osteopontin (OPN), a cytokine and one of the major members of the noncollagenous extracellular matrix proteins of bone. In this work, we found that the stimulation of the sympathetic tone by isoproterenol increased the level of OPN expression in the plasma and bone and that mice lacking OPN (OPN-KO) suppressed the isoproterenol-induced bone loss by preventing reduced osteoblastic and enhanced osteoclastic activities. In addition, we found that OPN is necessary for changes in the expression of genes related to bone resorption and bone formation that are induced by activation of the sympathetic tone. At the cellular level, we showed that intracellular OPN modulated the capacity of the β2-adrenergic receptor to generate cAMP with a corresponding modulation of cAMP-response element binding (CREB) phosphorylation and associated transcriptional events inside the cell. Our results indicate that OPN plays a critical role in sympathetic tone regulation of bone mass and that this OPN regulation is taking place through modulation of the β2-adrenergic receptor/cAMP signaling system (Proc Natl Acad Sci USA, 2011).

Publications

[Original articles]

1. Nagao M, Feinstein TN, Ezura Y, Hayata T, Notomi T, Saita Y, Hanyu R, Hemmi H, Izu Y, Takeda S, Wang K, Rittling S, Nakamoto T, Kaneko K, Kurosawa H, Karsenty G, Denhardt DT, Vilardaga JP, Noda M. Sympathetic control of bone mass regulated by osteopontin. ***Proc Natl Acad Sci U S A*** 108:17767-72, 2011.
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3. Hemmi H, Zaidi N, Wang B, Matos I, Fiorese C, Lubkin A, Zbytnuik L, Suda K, Zhang K, Noda M, Kaisho T, Steinman RM, Idoyaga J. Treml4, an Ig Superfamily Member, Mediates Presentation of Several Antigens to T Cells In Vivo, Including Protective Immunity to HER2 Protein. ***J Immunol*** 188:1147-55, 2012.
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Division of Advanced Molecular Medicine
Department of Molecular Cell Biology

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Various signaling molecules inducing the cell-growth and differentiation regulate morphogenesis and organogenesis of the vertebrate. The failure of these signal molecules has also been caused with induction of the diseases. Therefore, the elucidation of signal transduction network regulating generation and differentiation is important upon clarifying the mechanism of morphogenesis, organogenesis and diseases. We focus on the signal transduction network regulating the mechanisms of morphogenesis and organogenesis in developmental process.

WNK protein kinases, the causative genes of pseudohypoaldosteronism type II (PHAII) disease

WNK (with no lysine (K)) kinase family that has been recently identified serine/threonin protein kinase family conserved among many species. There are four mammalian WNK family members, and mutations in two of them, WNK1 and WNK4 have been linked to a hereditary form of human hypertension known as Pseudohy-poaldosteronism type II (PHAII). Previously, we identified that WNKs phosphorylated and activated SPAK/OSR1 kinases, which in turn regulated various ion co-transporters, such as NKCC1, NKCC2 and NCC. We also presented that the malfunction of this regulation caused the similar phenotypes of PHAII in mouse. However, this misregulation cannot cause all of pathological conditions of PHAII, such an intellectual impairment, dental abnormalities and impaired growth. This suggests that WNK is involved in the other signaling cascade. We started to look for the other interacting factor(s) of WNK using *Drosophila melanogaster*.

1. Evolutional conservation of WNK signaling pathway

Drosophila WNK (DWNK) could bind to and directly phosphorylate *Drosophila* OSR1 homologue Fray, as well as mammalian WNKs did OSR1. As DWNK, Fray, mammalian WNK1 and OSR1 were ectopically expressed at the posterior compartment of wing, all of these overexpressions caused similar phenotypes such as ectopic wing veins. Together with our previous results in mouse and nematode, these results suggest that WNK pathway is conserved among many species.

2. The downstream transcription factor

The mosaic clones of *DWNK* mutant, and the overexpression of the kinase-dead form of DWNK, which might work as a dominant negative, caused the defect of abdominal development. Since these phenotypes are similar to mutant of gene X, which encodes the transcription factor, these data suggest that the transcription factor X and WNK are genetically involved. When we simultaneously overexpressed the kinase-dead form of DWNK and the transcription factor X, the defect of abdominal development, which caused by the overexpression of the kinase-dead form of DWNK, was rescued. Furthermore, the phenotypes of mosaic clones of *DWNK* mutant were rescued by the overexpression of gene X. In *DWNK* mutant embryos, the abdominal expression of gene X was reduced. These results indicate that the transcription factor X works at the downstream of WNK signaling pathway.

Roles of IQGAP1 on the canonical Wnt signaling.

Wnt signaling plays important roles in multiple developmental events during embryogenesis. Canonical Wnt signaling is initiated by binding of the Wnt ligand to the cell-surface Frizzled and transmembrane LRP complex. This leads to the membrane recruitment and activation of Dishevelled (DVL), which inactivates the APC/Axin/GSK-3 complex in the cytoplasm, responsible for the degradation of β-catenin. As a result, β-catenin accumulates in the cytoplasm, translocates to the nucleus and associates with Tcf transcription factors, which activate the Wnt target genes. In *Xenopus*, Wnt signaling accompanied by β-catenin nuclear localization at the dorsal side is an important for axis formation during early embryogenesis. Ventral over-expression of *Xwnt-8*, *β-catenin* and *DVL2* induces a secondary axis and promotes expression of Wnt target genes, such as *Siamois*, *Xnr3* and *Xtwn*. DVL contains three conserved regions known as the DIX, PDZ and DEP domains. Both the DIX and PDZ domains are necessary for canonical Wnt inactivation of β-catenin degradation. In contrast, the DEP domain does not affect canonical signaling, but is involved in the planar cell polarity (PCP) pathway. DVL plays an additional role in the Wnt signaling pathway, by localizing to the nucleus and binding a complex containing β-catenin and Tcf, which in turn activates Wnt target genes in the nucleus. The subcellular localization of DVL, either on the cell membrane or in the nucleus, is important for understanding its function in Wnt signaling.

To identify novel proteins that may bind to DVL, we performed a high-throughput analysis of proteins that co-immunoprecipitated with human DVL1 in HEK 293 cells using direct nanoflow liquid chromatography-coupled tandem MS (LC-MS/MS). We identified several known DVL-binding proteins, such as CK1, CK2, Strabismus, Par1, Axin and PP2C. In addition, we identified IQGAP1 as a candidate protein that may physically interact with DVL1. IQGAP1 contains multiple protein-interacting domains: the CH (calponin homology) domain binds to F-actin, the WW domain binds to ERK2, the IQ repeat motifs bind to calmodulin and myosin light chain, and the Ras GAP-like domain binds to Cdc42 and Rac1. IQGAP1 is also known to bind to E-cadherin and β-catenin, and is involved in cytoskeltal reorganization and cell adhesion. On the other hand, IQGAP1 stimulates β-catenin-mediated transcriptional activation.

We investigated roles of IQGAP and DVL in the canonical Wnt signaling pathway, and we have already obtained the following results: [1] xIQGAP1, xDVL2 and β-catenin can form a complex, and each protein contributes to the nuclear localization of each other under the Wnt stimulation. [2] Depletion of xIQGAP1 by antisense morpholino oligonucleotides (*xIQGAP1*-MO) reduced expression of Wnt target genes induced by *Xwnt-8*.

We performed more analyses to further elucidate the mechanism of nuclear localization of Wnt components with IQGAP1, and obtained the following new results.

1, To identify novel proteins that may bind to IQGAP1, we performed a high-throughput analysis of proteins that co-immunoprecipitated with human IQGAP1 in HEK 293 cells using LC-MS/MS. We identified human Importin-β5 as a candidate protein that may physically interact with IQGAP1. We found that xImportin-β5 bound to xIQGAP1 but not xDVL2 and β-catenin in HEK293T cells.

2, Depletion of xImportin-β5 by antisense morpholino oligonucleotides (*xImportin-β5*-MO) decreased the nuclear localization of GFP fusion proteins of xDVL2, xIQGAP1 and β-catenin when co-expressed with *Xwnt-8*.

Publications

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2. Goto T., Asashima M. (2011). Chemokine ligand *Xenopus CXCLC (XCXCLC)* regulates cell movements during early morphogenesis. **Dev. Growth Differ.** 53, 971-981.

Division of advanced Molecular Medicine
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The final goal of our research is to understand molecular, cellular, and neuronal ensemble mechanisms underlying higher order brain functions including learning and memory. For that purpose, we combine molecular genetics, physiological and behavioral methods. The laboratory also studies the mechanism that underlies neuronal cell death and regeneration.

1. Functions of glutamate transporters in the brain

Glutamate is a major excitatory neurotransmitter and plays an important role in neuronal plasticity and neurotoxicity in the central nervous system. Glutamate transport proteins provide the mechanism by which synaptically released glutamate is inactivated and kept below toxic levels in the extracellular space. By now, five subtypes of high-affinity glutamate transporters have been identified in the mammalian brain. Our lab studies the physiological and pathological roles of glutamate transporter subtypes using subtype-specific knockout mice.

Glutamate transporters regulate normal synaptic network interactions and prevent neurotoxicity by rapidly clearing extracellular glutamate. GLT-1, the dominant glutamate transporter in the cerebral cortex and hippocampus, is significantly reduced in Alzheimer's disease (AD). However, the role GLT-1 loss plays in the cognitive dysfunction and pathology of AD is unknown. To determine the significance of GLT-1 dysfunction on AD-related pathological processes, mice lacking one allele for GLT-1(+/-) were crossed with transgenic mice expressing mutations of the amyloid-β protein precursor and presenilin-1 (AβPPswe/PS1ΔE9) and investigated at 6 or 9 months of age. Partial loss of GLT-1 unmasked spatial memory deficits in 6-month-old mice expressing AβPPswe/PS1ΔE9, with these mice also exhibiting an increase in the ratio of detergent-insoluble Aβ42/Aβ40. At 9 months both behavioral performance and insoluble Aβ42/Aβ40 ratios among GLT-1(+/+)/AβPPswe/PS1ΔE9 and GLT-1(+/-)/AβPPswe/PS1ΔE9 mice were comparable. These results suggest that deficits in glutamate transporter function compound the effects of familial AD AβPP/PS1 mutant transgenes in younger animals and thus may contribute to early occurring pathogenic processes associated with AD.

2. Role of the lateral habenula in the social avoidance behavior

Habenula is a phylogenetically conserved nucleus and plays critical role in regulating the neuronal activity of serotonergic and dopaminergic neurons in the brain (Aizawa et al., 2011). Recent studies reported that potentiation of the neural transmission and increase of glial glutamate transporter GLT-1 protein were observed in the helpless rats, a rodent model for the human depression. These facts indicate dysfunction of the habenula may play pivotal role in the pathophysiology of major depression.

To address this, we previously conducted anatomical and physiological analyses and revealed that 1) the habenula could be subdivided into several subnuclei based on the distinct gene expression patterns, and 2) the firing of the habenular neurons changed according to transition across different brain states. However, it remains unclear how the subregions of the habenula are involved in the depressive behavior.

In 2011, we examined the role of the habenula in the susceptibility to social stress which influences the depressive symptoms. In the chronic social defeat stress paradigm, seven-weeks old mouse was exposed to the larger aggressor mouse for ten days. This is supposed to mimic the condition of the patients with depression, since the defeated mouse starts to show avoidance from the aggressor (social avoidance test) and anhedonia in the sucrose preference test after chronic social defeat stress. The extent of social avoidance were varied even within wild-type and categorized into “susceptible” and “resilient” mice showing the less and more interaction with aggressor in the social avoidance test (upper panels in Fig.3A). Intriguingly, the functional mapping of the immediate early gene, c-Fos, in the brain revealed that greater activation of the lateral habenular neurons were observed in the resilient group than in the susceptible group (lower panels in Fig.3A and Fig.3B). This suggests that the lateral habenula may play pivotal role in determining the susceptibility to the stress and stress coping strategy. Dysfunction of the lateral habenula may lead to the depressive symptoms.

Furthermore, we developed a novel method which allows us to manipulate the cells in the habenula genetically. We focused on the Dbx1 gene expressed specifically in the habenular anlage in the epithalamus and generated double mutants expressing red fluorescent reporter. Dependent upon the timing of induction of the transgene, we were able to label either neurons or astrocytes in the habenula specifically. This enables us to reveal a novel role of the habenula in the social avoidance behavior when combined with optogenetic probes.

3. Glia- and neuron-specific functions of TrkB signaling during retinal degeneration and regeneration

Glia, the support cells of the central nervous system, have recently attracted considerable attention both as mediators of neural cell survival and as sources of neural regeneration. To further elucidate the role of glial and neural cells in neurodegeneration, we generated TrkB(GFAP) and TrkB(c-kit) knockout mice in which TrkB, a receptor for brain-derived neurotrophic factor (BDNF), is deleted in retinal glia or inner retinal neurons, respectively. Here, we show that the extent of glutamate-induced retinal degeneration was similar in these two mutant mice. Furthermore in TrkB(GFAP) knockout mice, BDNF did not prevent photoreceptor degeneration and failed to stimulate Müller glial cell proliferation and expression of neural markers in the degenerating retina. These results demonstrate that BDNF signalling in glia has important roles in neural protection and regeneration, particularly in conversion of Müller glia to photoreceptors. In addition, our genetic models provide a system in which glia- and neuron-specific gene functions can be tested in central nervous system tissues in vivo.

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[original papers]

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Department of Biodefense Research

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Our research projects focus on maintenance and failure of immunological homeostasis. Our goal is to define the mechanism of immune cell and tissue stem cell behavior under conditions of health and disease. To accomplish this goal, we are trying to clarify the molecular basis of induction and failure of immunological tolerance by focusing on immune cells and tissue stem cells in the bone marrow, skin, and intestine including its associated lymphoid tissues. On the basis of our findings, we will further pursue our research in the hope of developing new rational therapies for prevention and treatment of disease.

1. Regulatory mechanism in the gut-associated lymphoid tissues

Dendritic cells (DCs), composed of plasmacytoid DCs (pDCs) and conventional DCs (cDCs), are representative antigen presenting cells (APCs) and play integral roles in balancing tolerance to self-Ags and immunity to pathogens in peripheral lymphoid tissues. In the intestine, DCs are requested to keep the balance even more sharply such that DCs should be tolerogenic in the presence of numerous commensal bacteria while retain the capacity to respond to episodic pathogens. We have recently found prominent role for pDCs in mucosal T cell-independent (TI) IgA production (**Immunity** 34, 247-257 (2011)). The prominent IgA induction capacity was dependent on APRIL and BAFF production by pDCs. Importantly, pDC expression of APRIL and BAFF was dependent on stromal cell-derived type I IFN-signaling under steady-state conditions. Given that both APRIL and BAFF are important for B-cell maturation and survival, and that their overproduction is associated with cancer and autoimmunity, our findings suggest a possible molecular basis for the control of gut homeostasis and may lead to improvements in vaccination strategies and treatment for mucosal-related disorders. This study was introduced in Previews of the same issue (**Immunity** 34, 144-146 (2011))

2. Differentiation and function of dendritic cells

1) Identification of a clonogenic progenitor with prominent plasmacytoid DC differentiation potential.

DCs are divided into two major subsets. It is currently accepted that pDCs, characterized by a capacity of high type I IFN production, and cDCs in lymphoid tissues are continuously regenerated from hematopoietic stem cells through the macrophage and DC precursor (MDP) and common DC precursor (CDP), the latter is a DC-restricted developmental intermediate. Importantly, we have recently succeeded to identify another DC-restricted developmental intermediate with prominent pDC differentiation potential. Consistent with the potential, the newly identified DC pecursors express elevated level of E2-2 and IRF8, critical transcription factors for pDC differentiation and function (Fig.1). We are currently analyzing the details of this DC progenitor.

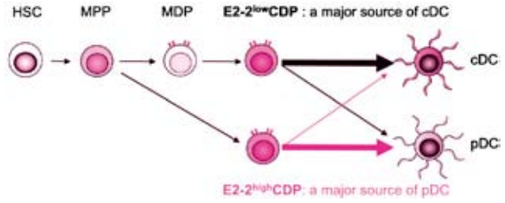

Fig.1 Identification of common DC precursor (CDP)

2) Identification of new tolerance induction machinery by DCs

An immune response is a double-edged sword. Immunologists assume that the host defence strategy, including the immune response, involves mechanisms that directly attack the pathogen to block its invasion or to eliminate it. However, the same immune response can often cause self-damage, i.e., bystander damage or immunopathology. To assure the survival of the host, the immune system must be equipped with machinery to optimize immune responses while fine-tuning the balance between host defence and self-damage, particularly under severe inflammatory conditions. In this respect, little is known about the fine-tuning machinery of the immune system. We have recently identified new DC-mediated tolerance mechanism to prevent excessive immune responses under severe infectious and inflammatory conditions, and we are currently analyzing the molecular mechanisms and its physiological relevance.

3) Understanding of immunological diseases on the basis of tissue stem cell disorder

Type I interferons (IFNs), a family of cytokines, are produced by mammalian cells and orchestrate numerous biological and cellular processes. Although it is well known that type I IFNs are essential for establishing the host antiviral state, their role in hematopoietic homeostasis remains unstudied. Importantly, we recently found that type I IFNs induce proliferation and exhaustion in hematopoietic stem cells (HSCs), and that interferon regulatory factor-2 (IRF2), a transcriptional suppressor of type I IFN signaling, preserves the self-renewal and multi-lineage differentiation capacity of HSCs (**Nat Med** 15, 696-700 (2009)). Based on our findings, we are currently trying to establish type-I IFN-based BM-transplantation without or with minimal irradiation for the treatment of congenital metabolic disorder, and type-I IFN-based treatment for chronic myelogenous leukemia (CML) and congenital disorder.

Publications

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[Presentation at international meetings]

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3. Ohteki T. Prominent fole for pDCs in mucosal IgA induction. The 6th Chiba University Global COE Symposium Immune System Regulation toward Disease Control. Chiba, Japan. 2011.11.30
4. Ohteki T. Role for plasmacytoid dendritic cells in gut IgA induction CFCD3rd International pDC Workshop Pasteur Institute, Paris, France. 2011.12.8

Department of Bio-informational Pharmacology

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Associate Professor Junko Kurokawa, Ph.D.
Assistant Professor Yusuke Ebana, M.D., Ph.D.

This laboratory focuses on understanding pathogenesis of intractable and common cardiovascular diseases using multidisciplinary approach (patch-clamp, cell biology, optical recording, genetic analysis, and computational analysis). Our ultimate goal is to improve diagnosis and management of intractable and common cardiovascular diseases.

1. Gender-specific medicine (GSM) for cardiovascular diseases

Susceptibility of several diseases and responsibility to various drugs and therapy exhibit gender-difference, and cardiovascular system show unique gender difference. We have previously reported that non-genomic actions of sex hormones play an important role creating gender-difference in cardiac arrhythmia. As a next step, we focused on the role of XY chromosome in gender difference.

(collaboration with Prof. Y. Kurihara in the University of Tokyo Graduate School of Medicine)

2. Pathogenesis of atrial fibrillation (AF)

Atrial fibrillation (AF) is the most frequent persistent arrhythmias, reaching more than 3.5 million patients in Japan. Associated cerebral infarction due to cardiogenic thrombosis (250,000 patients /year in Japan) causes reduced QOL and is one of the main causes of bedridden old people. We have taken following approaches to establish protection and treatment of atrial AF.

a. GWAS (genome-wide association study) in AF

We carry out most extensive GWAS (genome-wide association study) in Japan to determine gene polymorphisms associated with AF. In this year, we also participated in the international Meta-analysis called as CHARGE study. We found 12 SNPs associated with AF: among them, 6 SNPs were associated with both European/American and Japanese, 4 with European/American but not with Japanese, and 2 with Japanese but not with European/American.

(collaboration with Prof.. Nakamura Y in The Institute of Medical Science, The University of Tokyo, Dr. Tanaka T. in RIKEN, Dr. Sawabe M. in Tokyo Metropolitan Geriatric Hospital, and Department of Cardiology in this University)

b. Inflammatory and immunological mechanisms in atrial fibrillation

AF is a multifactorial disease, and inflammatory response is believed to play a role in linking between these risks and AF. We examined the relation between atrial dilatation, one of the most frequently found risk factor and inflammation. We found that stretch-induced ATP release and thereby recruitment of macrophages act as an initial factor to provoke atrial inflammation.

3. Pathogenesis of ventricular tachyarrhythmias and sudden cardiac death

Despite extensive effort by many researchers for years, ventricular tachycardia and fibrillation remain the main cause of sudden death, and the biggest challenge in arrhythmia research. Our laboratory approaches this issue using 2 genetically engineered mice.

a. Analysis of *NOS1AP* (NOS1 associated protein) KO mice

(collaboration with Dr. N. Kato in National Center for Global Health and Medicine)

b. KO mice for a transcription regulator specific to the His-Purkinje system

Recent clinical data implicate the importance of His-Purkinje system (HPS) in development of ventricular tachycardiac/fibrillation, and cardiac sudden death ("Purkinje arrhythmias"). We created mice deficient of a transcription regulator specifically expressed in HPS. Mice exhibited similar phenotype as Brugada syndrome (BrS) and/or early repolarization syndrome (ERS), including elevated J-wave, and right bundle branch block pattern in surface ECG, and greater arrhythmogeniety. This mouse could provide a mouse model of BrS and/or ERS.

(collaboration with Prof. N. Miura Hamamatsu University School of Medicine, Dr. Wataru Shimizu in National Center for Cardiovascular Diseases, and Dr. Akihiko Nogami in Yokohama Rosai Hospital)

4. Use of iPS cells for arrhythmia research

Traditional arrhythmia researches have been performed in cardiomyocytes of species other than human, or in cultured cells, in which human ion channel genes have been heterologously expressed. The milieu different from human cardiac myocytes (especially the lack of excitation-contraction coupling machinery) is the huge limitation for arrhythmia research. Cardiomyocytes differentiated from human iPS cells could overcome this critical limitation, and would bring marked advance in arrhythmias researches. We take following 2 approaches.

a. Establishment of human iPS-derived cardimyocytes (hiPS-CM) from familiar sudden death patients (LQT, Brugada syndrome)

We try to establish and characterize iPS cell-derived cardiomyocytes from human fibroblasts obtained from familiar sudden death patients (LQT, Brugada syndrome). We have able to establish iPS cells from LQT1, LQT2, LQT3, and Brugada syndrome. Our data showed that hiPS-CM from LQT patients maintain some of electrophysiological phenotype found in LQT patients' hearts. In addition, in hiPS-CM from LQT3 patient, we found that abnormal Ca^{2+} handling contributed to the arrhythmogenecity.

(collaboration with Prof. K. Fukuda in Keio University School of Medicine)

b. Drug screening system using human iPS cells-derived cardiomyocytes

(collaboration with Prof. Kenji Yasuda in Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, and Dr. Y. Kanda in National Institute of Health Sciences)

5. Use of state-of-art technology for cardiovascular research

a. Use of motion vector technology for in vitro analysis of cardiac contraction

To analyze cardiac contractility, one has to perform echocardiography or catheter measurement of intra-cardiac pressure/intra-cardiac volume in vivo. Thus, to examine possible cardiac toxicity of new drugs, one must wait until in vivo assay. Motion vector technology created by Sony Co. can non-invasively estimate contraction and relaxation speed of cardiac myocytes in vitro (Fig. 1). We verified using well-defined drugs that motion vector technology can assess drug's effects on contraction and relaxation of cardiac myocytes. We also confirmed that motion vector can be monitored simultaneously with electrical activity of cardiomyocytes (MEA), and also that this technology can be applied to the hiPS-CMs.

(collaboration with Dr. Akio Yasuda, Dr. Eriko Matsui, Dr. Tomohiro Hayakawa, Dr. Hatsune Uno, and Dr. Takeshi Kunihiro in Sony Co.)

b. Basic research for generation of 3-D simulator of cardiac electrical activity

(collaboration with Prof. Toshiaki Hisada, Prof. Seiryo Sugiura, and Dr. Junichi Okada in Graduate School of Frontier Sciences, the University of Tokyo)

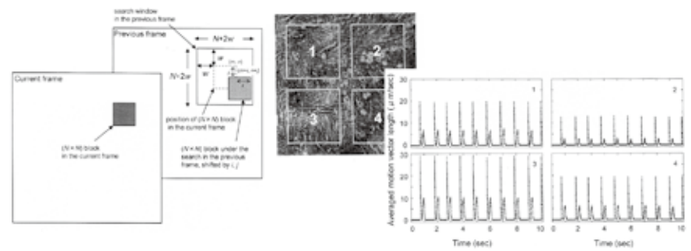

Fig.1 In vitro analysis of cardiomyocyte contraction by motion vector
Images of cultured rat neonatal myocytes were obtained at a speed of 1/125 frames/sec. Plotting speed of cardiomyocytes motion exhibited contraction and relaxation speed of cardiomyocytes.

Publications

[Original articles]

1. Sugiyama H, Nakamura K, Morita H, Akagi S, Tani Y, Katayama Y, Nishii N, Miyoshi T, Nagase S, Kohno K, Kusano FK, Ohe T, Kurokawa J, Furukawa T, Ito H. Circulating KCNH2 current-activating factor in patients with heart failure and ventricular tachyarrhythmia. *PLoS ONE*, 2011;6:e19897.
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Department of Stem Cell Regulation

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Our research is aimed to elucidate mechanisms by which multicellular organs, in particular the central nervous and hematopoietic systems, are developed. We have mostly focused on molecular regulation of neural stem cells and hematopoietic stem cells in view of cell-external cues such as cytokines as well as cell-intrinsic programs including chromatin modification. These projects have been performed, for instance by analyzing cross-interactions of transcriptional regulatory signaling pathways, which lead to spatio-temporally coordinated gene expression. Our major research subjects are as follows:

1) Molecular basis for the maintenance of neural stem cells
2) Regulation of the neural stem cell fate
3) Characterization of hematopoietic stem cells in fetal hematopoietic organs
4) Characterization of cancer stem cells and their niche
5) Epigenetic regulation of neural development

1. Epignenetic regulation of astrogliogenesis

Astrogliogenesis is prevented until the late gestational stage. Cytosine residue in the critical DNA element for STAT3, a downstream target of astrogliogenic cytokines, in an astroglial gene promoter is highly methylated until the mid-gestational stage and demethylated as the brain develops. We here show that expression of *Tet3*, whose gene product TET3 converts the 5-methylcytosine to 5-hydroxymethylcytosine and then leads to demethylation of this cytosine and/or hindrance of access of gene-silencing protein(s), increased in mouse brain in accordance with development. Forced expression of *Tet3* in the culture of mid-gestational neural stem cells which do not normally respond to astrogliogenic cytokines endowed these cells with responsive to such cues.

2. Epigenetic regulation of brain function

GASC1 encodes a histone H3 lysine 9 (H3K9) demethylating enzyme which leads to gene expression (Fig.1). We show that *GASC1* is expressed in post-mitotic neurons in the brain. A strain of mutant mouse showing significant reduction in the expression of *GASC1* was provided by Professor Inazawa. Our recent histological analyses showed no obvious histological abnormalities in the brain of *GASC1* mutant homozygous mice during development and adulthood. However, they exhibited impaired acquisition of skilled behavior in a rotarod test and poorer cognitive task performance on Barnes maze as compared with normal mice. The mutants also displayed hyperactivity in the open field test.

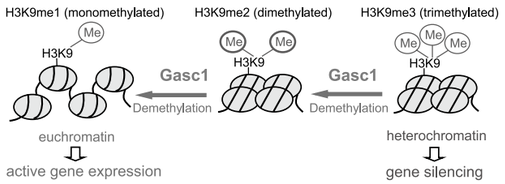


Fig.1 Gasc1 functions as a histone H3 lysine 9 (H3K9) demethylase and changes chromatin structure, leading to active gene expression. *gasc1* mutant mice exhibit abnormal behavior that resembles human neurological/psychiatric symptoms.

3. Analysis of astroglial cell sub-lineages in the developing central nervous system

In the central nervous system, neurons and glial cells cooperatively play critical and important roles in brain function and interact with each other. Among the glia, astroglial cells are known to exhibit various functions, particularly in the regulation of neuronal survival, recycling of neurotransmitters, formation of blood-brain barrier, process of synaptogenesis, and modulation of synaptic activities. When and how astrogilal cells are specified to exhibit such diverged functions has been poorly understood. One of our hypotheses is that the distinct brain domains may contribute to astroglial cell diversification. If so, there may be some functional differences between the dorsal and ventral telencephalic neuroepithelium-derived astrocytes. To challenge this question, we have established a mouse line in which only a sub-lineage of astrocytes express green fluorescent protein. With this line of mice, we purified astrocytes from different brain domains and did gene expression profiling on these cells.

4. Maintenance of immature phenotype of the hematopoietic cell clusters in the aorta-gonad-mesonephros region by Sox17 family proteins

Sry-related high mobility group box (Sox)17 is a marker of endodermal cells and a transcriptional factor containing a DNA binding domain. We examined the function of Sox17 in the hematopoiesis in the aorta-gonad-mesonephros (AGM) region, from which definitive hematopoiesis firstly arises in the mouse embryo. Sox17 proteins were expressed in endothelial cells lining dorsal aorta and hematopoietic cell cluster (Fig.2). Overexpression of Sox17 in E10.5 AGM CD45^{low}c-Kit^{high} cells, in which we have demonstrated the high multilineage hematopoietic activity, led to form cell clusters with a ball-like structure. These ball cells maintained the immature morphology and had a high ability to form hematopoietic colonies in vitro. After 8 passages of the coculture with stromal cells, Sox17-infected cells were capable of maintaining the ability of producing ball-like cells with mix-lineage colony forming ability in semi-sold media. These results suggested that Sox17 have roles in maintaining the undifferentiated states in hematopoietic progenitor in the AGM region.

5. Characterization of tumor stem cells and their niche

Tumor stem cells (TSCs) are primarily responsible for tumor maintenance and relapse, and thus considered as a potential target to eradicate tumors. Since TSCs self-renew in a special microenvironment (niche), it has been proposed that disruption of niche could significantly inhibit the tumor growth. C6 rat glioma contains a subpopulation of TSCs, which is enriched using the Hoechst 33342 side population (SP) technique. SP in C6 is tumorigenic, but a majority of main population (MP) is not. We found that a portion of TSCs differentiate into vascular endothelial cells (VECs) to compose the vascular niche. In mice brain intracranially transplanted with SP cells, extracellular matrix proteins are specifically expressed around tumor blood vessels, which are composed of SP-derived and host-derived VECs. Although host-derived VECs have SP phenotype, SP-derived VECs mostly have MP phenotype with more accumulative and resistant properties for anti-cancer drugs, suggesting that SP-derived VECs may confer some survival advantages as a drug barrier for tumor cells. Our study provides important insights into the fundamental aspect of tumor development from TSCs and the functional multiformity of tumor vessels. In addition to this line of projects, we are in search for artificail niche condition which supports TSC maintenance by using microarrays of hundreds of synthetic polymers that our collaborator Professor Bradley provided us. We identified one candidate polymer that can separate highly tumorigenic cells among the SP cells (Fig.3).

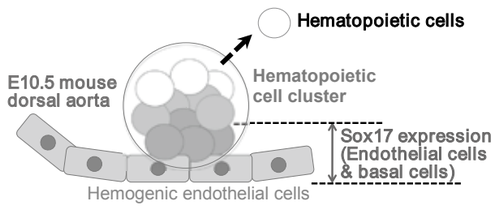


Fig.2 Sox17 expression is observed in the endothelial cells and part of the hematopoietic cell cluster present in the dorsal aorta in the aorta-gonad-mesonephros (AGM) region.

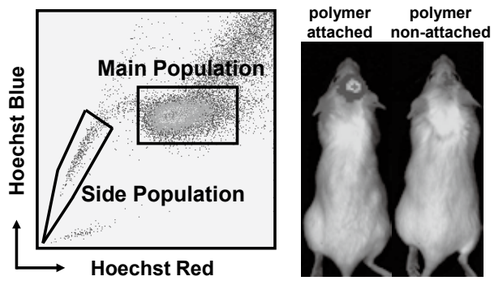


Fig.3 Detection of side population (SP) in C6 glioma cells by Hoechst staining (left) and bioluminescent images (right) of mice transplanted with two subtypes of SP cells; polymer-attached and -non-attached cells.

Publications

[Original Article]

1. Yamasaki S, Nobuhisa I, Ramadan A, and Taga T: Identification of a yolk sac cell population with hematopoietic activity in view of CD45/c-Kit expression. Develop. Growth Differ. 53:870-877, 2011
2. Nobuhisa I, Yamasaki S, Ramadan A and Taga T: CD45^{low}c-Kit^{high} cells have hematopoietic properties in the mouse aorta-gonad-mesonephros region. Exp. Cell Res. In press

[Review and Book]

1. Tabu K, Taga T and Tanaka S. "Glioma Stem Cells" Molecular Targets of CNS Tumors, Miklos Garami (Ed.) (Intech) 151-176, 2011
2. Tabu K, Taga T and Tanaka S. "Tumor Stem Cells: CD133 Gene Regulation and Tumor Stemness" Stem Cells and Cancer Stem Cells, Volume 2, Part 2 (Springer) 145-153, 2011

Frontier Research Unit
Laboratory of Oxygen Biology

Associate Professor　Koh Nakayama, Ph.D.

Aim of the Research

Study in our laboratory aims to understand how changes in the ambient oxygen concentration affects our body function. It is well known that oxygen is required for the efficient energy production in mitochondria. Furthermore, recent studies revealed the role of oxygen, particularly a hypoxic condition, involved in the developmental processes, tumorigenesis, and stem cell proliferation. Our goal is to understand the molecular mechanism of hypoxia response and establish new tools for cancer therapy and regenerative medicine.

Subjects of Research

1. Signal transduction of hypoxia response

Hypoxia-inducible factor (HIF)-α is a transcription factor which plays a central role during hypoxia response by altering multiple cellular functions including metabolism, respiration, and cell growth. HIF-α is actively degraded during normoxic condition, whereas it is stabilized and activated under hypoxic conditions. PHD is a HIF-prolyl hydroxylase which hydroxylates and regulates the expression level of HIF-α. There are 3 PHDs indentified, which are named PHD1, 2, and 3. These proteins hydroxylate HIF-α to negatively regulate its expression, however, it is suggested to have substrates besides HIF-α. We have been focusing on PHD3, and studying hypoxic cell signaling pathways which are connected to the HIF-dependent and -independent pathways.

2. Regulation of hypoxic cell death by PHD3-PRP19 interaction

Our recent study demonstrated the formation of 'hypoxia complex' under hypoxic conditions which consists of PHD3 as well as other unidentified proteins (Fig.1). We purified the complex and identified the components of the complex by proteomics analyses. The components include enzymes regulating energy metabolism, cytoskeletal protein, and proteins regulating translation. Out of these proteins, we focused on spliceosomal protein PRP19 as a component of the complex. PRP19 efficiently interacted with PHD3 under hypoxic conditions. PHD3-PRP19 interaction inhibited the caspase activation caused by PHD3, thus suppressed the cell death under hypoxic conditions. PHD3-PRP19 interaction was required for preventing the hypoxic cell death, since the PRP19 deletion mutant which lacks the PHD3 interaction domain did not show such activity.

PRP19 appears not to have any effect on HIF pathway. Therefore, we are currently characterizing this HIF-independent cell survival signal during hypoxia response further by utilizing microarray analyses.

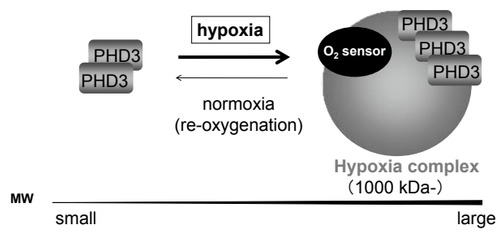


Fig.1 Characterization of Hypoxia Complex

Presentation at the meetings

Koh Nakayama
Regulation of hypoxic cell death by prolyl-hydroxylase PHD3 and PRP19
The 6th Network symposium of the Research Institutes, June 9th, Tokyo

Koh Nakayama
Hypoxic regulation of gene expression by Pre-mRNA processing factor (PRP) 19
The 84th Annual Meeting of the Japanese Biochemical Society, Sep. 22nd, Kyoto

Koh Nakayama
Regulation of matrix metalloproteinase *MMP1* gene expression under prolonged hypoxic conditions
The 34th Annual Meeting of the Molecular Biology Society of Japan, Dec 15th, Yokohama

Division of Pathophysiology

【Aim and Scope】

Research purpose of the Division of Pathophysiology is to understand the fundamental mechanisms underlying biological phenomena and their abnormalities in the disease conditions including intractable diseases and to develop new diagnostic or therapeutic tools for various diseases of which curative treatment is difficult. Topics of research projects in each Department are as follows;

【Neuropathology】

- Elucidation of a new cellular function of Ataxin-7, the causative gene for SCA7.
- Confirmation of therapeutic effect of Ku70 in Drosophila Huntington's diseases models.

【Pathological Cell Biology】

- Identification of a novel function of Beclin 1 in apoptotic cell clearance.
- Discovery of spatial coupling of catabolic and anabolic machinery (TASCC) in senescent cell.

【Developmental and Regenerative Biology】

- Elucidation of physiological roles of Stress-activated protein kinase MKK7 in the developing mouse cerebral cortex.
- Characterization of triglyceride and phospholipid species in regenerating mouse liver by Imaging mass spectrometry.

【Stem Cell Bilogy】

- Identification of an essential role of hair follicle stem cells for melanocyte stem cell maintenance by serving as niche cells.
- Identification of the roles of collagen XVII for pigmented hair growth through stem cell maintenance.

【Immunology】

- Elucidation of novel B lymphocyte tolerance mechanism that takes place in splenic marginal zone.
- Development of a novel method to efficiently introduce functional proteins into cells that are resistant to conventional methods (B cell receptor).

【Molecular Pathogenesis】

- Dilated cardiomyopathy-associated BAG3 mutations increased the sensitivity of cardiomyocytes to metabolic stress-induced apoptosis.
- ULBP4/RAET1, a ligand of NKG2D receptor, is highly polymorphic in Old World monkeys.

【Virus Research Unit】

- Establishment of a chronic active EBV infection model using NOG mice.
- Development of an exhaustive and quantitative pathogen microbes screening system capable of screening dozens of virus, bacteria and protozoa simultaneously.

Department of Neuropathology

Professor	Hitoshi Okazawa	Associate Professor	Kazuhiko Tagawa
Adjunct Lecturer	Nobuyuki Nukina, Masaki Sone, Toshiki Uchihara		
Assistant Professor	Takuya Tamura		
Project Assistant Professor	Hikaru Ito, Tsutomu Oka, Toshikazu Sasabe, Chisato Yoshida		
Technicians	Tayoko Tajima, Chie Iinuma, Chiharu Mizoi, Unno		
Secretary	Reiko Kikuchi		
Graduate Students	Yoko Nakamura, Min Xu, Chan Li, Hong Zhang, Tomomi Imamura, Keisuke Kurosu		
Research Trainees	Ei Mou		

Research contents

Our research aims are: 1) to elucidate molecular mechanisms underlying neurodegenerative diseases and to develop effective therapeutic approaches based on the information obtained; 2) to uncover the mechanisms of mental retardation (MR) influenced by a key regulator of neurodegenerative diseases, PQBP1; 3) to study mechanisms of stem cell differentiation through characterization of a transcription factor, Oct-3/4. Progress along 1) in this year will be described in the following.

1) Elucidation of a new cellular function of Ataxin-7, the causative gene for SCA7

The spinocerebellar ataxia type 7 (SCA7) gene product, Ataxin-7 (ATXN7), localizes to the nucleus and has been shown to function as a component of the TFTC/STAGA transcription complex, although cytoplasmic localization of ataxin-7 in affected neurons of human SCA7 patients has also been detected. This year, we defined a physiological function for cytoplasmic ATXN7. Live imaging revealed that the intracellular distribution of ATXN7 dynamically changes and that ATXN7 distribution frequently shifts from the nucleus to the cytoplasm. Immunocytochemistry and immunoprecipitation demonstrated that cytoplasmic ATXN7 associates with microtubules (Fig.1), and expression of ATXN7 stabilizes microtubules against nocodazole treatment, while ATXN7 knock-down enhances microtubule degradation. Interestingly, normal and mutant ATXN7 similarly associated with and equally stabilized microtubules. Taken together, these findings provide a novel physiological function of ATXN7 in the regulation of cytoskeletal dynamics, and suggest that abnormal cytoskeletal regulation may contribute to SCA7 disease pathology.

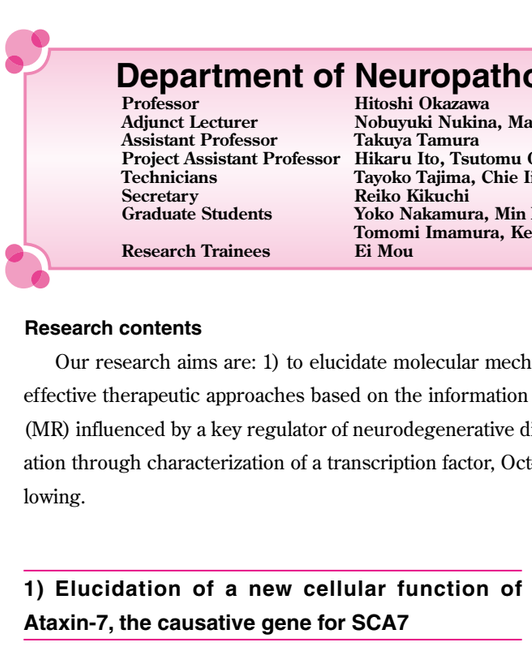


Fig.1.Normal and mutant ataxin-7 are co-localized with alpha-tubulin. Immunoprecipitation also supported interaction between ataxin-7 and alpha-tubulin.

2) Confirmation of therapeutic effect of Ku70 in Drosophila Huntington's diseases models

DNA damage accumulates in genome DNA during the long life of neurons, thus DNA damage repair is indispensable to keep normal functions of neurons. We previously reported that Ku70, a critical molecule for DNA double strand break (DSB) repair, is involved in the pathology of Huntington's disease (HD). Mutant huntingtin (Htt) impaired Ku70 function via direct interaction, and Ku70 supplementation recovered phenotypes of a mouse HD model (Enokido et al., JCB 2010). In this year, we generated multiple Drosophila HD models that express mutant huntingtin (Htt) in eye or motor neuron by different drivers and show various phenotypes. In such fly models, Ku70 co-expression recovered lifespan (Fig.2), locomotive activity and eye degeneration. In contrast, Ku70 reduction by heterozygous null mutation or siRNA-mediated knock down accelerated lifespan shortening and locomotion disability. These results collectively support that Ku70 is a critical mediator of the HD pathology and a candidate therapeutic target in HD.

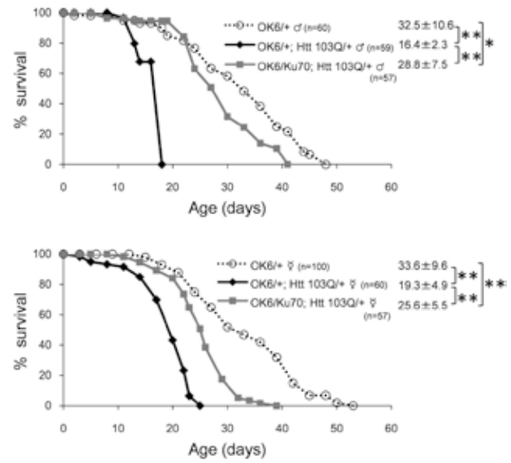
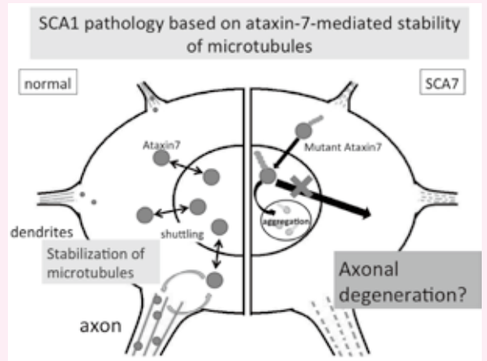


Fig.2. Co-expression of Ku70 recovers lifespan shortening of Drosophila expression mutant huntingtin in motor neurons by OK6-driver.

Highlight

Aggregation of mutant Ataxin-7 inhibits cytoplasmic localization of ataxin-7, which finally leads to instability of microtubules.



Publications

1. Oka, T., Tagawa, K., Ito, H. and Okazawa, H. (2011). Dynamic Changes of the Phosphoproteome in Postmortem Mouse Brains. ***PLoS One.*** 6, e21405. doi:10.1371/journal.pone.0021405
2. Tamura, T., Sone, M., Iwatsubo, T., Tagawa, K., Wanker, E.E. and Okazawa, H. (2011). Ku70 alleviates neurodegeneration in Drosophila models of Huntington's disease. ***PLoS One.*** 6, e27408. doi: 10.1371/journal.pone.0027408
3. Nakamura, Y., Tagawa, K., Oka, T., Sasabe, T., Ito, H., Shiwaku, H., La Spada, A.R. and Okazawa, H. (2012). Ataxin-7 associates with microtubules and stabilizes the cytoskeletal network. ***Hum Mol Genet.*** 21 (5): 1099-1110. doi: 10.1093/hmg/ddr539
4. Ress, M., Gorba, C., Gorba, C., de Chiara, C., Bui, T.T.T., Garcia-Maya, M., Drake, A.F., Okazawa, H., Pastre, A., Svergun, D. and Chen, Y.W. (2012). The solution model of the intrinsically disordered polyglutamine tract binding protein-1 (PQBP-1). ***Biophys J.*** in press

Department of Pathological Cell Biology

Professor	Shigeomi SHIMIZU
Junior Associate Professor	Akimitsu KONISHI
Tokunin Junior Associate Professor	Tatsushi YOSHIDA
Assistant professor	Satoko ARAKAWA
Tokunin Assistant Professor	Michiko MUROHASHI, Leishuku LI, Shinya HONDA, Yuya NISHIDA

This laboratory mainly focuses on understanding the molecular mechanisms of autophagy, programmed cell death, and the mitochondria-related diseases. We take biochemical, genetical, cellular biological approaches to elucidate them. The aims of this laboratory are to develop new strategies to control autophagy and cell death and to apply them to various diseases.

〈**Research Projects**〉

1, Discovery of Atg5/Atg7-independent alternative macroautophagy.

Macroautophagy is a process that leads to the bulk degradation of subcellular constituents through the creation of autophagosomes/autolysosomes. This mechanism is involved in the turnover of cytoplasmic components. Accumulating studies have shown that certain Atg genes, including Atg5, Atg6 (also called Beclin-1), and Atg7, are essential for induction of macroautophagy. However, recently we found that cells lacking Atg5 or Atg7 can still form autophagosomes/autolysosomes when subjected to certain stresses. Although lipidation of LC3 (LC3-II formation) is generally considered to be a good indicator of macroautophagy, it did not occur during the ATG5/ATG7-independent alternate macroautophagy. We also found that this alternative macroautophagy was regulated by several autophagic proteins, including Ulk1 and Beclin-1. In vivo, ATG5-independent alternate macroautophagy was detected in several embryonic tissues. These results indicate that mammalian macroautophagy can occur via at least two different pathways, which are an ATG5/ATG7-dependent conventional pathway and an ATG5/ATG7-independent alternate pathway. This year, we identified several molecules involving alternative macroautophagy and generated several different knockout mice.

2, Discovery of a novel function for Beclin1: regulation of the apoptotic cell engulfment.

Efficient apoptotic cell engulfment is important for both tissue homeostasis and immune response in mammals. Recently we found that Beclin 1 (a regulator of autophagy) is required for apoptotic cell engulfment. The engulfment process was largely abolished in Beclin 1-knockout cells (Fig. 1A). Beclin 1 was recruited to the early phagocytic cup along with the generation of phosphatidylinositol-3-phosphate and Rac1, which regulates actin dynamics in lamellipodia. No lamellipodia were formed in Beclin 1-knockout cells (Fig. 1B), and Beclin 1 knockdown completely inhibited the promotion of engulfment by ectopic expression of Rac1. Beclin 1 was co-immunoprecipitated with Rac1. These data indicate that Beclin 1 coordinates actin dynamics and membrane phospholipid synthesis to promote efficient apoptotic cell engulfment.

3, Discovery of special coupling of mTor and autophagic vacuoles.

Protein synthesis and autophagic degradation are regulated in an opposite manner by mTor, whereas under certain conditions it would be useful if they occurred with coupling. We discovered uncharacterized cellular compartment containing mTor and autolysosomes at the trans-side of Golgi apparatus during cellular senescence (Fig. 2A). We named this compartment "Tor-autophagy spatial coupling compartment (TASCC)". Both autophagic degradation and cytokine production were simultaneously occurred in TASCC (Fig. 2B).

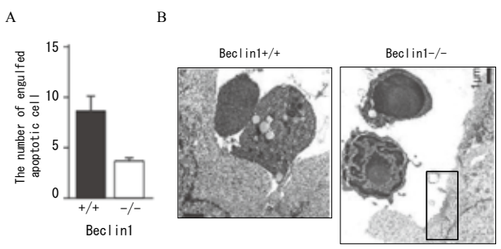


Fig.1 Inhibition of apoptotic cell engulfment by Beclin 1 –/– ES cells.
A. Apoptotic cell engulfment in Beclin 1 +/+ and –/– ES cells were quantified. Values represent mean ± SD (n = 3).
B. EM observation of apoptotic thymocyte engulfment by Beclin 1 +/+ and –/– ES cells. ES cells were incubated with apoptotic thymocytes for 3 hours and subjected to EM analysis. Arrows indicate apoptotic thymocytes. Arrowheads and box indicate polymerized actin filaments.

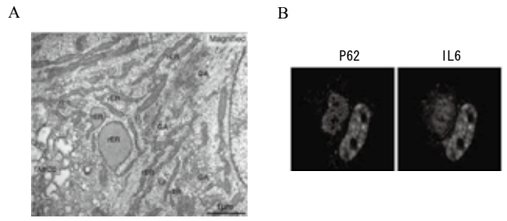


Fig.2 Discovery of special coupling of mTor and autophagic vacuoles.
A. "Tor-autophagy spatial coupling compartment (TASCC)" is localized at the trans-side of Golgi apparatus in the ras-senescent cell. GA, Golgi apparatus. Note highly enlarged rER with homogenous electron density are located near the TASCC.
B. Co-localization of autophagy (p62) and cytokine production (IL6) in TASCC.

4, Molecular mechanisms of programmed cell death

Programmed cell death is the essential mechanisms for selective elimination of cells. It plays an integral part in a variety of biological events, including morphogenesis and removal of harmful cells. Previously, apoptosis is considered as the only form of programmed cell death. Recent studies, however, have provided evidence that there is another mechanism of programmed cell death called non-apoptotic cell death. Therefore, in order to understand the role of cell death in multicellular organisms, it would be required to elucidate all the molecular mechanisms of cell death. Our final goal is to understand the role of programmed cell death in humans.

A, Analysis of apoptosis mechanism

This year, we searched a molecule regulating Bcl-2 function using yeast genetic screening method.

B, Analysis of autophagic cell death

Recently, we found that apoptosis-resistant cells die by autophagic cell death in response to various death stimuli. This year, we analyzed physiological role of autophagic cell death using apoptosis-resistant mice (manuscript submitting).

5, Analysis of mitochondrial diseases

Mitochondria play an essential role in both cell survival and cell death. In both cell function, mitochondrial membrane permeability is crucial. Therefore, we are focusing on channel proteins located on mitochondrial membrane. We are also focusing on mitochondrial diseases. The motor neuron degeneration 2 (mnd2) mouse is considered to be an animal model of Parkinson disease (PD). Mnd2 mice possess a non-functional missense mutation $^{Ser}276^{Cys}$ in the mitochondrial protease HtrA2/Omi. We are trying to prolong the life of these mice.

List of Publications

[Original paper]

1. Yoshioka Y, Shimizu S, Ito T, Taniguchi M, Nomura M, Nishida T, Sawa Y. p53 Inhibits Vascular Endothelial Growth Factor Expression in Solid Tumor. ***J Surg Res.*** *in press* 2011
2. Hikita H, Takehara T, Kodama T, Shimizu S, Shigekawa M, Hosui A, Miyagi T, Tatsumi T, Ishida H, Li W, Kanto T, Hiramatsu N, Shimizu S, Tsujimoto Y, Hayashi N. Delayed-onset caspase-dependent massive hepatocyte apoptosis upon Fas activation in Bak/Bax-deficient mice. ***Hepatology*** 54: 240-251, 2011.
3. Narita M, Young ARJ, Arakawa S, Samarajiwa SA, Nakashima T, Yoshida S, Hong SK, Berry LS, Reichelt S, Ferreira M, Tavaré S, Inoki K, Shimizu S, Narita M. Spatial coupling of mTOR and autophagy augments secretory phenotypes. ***Science*** 332: 966-970, 2011
4. Yamasaki T, Kawasaki H, Arakawa S, Shimizu K, Shimizu S, Reiner O, Okano H, Nishina S, Azuma N, Penninger JM, Katada T, Nishina H. Stress-activated protein kinase MKK7 regulates axon elongation in the developing cerebral cortex. ***Journal of Neuroscience*** 31: 16872-16883, 2011
5. Konishi A, Arakawa S, Yue Z, Shimizu S. Involvement of Beclin 1 in the engulfment of apoptotic cells. ***J. Biol. Chem.*** in press

Department of Developmental and Regenerative Biology

Professor Hiroshi Nishina, Ph.D.
Associate Professor Jun Hirayama, Ph. D.
Assistant Professor Yoichi Asaoka, Ph.D.
Assistant Professor Tokiwa Yamasaki, Ph.D.
Assistant Professor Shoji Hata, Ph.D.
Assistant Professor Mamiko Iwatsuki, M.D. & Ph.D.

Our goal is to define the molecular basis for the mechanism of organ formation, regeneration, and maintenance using knockout mice and mutant fishes. To accomplish this goal, we have focused on defining signaling molecules and pathways that regulate liver formation and maintenance. Our study will provide new insights into understanding of the precise molecular mechanisms that underlie organ failures found in human diseases and will lead to the development of new rational therapy for the diseases.

1. Activation mechanism and physiological roles of stress-activated MAP kinase, SAPK/JNK

Stress-activated protein kinase (SAPK)/c-Jun NH_2-terminal kinase (JNK) is activated by many types of cellular stresses and extracellular signals. We showed that two JNK activators, MKK4/SEK1 and MKK7, are required for full JNK activation and fetal liver formation. MKK4-deficient embryos die between E10.5 and E12.5 with impaired liver formation and massive apoptosis. MKK7-deficient embryos die between E11.5 and E12.5 with similar defects in liver formation. These results indicate that MKK4 and MKK7 cannot substitute for one another in vivo and that both are important for hepatoblast proliferation and survival during mouse embryogenesis. Furthermore, we found that the Tyr and Thr residues of JNK are sequentially phosphorylated by MKK4 and MKK7, respectively. We propose the hypothesis that the strong or weak activation of SAPK/JNK is induced by MKK4 and MKK7 to regulate the cell fate.

2. Mutations affecting liver development and function in Medaka, Oryzias Latipes

The liver is an organ with vital functions, including processing and storage of nutrients, maintenance of serum composition, detoxification and bile production. Recently, several genes that are crucial for liver formation and function have been isolated in mice and confirmed by reverse genetics. Although a reverse genetic approach is powerful in characterizing function of known genes, knowledge of genes in liver formation and disease is still limited. Therefore, identifying mutations affecting these aspects will uncover genes required for these processes. Systematic forward genetic screens for mutations affecting liver formation and function such as hepatic bud formation, liver morphogenesis, bile color in the gall bladder, lipid metabolism, and liver laterality have been carried out in Medaka, *Oryzias latipes*. One of the mutations, *hio* exhibits a profound (but transient) defect in liver specification that resembles the liver formation defect found in the zebrafish *prometheus* (*prt*) mutant, whose mutation occurs in the *wnt2bb* gene. Positional cloning revealed that the *hio* mutation affects the *raldh2* gene encoding retinaldehyde dehydrogenase type2 (RALDH2), the enzyme principally responsible for retinoic acid (RA) biosynthesis. Interestingly, in *hio* mutants, expression of *wnt2bb* in the lateral plate mesoderm (LPM) directly adjacent to the liver-forming endoderm was completely lost. Our data reveal the unexpected finding that RA signaling positively regulates the *wnt2bb* gene expression required for liver specification.

3. A regulatory link between the circadian clock and photoreactivation in zebrafish

The circadian clock enhances the physiological efficiency and survival of an organism, and thus disruption of the circadian clock in humans has been linked to profound effects on health. We have used zebrafish as an attractive vertebrate model suitable for the examination of the light signaling pathway and its impact on the circadian clock. In zebrafish, an acute light pulse entrains oscillations of clock gene expression to a new light-dark cycle. Zebrafish have also been used to study the light-dependent triggering of DNA repair via photoreactivation. We reported that light stimulates the ERK/MAPK signaling pathway and then increases expression levels of the *z64Phr* gene associated with photoreactivation, as well as the *zCry1a* gene associated with the circadian clock. Thus, light-dependent DNA repair and the entrainment of the circadian clock are governed by shared regulatory pathways.

Highlight

Our group has generated *Mkk7*$^{flox/flox}$ *Nestin-Cre* mice. Unlike *Mkk4*$^{flox/flox}$ *Nestin-Cre* mice, which survive until age 3 weeks, *Mkk7*$^{flox/flox}$ *Nestin-Cre* mice die at birth without breathing. Like *Mkk4*$^{flox/flox}$ *Nestin-Cre* mice, JNK activation is reduced to 20% of normal in the developing brain of *Mkk7*$^{flox/flox}$ *Nestin-Cre* mutants, and a delay in neuronal migration in the cerebrum is observed. However, other phenotypes do not overlap between *Mkk7*$^{flox/flox}$ *Nestin-Cre* and *Mkk4*$^{flox/flox}$ *Nestin-Cre* mice. At E18.5, *Mkk7*$^{flox/flox}$ *Nestin-Cre* mice display enlarged brain ventricles, diminished striatum, decreased forebrain axon tracts, and reduced corticofugal axons; none of these defects has been found in *Mkk4*$^{flox/flox}$ *Nestin-Cre* mice. In addition, ultrastructural alterations such as abnormal accumulations of filamentous structures and autophagic vacuoles are observed in *Mkk7*$^{flox/flox}$ *Nestin-Cre* brain but not in *Mkk4*$^{flox/flox}$ *Nestin-Cre* brain. Thus, *Mkk7* has unique functions in the developing brain that differ from those of MKK4. Differences between MKK7 and MKK4 functions also appear at the molecular level. In *Mkk4*$^{flox/flox}$ Nestin-Cre brain, phosphorylation levels of MAP1B are reduced but DCX phosphorylation is not altered. In contrast, phosphorylation levels of both MAP1B and DCX are decreased in *Mkk7*$^{flox/flox}$ *Nestin-Cre* brain, suggesting that the MKK7-JNK and MKK4-JNK signaling modules in this organ are not identical. In line with this hypothesis, the scaffold protein JIP1 binds to JNK, MKK7 and DCX but not to MKK4. We therefore propose that differences in scaffold proteins and/or substrates involved in the MKK7-JNK versus MKK4-JNK pathways could cause the phenotypic divergence observed between *Mkk7*$^{flox/flox}$ Nestin-Cre and *MKK4*$^{flox/flox}$ *Nestin-Cre* mice (Fig.1).

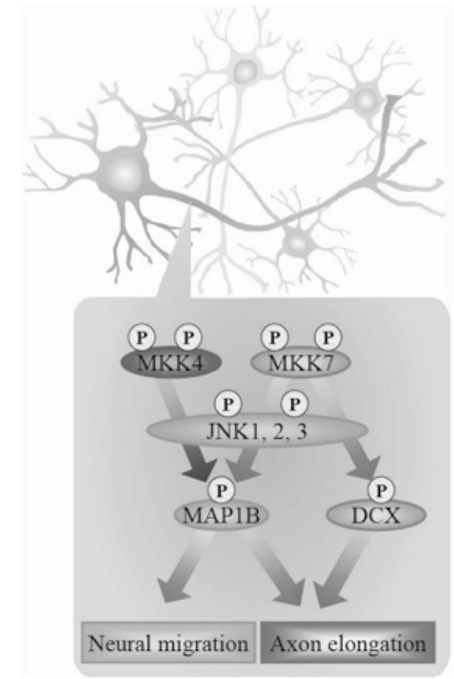


Fig.1. MKK4 and MKK7 have different functions in the developing brain.

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Department of Stem Cell Medicine

Professor	**Emi K. Nishimura, M.D., Ph. D.**
Assistant Professor	**Takahiro Aoto, D.V.M., Ph.D.**
Assistant Professor	**Hiroyuki Matsumura, Ph. D.**

Stem cell systems play fundamental roles in tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue homeostasis driven by stem cell systems and to apply the knowledge to better understand the mechanisms underlying specific tissue decline, cancer development and other diseases associated with ageing. We further aim to apply this knowledge to regenerative medicine using somatic stem cells and the treatment of cancer as well as other age-associated diseases.

1) Identification of stem cells in the skin.

The skin is the largest organ in the body. Hair follicles in the skin constantly renew themselves by alternate phases of growth, regression and rest. During this process, mature melanocytes (pigment cells) in hair follicles are replaced by a new cell population every hair cycle. We previously identified the source of those melanocytes, "melanocyte stem cells" (MCSC), which are located in the hair follicle bulge and supply mature melanocytes required for hair pigmentation (Nishimura EK et al. Nature 2002). We are currently trying to identify melanocyte stem cells in hairless areas of skin. This year we discovered an unprecedented melanocytic population in the mouse footpad skin. We are currently trying to identify the population and test whether those cells satisfy the criteria for somatic stem cells.

2) Mechanisms of stem cell maintenance

To understand the mechanisms underlying MCSC maintenance, we hypothesized that the hair graying phenotype is caused by incomplete maintenance of MCSCs. To test this, we took advantage of *Bcl2* deficient mice and *Mitf-vit* mutant mice, both of which show irreversible hair graying phenotypes. *Mitf* encodes a transcription factor of the bHLH Zip type and is known as a master regulator of melanocyte development and *Bcl2* is one of the target genes of MITF. We found that defective maintenance of MCSCs underlie the hair graying phenotype in both *Bcl2* deficient and *Mitf-vit* mutant mice. In other words, these findings reveal that *Mitf* and *Bcl2* are essential intrinsic genes involved in MCSC maintenance to prevent hair graying (Nishimura EK et al. Science 2005) (Fig.1).

While we previously found that the niche microenvironment plays a dominant role in MSC fate determination (Nishimura EK et al. 2002), the identity of the niche cells and the underlying molecular mechanisms have been largely unknown. Melanocyte stem cells (MCSC) and hair follicle stem cells (HFSC), which are originally derived from a completely different developmental origin, are located in the bulge area of mammalian hair follicles. Our recent study published in *Cell Stem Cell* revealed that HFSCs provide a functional niche for MCSCs through transforming growth factor β (TGF-β) signaling to prevent premature hair graying. To explore the

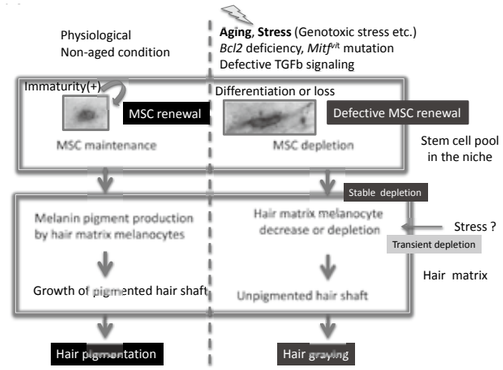

Fig.1

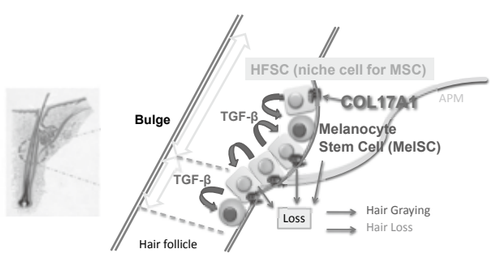

Fig.2

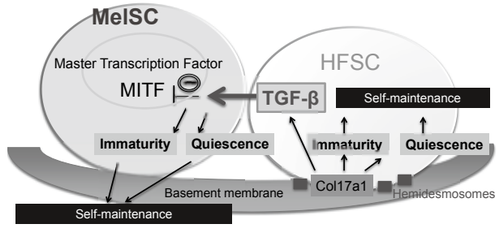

Fig.3

roles of HFSCs as niche cells, we have focused on Collagen XVII (Col17a1/BP180/BPAG2), a hemidesmosomal transmembrane collagen and transforming growth factor β1/2 (TGF-β1/2), both of which are preferentially highly expressed by HFSCs. First, to examine the possible involvement of these two molecules in MCSC maintenance, we analyzed deficient mice of *Col17a1* gene and *Tgfbr2* gene. *Tgfbr2* null mice show progressive hair graying but not hair loss, while *Col17a1* deficient mice show premature hair loss as well as premature hair graying. Analysis of HFSCs and MCSCs of the *Col17a1* null mice showed that *Col17a1* is critical for maintenance not only of HFSCs but also of MCSCs, which do not express *Col17a1* but directly adhere to HFSCs through maintaining their quiescence and immaturity (Fig.2). This potentially explains the mechanism underlying hair loss in human *COL17A1* deficiency. Interestingly, *Col17a1*-deficient mice show defective TGF-β production by HFSCs. TGF-β signaling is activated in MCSCs when they reenter the quiescent non-cycling state during hair cycles. Therefore, we analyzed MCSCs in conditional *Tgfbr2* deficient mice in which *Tgfbr2* gene that encodes TGF-β type II receptor is knocked out specifically in the melanocyte lineage and found that *Tgfbr2* is essential for the maintenance of MCSC immaturity and quiescence to prevent hair graying[3]. These data indicate that HFSC-derived TGF-β is a critical niche factor that regulates MSC immaturity and quiescence. Finally, forced expression of *COL17A1* in basal keratinocytes, including HFSCs, in *Col17a1* null mice rescues MCSCs from premature differentiation and restores TGF-β signaling, demonstrating that HFSCs function as a critical regulatory component of the MSC niche through TGF-β signaling (Fig.3). The interactions between different lineages of stem cells thus turned out to be crucial for cyclic regenerative growth of pigmented hair and point to a complex but efficient crosstalk in stem cell niches. The maintenance of somatic stem cell populations by another type of somatic stem cells in a coherent cell mass with might be a recurring strategy for somatic stem cell maintenance.

3) Mechanisms for MSC ageing and quality control of stem cell pools.

Physiological hair graying is the most obvious outward sign of aging even in normal mammals. We previously demonstrated that physiological hair graying is caused by incomplete self-renewal/maintenance of MSCs (Nishimura EK et al. 2005) (Fig.1). However, it was still not known what causes the self-renewal of MSCs to become defective during the course of ageing. We recently found that genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. Stem cell differentiation but not stem cell apoptosis nor senescence turned out to be the major fate of MSCs under irreparable/excessive genotoxic stress or with ageing. Our findings indicated that a "stem cell renewal checkpoint" exists to maintain the quality of the melanocyte stem cell pool (Inomata K., Aoto T. et al. Cell 2009) (Fig.1).

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Invited lecture/presentation at international meetings

1. Emi K. Nishimura: Stem Cell Regulation by Stem Cel: 21th International Pigment Cell Conference(IPCC):(Bordeaux, France) September 22th, 2011
2. Emi K. Nishimura: Hair Follicle Stem Cell Provide a Functional Niche for Melanocyte Stem Cells: ISSCR 9th Annual Meeting: (Tronto, Canada), Jun 16th, 2011

Presentation at international meetings

1. Takahiro Aoto, Natsuko Okamoto, Yoshiki Miyachi, Emi K.Nishimura : Identification of eccrine gland melanocyte stem cells in mouse acral Melanoma : ISSCR 9th Annual Meeting:(Tronto, Canada), Jun 16th, 2011
2. Natsuko Okamoto, Takahiro Aoto, Yoshiki Miyachi, Emi K.Nishimura : Identification of eccrine gland melanocyte stem cells in mouse acral skin as a potential source of acral melanoma : The 9th Stem Cell Research Symposium:(Tokyo) May 13th, 2011

Laboratory of Immunology School of Biomedical Sciences
Department of Immunology Medical Research Institute

Professor	Takeshi Tsubata, M.D., Ph.D.
Associate Professor	Takahiro Adachi, Ph.D.
Assistant Professor	Kozo Watanabe, Ph.D.
Assistant Professor	Naoko Matsubara, Ph.D.
	Yusuke Kishi, Ph.D.

We are currently conducting the following four projects. These projects are closely related each other.

1) Elucidation of the mechanisms for rapid activation of memory B cells, and drug development for host defense against infection by inducing rapid immune responses.
2) Elucidation of the mechanisms for selection of self-reactive B cells, and functional defects of B cells in autoimmune diseases
3) Cell biological study on B cell signaling
4) Elucidation of the mechanisms for the regulation of acquired immunity by glycan ligands, and development of novel methods to control acquired immunity by modulated glycan ligands.

1. A novel tolerance mechanism that tolerizes pathogenic B cells reactive to the RNA-related Sm antigen

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases, and characterized by production of autoantibodies to various nuclear components such as anti-DNA antibody. The autoantibodies to RNA-associated antigens such as the Sm antigen are demonstrated to be pathogenic in SLE. We demonstrated that anti-Sm B cells are deleted by a novel tolerance mechanism distinct from those tolerize other self-reactive B cells including anti-DNA B cells (See Highlights). This finding may enhance our understaning of the pathogenesis of SLE, and contribute to the development of new therapies for SLE.

2. Cationic nanogel-mediated delivery of proteins to cells that are resistant to protein delivery by conventional methods

Fusion proteins containing protein transduction domain (PTD) are widely used for intracellular delivery of exogenous proteins. PTD-mediated delivery requires expression of heparan sulfate on the surface of the target cells. However, some of metastatic tumor cells and primary lymphocytes poorly express heparan sulfate. To address protein delivery to cells with low heparan sulfate, we mixed GFP protein with nanosize hydrogels formed by cationic cholesteryl group-bearing pullulans (cCHP) and then cells were incubated with this complex. By this method, GFP protein is efficiently delivered to both myeloma cells and mouse primary lymphocytes probably by induction of macropinocytosis although these cells are resistant to PTD-mediated protein delivery as a consequence of poor heparan sulfate expression. To regulate function of the cells expressing poor heparan sulfate, we delivered anti-apoptotic protein Bcl-xL by cCHP. By Bcl-xL protein delivery with cCHP, primary CD4+ T cells and myeloma cells showed markedly suppressed staurosporine-induced apoptosis, establishing functional regulation of cells by proteins delivered by cCHP. In addition, cCHP showed prominent efficiency in cell function regulation compared with recently developed other type of tested protein delivery reagents, cationic liposome and a PTD-based amphiphilic peptide carrier. Thus, cCHP nanogel is a useful tool to deliver proteins for development of new cancer therapy and immune regulation (Fig.1). This study was done in collaboration with Prof. Akiyoshi.

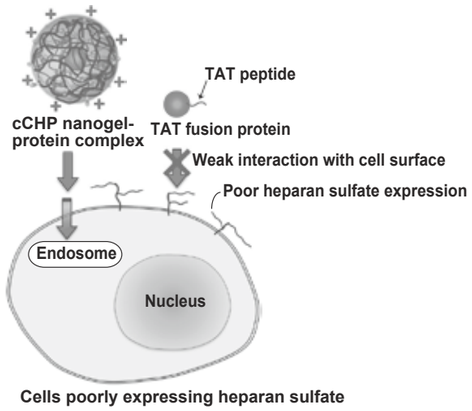

Fig.1. Schematic illustration of protein delivery by cCHP and TAT-peptide in cells poorly expressing heparan sulfate.

3. Regulation of B cell activation by membrane-bound lectins.

Various lectins are expressed on immune cells, suggesting that glycans play crucial roles in the regulation of immune responses. However, the roles of glycans are not well understood in acquired immune responses. We are assessing the roles of glycans in antibody responses by focusing on membrane-bound lectins that regulate antibody responses. CD22 is one of such lectins expressed primarily in B cells. CD22 recognizes sialic acid, and regulates B cell signaling. As CD22 regulates time course of antibody responses, which is crucial for infection immunity, we developed in collaboration with Professors Ishida and Kiso (Gifu University) modified sialic acids that bind to mouse CD22 with high affinity (Abdu-Allah et al. 2011). Such compounds may be useful for drug development for immune regulation.

Highlight

Novel tolerance mechanism for self-reactive B cells that are pathogenic in SLE

B cells are generated in the bone marrow, and migrate to the peripheral lymphoid organs such as spleen and lymph nodes after maturation. When self-reactive B cells are generated in the bone marrow, they are immediately deleted or inactivated leading to maintenance of self-tolerance. Such tolerance mechanisms are called central tolerance and are already well characterized.

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases, and characterized by production of autoantibodies to various nuclear components including DNA and histone. The autoantibodies to RNA-associated antigens such as the Sm antigen are demonstrated to be pathogenic in SLE. Although anti-DNA B cells were shown to be inactivated or deleted by central tolerance mechanisms, little is known about how anti-Sm B cells are tolerized. We demonstrated that anti-Sm B cells somehow escape central tolerance mechanisms, and migrate to splenic marginal zone, where they undergo apoptosis. We further demonstrated that apoptosis of anti-Sm B cells in spleen is reversed by excess CD40L (Fig.2), which is often found in SLE. Thus, our finding demonstrates the crucial role of the B cell self-tolerance in splenic marginal zone in preventing development of SLE, and strongly suggests that excess CD40L contributes to the pathogenesis of SLE by perturbing this novel tolerance mechanism for anti-Sm B cells.

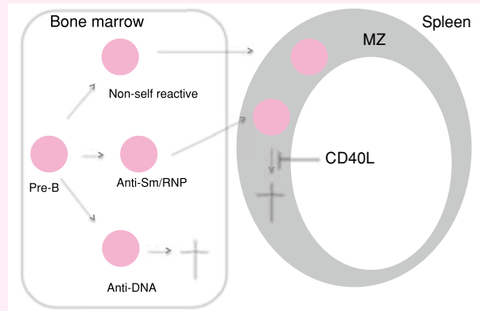

Fig.2. Novel tolerance mechanism for anti-Sm B cells.
Anti-DNA B cells are deleted in the bone marrow. In contrast, anti-Sm B cells that play a crucial role in development of SLE somehow escape the tolerance in the bone marrow, migrate to the splenic marginal zone, and undergo apoptosis. Apoptosis of anti-Sm B cells is reversed by excess CD40L, which is often seen in SLE.

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Division of Pathophysiology
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Assistant Professor	Takuro Arimura, D.V.M., Ph.D.
Research Associate	Taeko Naruse, Ph.D.

Genetic factors, i.e. functional diversity of human genome mainly due to mutations or polymorphisms, are less involved in the etiology and pathogenesis of human diseases. Contribution of gene mutations in the disease development is quite large in monogenic diseases showing simple Mendelian inheritance, while multiple gene mutations along with environmental factors are involved in the pathogenesis of multi-factorial diseases. Our goal is to decipher the disease-related genes for intractable or incurable diseases for which etiology and/or pathogenesis are not fully understood and, in addition, to develop novel therapeutic or preventive strategies for the intractable diseases based on the knowledge of disease-related genes.

1. Molecular mechanisms for hereditary cardiomyopathy

Gene mutations cause hereditary cardiomyopathy including hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). This year we systematically searched for mutations in the genes for sarcomere contractile elements and found mutations in about 47% of familial HCM patients. Distribution of HCM-causing mutations was largely different in geographic regions of Japan. In addition, we have revealed that BAG3 gene mutations cause DCM via impairing assembly of sarcomere Z-discs and increased sensitivity to metabolic stress-induced apoptosis (see Highlight).

2. Molecular mechanisms for atherosclerosis

A genome-wide screening of loci for myocardial infarction (MI) identified a promoter SNP of MKL1, which induced higher expression, as the disease gene for coronary artery disease (CAD). We generated transgenic mouse lines expressing human MKL1 under the CD68 promoter. In addition, because it was reported that periodontitis is a risk factor for vascular diseases including atherosclerosis and Burger disease, we investigate polymorphisms in the genes involved in innate immunity, MYD88 and TLR2, in various disease, we found that they were associated with Buerger disease and aggressive periodontitis, respectively.

3. Molecular mechanisms for arrhythmia

We revealed that SCN5A mutations found in patients with idiopathic ventricular fibrillation was associated with early reporalization.

4. Analysis of MHC in human and old world monkeys

We revealed that the MHC class I genes of Crab-eating macaques, Mafa-A and Mafa-B, consisted of specific haplotypes with multiple alleles on the same chromosomes. Of note was that we found unique haplotypes containing two major Mafa-A alleles. In addition, NFKBIL1 gene was found to regulate alternative splicing of human and viral genes, which may be involved in the immunological host-pathogen interaction.

5. Genome diversity in association with HIV/AIDS

We are investigating polymorphisms in several immune-related genes in association with the susceptibility/resistance to HIV/AIDS in humans and response to SIV vaccine in primate models. We found that individual difference in response to SIV vaccine was in part controlled by genome diversities in MHC and MHC-related molecules.

Highlight

By candidate gene approach, we identified BAG3 mutations in patients with dilated cardiomyopathy (DCM). Introduction of wild-type or mutant BAG3 gene in rat primary cardiomyocytes revealed that DCM-associated mutations, R218W and L462P, impaired sarcomerogenesis, whereas such abnormality was not induced by myofibrillar myopathy-associated mutation, P209L, or a disease-non-associated polymorphism, R258W (Fig.1).

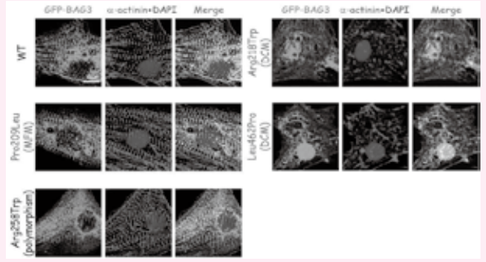

Fig.1. DCM-associated BAG3 mutations impaired the sarcomerogenesis
GFP-tagged BAG3 gene of wild-type or mutant was introduced into primary rat cardiomyocytes. GFP signals indicate the localization of BAG3 in cardiomyocytes which were stained for Z-discs (alpha-actinin) and nuclei (DAPI). Note that BAG3s with DCM-associated mutations were accumulated in nuclei.

In addition, the DCM-associated BAG3 mutations induced apoptosis of rat cardiomyocytes. To validate the effect of BAG3 mutation in apoptosis, wild type of mutant BAG3 gene was introduced into H9c2 cells and it was found that doxorubicin-induced apoptosis was specifically increased by the DCM-associated BAG3 mutations (Fig.2).

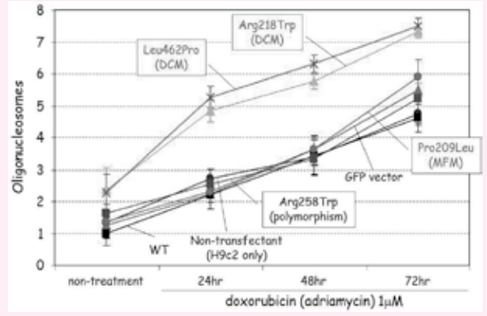

Fig.2. Susceptibility to doxorubicin-induced apoptosis was increased by DCM-associated BAG3 mutations in H9c2 cells.
Wild type or mutant BAG3 construct was introduced into H9c2 cells and the cells were treated with doxorubicin for 24, 48, and 72 hrs. Amounts of oligosomes were quantified as indicatives of apoptosis.

Publications

(#; equal contribution, *; corresponding author)

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Frontier Research Unit
Virus Research Unit

Associate Professor　Norio Shimizu, PhD

The goals of our research unit are: the elucidation of the development mechanism of Epstein-Barr virus (EBV) infection, the employment of immunodeficiency animals for the creation of virus research models and development of an exhaustive pathogenic microbial screening system.

1. Development of novel EBV infection animal models using the NOG mice

a) The functional human immune system is reconstituted in NOD/Shi-*scid*/IL-2R γ null(NOG) mice that receive hematopoietic stem cell transplants. The NOG mouse is the most comprehensive small-animal model of EBV infection described to date and should facilitate studies of the pathogenesis, prevention, and treatment of EBV infection.
b) We developed a xenograft model of chronic active EBV infection (CAEBV) by transplanting patient's PBMC to NOG mice. In this model, EBV-infected T or NK cells proliferate and infiltrate major organs showing histological characteristics of CAEBV.

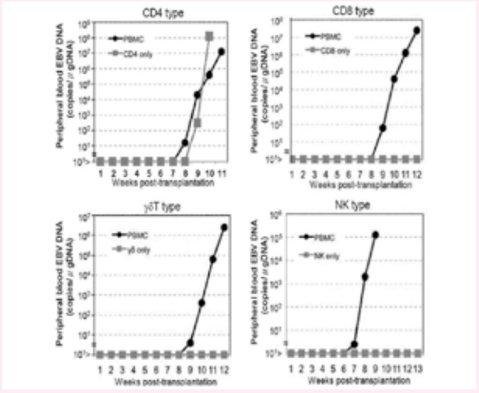
2. Development of an exhaustive pathogenic microbe screening system

We aim to establish an exhaustive pathogenic microbe screening system. We have modified our exhaustive virus screening system (capable of screening dozens of viruses simultaneously) which is currently under development so that in addition to viruses, it can also detect various other kinds of pathogens such as bacteria and protozoa. Other goals are to improve the sensitivity of the viral screening system and put it to practical use by conducting clinical microbiological investigations.

Highlight

Developent of a xenograft model of CAEBV using NOG mice

Engraftment of EBV-infected T (CD4, CD8, $\gamma\delta$) or NK cells in NOG mice following transplantation with PBMC of patients with CAEBV. The EBV DNA levels in the peripheral blood of recipient mice were measured by real-time PCR analysis.



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Division of Medical Genomics

【Medical Genomics】

Over the last decade, remarkable strides have been made in human genome science. The complete human genome sequences have been available through the public databases, and many high throughput technologies have been developed in the human genome project. In the post-sequence era we have excellent opportunities to identify genetic or epigenetic changes that are responsible for diseases. The missions of the Division of Medical Genomics are to understand genomic, epigenomic and proteomic changes underline the initiation and progression of human disease, and to elucidate the pathogenesis of intractable diseases. Our goal is to translate our research discoveries into the clinical setting.

【Molecular Cytogenetics】

The principal aim of our department is to understand the molecular basis underlying cancer and genetic diseases including chromosome aberration syndromes. In 2009 we contributed as follows;

1. Identification of novel genes responsible for cancer and unknown genetic diseases by an integrative genomic and epigenomic approach.
2. Development of practical tools for diagnosis in personalized medicine of cancer and genomic disorders in the clinical setting.
3. Exploring molecular targets for the development of drugs in personalized cancer medicine.

【Biochemical Genetics】

Our lab is focusing on basic transcriptional mechanism and its biological role in pathogenesis of human disease.

1. Genome-wide system biology of stress response transcription factor ATF3 in human cancer and p53/Atf3 knockout mice revealed novel ATF3 targets involved in cancer therapy.
2. Role of FCP1 or Elongin A in transcription cycle was studied.
3. Biological role of histone methyltransferase ASH1L was investigated.

【Molecular Genetics】

Our research is directed at understanding the molecular mechanisms of apoptosis in response to DNA damage and genome stability through DNA damage repair in breast cancers.

1. Identification of human nonmuscle myosin heavy chain (NMHC) IIC as the BRCA2-associated protein.
2. Regulation of Mdm2, negative regulator of p53, by PKCdelta in the apoptotic response to DNA damage.
3. Identification of Evi-1 as a novel effector of PKCdelta in the apoptotic response to DNA damage.

【Molecular Epidemiology】

1. We identified that Fucosyltransferase 8 (*FUT8*) gene Thr267Lys polymorphisms is associated with pulmonary emphysema by employing pathological and clinical samples.
2. Using genome-wide DNA methylation analysis we revealed that genistein, a phytoestrogen product of soy, affects promoter methylation patterns during embryonic stem cell differentiation, *in vitro*.
3. We have shown that oral administration of genistein to ovariectomized (OVX) mice induced hypomethylation and transcription activation of steroid factor-1 (*sf1*) promoter in endometrial cells, leading to proliferation of the endometrium.

【Functional Genomics】

【Epigenetics】

1. We have identified *SIRH12* (sushi-ichi retrotranspson homologue 12) gene that is derived from a marsupial-specific domestication event. *PEG10/SIHR1* is conserved in both eutherians and marsupials but *PEG11/SIRH2* and *SIRH3-SIRH11* are eutherian-specific genes. It is now clear that the eutherians and the marsupials have different sets of *SIRH* genes probably contributed to diversification of these two groups of mammals.
2. We have succeeded the efficient production of somatic cloned mice with *Xist* knockdown method by collaboration with Dr. Ogura's group at RIKEN BioResource Center. It is a great step toward the practical use of the somatic cloning method in many domesticated animals.
3. Intracytoplasmic sperm injection (ICSI), one of the human assisted reproductive technologies (ART), has long raised concerns about its influence on development. Pre- and postnatal effects of ICSI were assessed using comprehensive transcriptome and phenotypic analyses in mice under strict conditions. We have demonstrate that, in contrast to *in vitro* fertilization (IVF), ICSI induces distinct long-lasting transcriptome change that remains at the neonatal stage.

【Bioinformatics】

1. In order to identify predictive factors for HCC recurrence in patients with early HCC, we have been conducting a joint research with Department of Hepato-Biliary-Pancreatic of Surgery of TMDU. Our analysis revealed that expression of CYP1A2 in non-cancerous tissues was strongly correlated with gene sets associated with peroxisome function.
2. By comparing the olfactory receptor gene repertories among five primate species, we showed that the hypothesis that the loss of olfactory receptor genes occurred due to the acquisition of the full trichromatic vision was not supported.
3. We constructed i2b2 database with 392 clinical patients' data collected in the university hospital of Tokyo Medical and Dental University. We also transferred 8,580 English and 54,579 Japanese descriptions into i2b2.
4. We sequenced 142 *env* genes collected from Japanese patients infected with HIV-1 in the 1980s and in the 2000s and examined the diversity change and potential adaptive evolution of the virus. The results indicate that it might have become easier for the HIV to infect a new host and to develop into AIDS now than 20 years ago.
5. We developed a novel algorithm to decompose a large complex network into several small sub-networks. By using the algorithm, we decomposed the human PIN into several small simple sub-networks and then mapped drug-target genes on to the sub-networks. We found that a sub-network contains majority of drug targets.
6. We have been conducting systems pathology studies on cancer, metastasis (epithelial-mesenchymal transition: EMT), and neurodegenerative disease (Alzheimer's disease) using large-scale molecular biology data, so-called omics data.

Division of Medical Genomics
Department of Molecular Cytogenetics

Professor	Johji Inazawa M.D., Ph.D.
Associate Professor	Ken-ichi Kozaki D.D.S., Ph.D.
Tokunin Lecturer	Shin Hayashi M.D., Ph.D.
Assistant Professor	Jun Inoue Ph.D.

The principal aim of Department of Molecular Cytogenetics is to understand the molecular mechanism underlying cancer and genetic diseases including multiple congenital anomalies and/or mental retardation (MCA/MR). Our research interests are as follows; (1) Identification of genes responsible for cancer and unknown genetic diseases, (2) Development of innovative techniques for detection of genomic and epigenomic aberrations underlying the pathogenesis of cancer and genomic disorders, and (3) Establishment of practically useful modalities for diagnosis and therapy in personalized medicine in cancer and other intractable diseases. It is our goal to bridge the gap between basic and clinical research for the benefit of each of the patients.

1. Identification of genes responsible for cancer by integrative genomics and epigenomics

For the last decade we performed Comparative genomic hybridization (CGH) analysis in over 2000 cases of various types of cancer and cell lines, and we constructed CGH database that is available through the internet (http://www.cghtmd.jp/cghdatabase/index.html). Through those CGH analyses we detected a number of novel and nonrandom amplifications in various tumors and identified target genes within the amplicons, such as GASC1 (Gene Amplified in Squamous Cell Carcinoma 1) and *cIAP1* in esophageal squamous cell carcinomas (ESCs), respectively. The former is a demethylase for tri- or dimetylated lysine 9 on histone H3. Some of them are being now focused as the target for the molecular target therapy.

2. Identification of cancer-related micro RNAs

MicroRNAs (miRNAs) have started a revolution in molecular biology and emerged as key players in the cancer process. We successfully identified novel tumor-suppressive miRNAs (TS-miRNAs), *miR-137* and *miR-193a* or *miR-124* and *miR-203*, as an intergenic TS-miRNA frequently silenced by tumor-specific DNA hypermethylation using expression-based screening in oral squamous cell carcinoma (OSCC) or methylation-based screening in hepatocellular carcinoma (HCC), respectively, and also revealed these mechanisms including these targets. Based on these studies, we have considered that the tumor-specific DNA hypermethylation of CpG islands located immediately 5'-upstream of miRNA genes is a useful landmark to explore novel TS-miRNAs silenced epigenetically in cancer cells, similar to classical tumor suppressor genes. Recently, by using function-based screening of candidate TS-miRNAs, we identified *miR-218* and *miR-152* as intragenic TS-miRNAs silenced through CpG island hypermethylation in OSCC and endometrial cancer (EC), respectively. Moreover, our studies showed that *Rictor* was a novel direct target of these two TS-miRNAs, *miR-218* and *miR-152*. Rictor, together with mTOR, forms mTOR complex 2 (mTORC2), and the Rictor-mTOR complex directly regulates the phosphorylation of Akt at Ser-473, resulting in cell growth.

3. Cancer pathogenesis relevant to impaired autophagy

Neuroblastoma (NB) is a malignant tumor consisting of undifferentiated neuroectodermal cells from the neural crest and the most common solid tumor in children.. We demonstrated that LAPTM5 (lysosomeal-associated protein multispanning membrane 5) was localized in intracellular vesicles and the accumulation of LAPTM5-positive vesicles was closely associated with cell death with the impaired autophagy during tumor regression of NB. We recently found that the expression level of LAPTM5 protein was negatively regulated through ubiquitination by ITCH, an E3 ligase, and the inhibition of ITCH expression enhanced the LAPTM5-mediated cell death in NB cells. The impairment of autophagy contributes to tumorigenesis. We recently found that the human LC3 microtubule-associated protein 1 light chain 3 (MAP1LC3: LC3) gene family consists of five members, LC3A (variant-1: v1 and -2: v2), LC3B, LC3B2, and LC3C, and LC3Av1 was also associated with autophagy as well as LC3B. Interestingly, LC3Av1 was frequently inactivated at the transcriptional level in various human cancer cell lines and its inactivation was due to aberrant DNA methylation in ESC cell lines and primary tumors, suggesting that the impairment of autophagy through inactivation of LC3Av1 may contribute to tumorigenesis.

4. Molecular cytogenetic investigation of MCA/MR

Array CGH (aCGH) is one of the most powerful tools to detect cryptic pathogenic copy number aberrations (pCNVs) in genomic disorders including multiple congenital anomalies and mental retardation (MCA/MR). We explored pCNVs in 646 patients with clinically uncharacterized MCA/MR, whose karyotypes showed normal according to conventional cytogenetics, for two-stage screening using two types of bacterial artificial chromosome-based microarray. The first screening using a targeted array detected pCNV in 69 of 646 cases (10.7%), whereas the second screening of the 515 cases negative in the first screening using a genome-wide high-density array detected pCNVs in 61 cases (11.8%), indicating the efficient application of aCGH in the clinical setting. Moreover, we performed aCGH with our in-house X-chromosome tiling array to explore pCNVs in 173 families having patient(s) with X-linked mental retardation (XLMR) of unknown etiology, and successfully detected pCNVs on chromosome X in 13 families (7.5%).

5. Construction of copy number variants (CNVs) database in healthy Japanese population

Recently we have constructed the MCG CNV Database, which provides copy number variants (CNVs) detected in 100 trios of healthy Japanese parents and one child by our in-house BAC arrays and SNP array (illumina). The MCG CNV Database (Fig.1) shows an incidence of CNVs in the Japanese healthy population and can be of assistance to estimate a pathogenicity of a CNV(s) detected in subjects having possible involvement of cryptic chromosome CNVs behind their pathogenesis.

Fig.1. The banner of MCG CNV database. http://www.cghtmd.jp/CNVDatabase

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Division of Medical Genomics
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Assistant professor	Katsuya Takenaka, Ph.D.
Assistant professor	Naoe Taira, Ph.D.

Our research is directed at understanding how cells recognize, assess and respond to lesions in DNA and the molecular mechanisms by which a damaged cell can distinguish between continued proliferation, permanent growth arrest and suicide. BRCAs, products of hereditary breast cancer genes, are associated with genome stability through DNA damage repair. We analyze functions of BRCAs and other related proteins to reveal the mechanism of breast carcinogenesis.

1. Functional analysis of the BRCA2 gene.

1) ATPase activity of non-muscle myosin IIC is regulated by BRCA2 in the midbody ring

The tumor suppressor gene BRCA2 is involved in homologous recombinational DNA repair that contributes to structural chromosomal stability. BRCA2 also participates in the regulation of centrosome and cytokinesis. BRCA2 is localized to the midbody during cytokinesis and interacts with the human non-muscle myosin heavy chain (NMHC) IIC. The biochemical activity of NMHC IIC originates from its actin-activated Mg ATPase activity. However, the specific function of BRCA2 in regulating the biochemical activity of NMHC IIC in the midbody is unclear.

To examine the function of BRCA2 in the regulation of NMHC IIC, we analyzed the effect of BRCA2 on the actin-activated Mg ATPase activity of NMHC IIC. BRCA2 and NMHC IIC were isolated by immunoprecipitation from BRCA2-FLAG or HA-NMHC IIC-transfected COS7 cells. The actin-activated Mg ATPase activity of NMHC IIC was analyzed by incubation of the immunoprecipitated HA-NMHC IIC in the presence and absence of BRCA2-FLAG. Mg ATPase was activated by the addition of BRCA2-FLAG in a dose-dependent manner. In contrast, Mg ATPase was not activated in the absence of BRCA2-FLAG. Based on these results, we suggest that Mg ATPase activity was likely to be caused by a complex formation between NMHC IIC and BRCA2.

Furthermore, we demonstrated that BRCA2 and NMHC IIC localized to the midbody ring (Flemming body) during cytokinesis. Time-lapse imaging and performed to reveal the ring structure showed that the midbody ring was dynamic and removed from side to side of the midbody. We hypothesized that the midbody ring is composed of NMHC IIC. To test this prediction, we attempted the *in vitro* reconstitution of the midbody ring using recombinant NMHC IIC. We expressed recombinant NMHC IIC-GFP in COS7 cells and placed a few drops of the cell lysates on a cover glass. The lysates were analyzed using high-resolution deconvolution microscopy, which demonstrated that NMHC IIC-GFP was a part of a unique ring-like structure, when both ATP and Mg^{2+} were added. The ring was 1.5-2.0 mm in diameter. However, in the presence of blebbistatin, which is an inhibitor of the myosin II ATPase activity, NMHC II failed to organize into a ring-like structure. The ring-like structure was also not observed in the presence of ATP or Mg^{2+} alone. Next, we co-expressed recombinant NMHC IIC-GFP and BRCA2-FLAG in COS7 cells and performed immunofluorescence microscopy using anti-GFP and anti-BRCA2 antibodies. The colocalization of the fluorescent signals derived from the anti-GFP and anti-BRCA2 antibodies suggested that the ring-like structure was composed of NMHC IIC-GFP and BRCA2. (Fig.1)

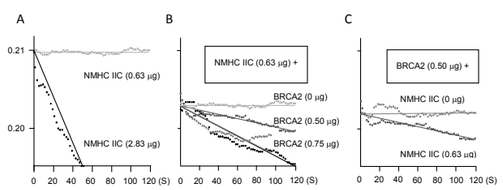

Fig.1. ATPase activity of non-musle myosin IIC is regulated by BRCA2

2. Regulatory mechanisms of tumor cells in the apoptotic response to DNA damage

1) PKCdelta regulates Mdm2 independently negative regulator of p53 in the apoptotic response to DNA damage.

Previous studies have demonstrated that PKCdelta transactivates p53 in response to DNA damage. These findings led us to determine if Mdm2, a nuclear phosphoprotein and negative regulator of p53, could also be a PKCdelta-modulated substrate. We discovered that inhibition of PKCdelta down-regulates Mdm2 protein expression regardless of p53 status. Given that Mdm2 mRNA change was detected in p53-proficient, but not -deficient cells, PKCdelta affected Mdm2 on the post-translational level. Interestingly, treatment of MG132 restored Mdm2 expression to the steady-state level. Further investigation showed that PKCdelta inhibited Mdm2 ubiquitination in p53-deficient cells and loss of PKCdelta resulted in an increase in Mdm2 proteosomal degradation. Moreover, P300/CBP-associated factor (PCAF), an ubiquitin ligase 3 for Mdm2, was observed to participate in Mdm2 ubiquitination by PKCdelta inhibition and knock down of PCAF rescued Mdm2 diminution. Finally, as shown for PKCdelta, Mdm2 was also required to exert pro-apoptotic response caused by genotoxic agents in p53-null cells. In addition, overexpression of Mdm2 restored inhibitory effect of apoptosis in cells silenced for PKCdelta. Taken together, we conclude that PKCdelta regulates Mdm2 expression distinctively of p53 pathway by affecting Mdm2 ubiquitination and maintenance of Mdm2 expression by PKCdelta is important to ensure normal genotoxic cell death response in human cancer cells.

2) Identification of Evi-1 as a novel effector of PKCdelta in the apoptotic response to DNA damage.

To explore the apoptosis mechanism that PKCdelta modulates, we sought to uncover transcription factor targets of PKCdelta by devising a screening strategy that utilizes ChIP-cloning and microarray analysis. Transcription factor candidates were generated with the application of public access data-mining tools and this resulted in the identification of Evi-1 as a novel PKCdelta-mediated DNA damage responsive molecule. The results demonstrated that PKCdelta is constitutively associated with Evi-1. PKCdelta regulated Evi-1 to activate PLZF transcription upon genotoxic stress. Furthermore, both Evi-1 and PLZF were associated with DNA damage-stimulated apoptosis. Taken together, we have discovered a novel regulation of Evi-1, which transactivates PLZF, by PKCdelta to induce cell death in response to genotoxic stress.

3. Analyses of molecular domains of translesion DNA polymerases by introducing a point mutation by homologous recombination in vertebrates.

A chicken B-lymphocyte line DT40 is characterized by a high efficiency of gene targeting and phenotypic stability. Utilizing this feature, we established a method to introduce a point mutation on genome to see functions of molecular domains of translesion polymerases *in vivo*. An *in vitro* study revealed that Pro1880 of Rev3 is responsible for the binding to Rev7, another Pol ζ subunit. We substituted Pro^{1880} to Phe by homologous recombination in DT40 cells. A cell line of *REV3* P1880F showed sensitivity to UV and cisplatin, an intrastrand crosslinker, whereas the sensitivity of *REV3* I1877A line, which must be unable to bind to Mad2, was the same as that of WT cells. The sensitivities of the P1880F line were not as strong as those of *REV3* knock-out cells. This raises the possibility either that other amino acid residues may also be responsible for the binding to Rev7 *in vivo*, or that Rev3 P1880F mutants may be able to bind to Rev7 weakly *in vivo*. Both hypotheses will be examined.

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Department of Molecular Epidemiology

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Many common chronic diseases are multifactorial in that they are caused by multiple genetic and environmental factors. By applying the technology and information of human genome to epidemiological studies, we aim to clarify the role of genetic polymorphisms, epigenetic changes, as well as their interaction with environmental factors, which may contribute to the development of these diseases.

1. Association of fucosyltransferase 8 (FUT8) polymorphism Thr267Lys with pulmonary emphysema

The fucosyltransferase 8 gene (FUT8) encodes an enzyme that transfers fucose to the innermost N-acetylglucosamine unit of N-glycan chains. Recent study showed that fut8-deficient mice develop pathological and physiological phenotypes resembling pulmonary emphysema (PE). The role of FUT8 in human PE is not known. A non-synonymous single-nucleotide polymorphism at the amino-acid position of 267 in FUT8 (rs35949016; C/A, C allele=Thr, A allele=Lys) was genotyped in a total of 1149 consecutive autopsies of elderly Japanese. A following study included 182 outpatients with chronic obstructive pulmonary disease, whose emphysematous changes were assessed quantitatively as the percentage of low attenuation area (%LAA) by high-resolution computed tomography. PE was detected in 163 of 1149 autopsy subjects (14.2%). Comparison of patient with vs without PE indicated that the FUT8 A allele was associated with PE (AA+AC vs CC; odds ratio=1.74, 95% confidence intervals=1.19-2.56, P=0.005). In the clinical study, presence of the FUT8 A allele significantly correlated with %LAA after adjustment (AA+AC vs CC=37.5 ± 14.7 vs 32.7 ± 13.9, P=0.02). The FUT8 gene Thr267Lys polymorphism is associated with human PE, and the Lys allele is the risk. The core fucosylation might be involved in the molecular pathogenesis of human PE.

2. Polymorphisms of the formylpeptide receptor gene (FPR1) and susceptibility to stomach cancer in 1531 consecutive autopsy cases.

Formylpeptide receptor (FPR1) is involved in inflammation, which is important in the pathogenesis of diverse conditions, including common diseases and cancers. To date, little is known about the relationships between FPR1 and such diseases, aside from the fact that FPR1 is related to periodontitis, which is implicated in systemic diseases such as stomach cancer. We hypothesized that FPR1 polymorphisms related to periodontal disease may confer susceptibility to stomach cancer. Two single nucleotide polymorphisms (SNPs) in the second extracellular region and C-terminus of the formylpeptide receptor gene were analyzed in 1531 consecutive autopsy cases in the Japanese elderly. The tri-allelic SNP of rs1042229 was detected by modified melting temperature analysis. Homozygous K alleles of rs1042229 were associated with stomach cancer (Odds ratio [OR]=1.62, confidence interval [CI]=1.05-2.48, p=0.028). In the analysis of the recessive model of the K allele, FPR1 was associated with a high risk of stomach cancer (OR=1.73, CI=1.15-2.55, p=0.0075). The risk allele for stomach cancer pointed in the same direction as periodontitis. This is the first study to evaluate polymorphisms of the FPR1 gene in stomach cancer to find a positive association between these polymorphisms and stomach cancer. Further studies on the relationship between stomach cancer and the FPR1 gene are warranted.

3. Association of the catechol-O-methyl transferase gene Val158Met polymorphism with blood pressure and prevalence of hypertension: interaction with dietary energy intake.

Previous studies of a functional variant of the catechol-O-methyl transferase (COMT) gene, Val158Met, have provided inconsistent results with regard to blood pressure or hypertension. We examined the effect of this variant, the considering environmental factors of daily salt and energy intakes. A total of 735 Japanese men (mean age, 47 years) were recruited from two separate occupational cohorts from Kanagawa and Kyoto prefectures. Participants were genotyped for the presence of COMT Val158Met (rs4680, G/A). Daily salt and energy intakes were evaluated by the food frequency questionnaire (FFQ). Met/Met carriers had higher adjusted systolic blood pressure (SBP) (+4.79 mm Hg, P < 0.001) and diastolic blood pressure (DBP) (+2.33 mm Hg, P = 0.001) than Met/Val or Val/Val carriers. There was a significant association between being a Met/Met carrier and having a higher prevalence of hypertension (odds ratio = 2.448, 95% confidence interval = 1.426-4.205, P = 0.001). When salt and energy intakes were dichotomized, the effect of Val158Met on hypertension was observed only in the high-energy intake group, and was equivalent between low- and high-salt groups. The Met allele of COMT Val158Met is associated with higher blood pressure and higher prevalence of hypertension in Japanese men, and energy intake may interact with this effect.

4. Genistein promotes DNA demethylation of the steroidogenic factor 1 (SF-1) promoter in endometrial stromal cells.

It has recently been demonstrated that genistein (GEN), a phytoestrogen in soy products, is an epigenetic modulator in various types of cells; but its effect on endometrium has not yet been determined. We investigated the effects of GEN on mouse uterine cells, in vivo and in vitro. Oral administration of GEN for 1 week induced mild proliferation of the endometrium in ovariectomized (OVX) mice, which was accompanied by the induction of steroidogenic factor 1 (SF-1) gene expression. GEN administration induced demethylation of multiple CpG sites in the SF-1 promoter; these sites are extensively methylated and thus silenced in normal endometrium. The GEN-mediated promoter demethylation occurred predominantly on the luminal side, as opposed to myometrium side, indicating that the epigenetic change was mainly shown in regenerated cells. Primary cultures of endometrial stromal cell colonies were screened for GEN-mediated alterations of DNA methylation by a high-resolution melting (HRM) method. One out of 20 colony-forming cell clones showed GEN-induced demethylation of SF-1. This clone exhibited a high proliferation capacity with continuous colony formation activity through multiple serial clonings. We propose that only a portion of endometrial cells are capable of receiving epigenetic modulation by GEN.

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Department of Biochemical Genetics Medical Research Institute and Genome Structure and Regulation School of Biomedical Science

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Scope of research :

Transcriptional regulation is one of the most important processes by which genome information is expressed from DNA to mRNA to protein. The faithful synthesis of mRNA is achieved by transcriptional machinery comprised of RNA polymerase II, basal factors and many other protein factors, whose dysfunction is implicated in various human diseases. Our research interest is focused on the basic mechanism of transcription cycle and implication of early response transcription factors in determining cell fate in stress response. We are also studying on the mechanism of cell cycle arrest of terminally differentiated cardiac cells and its re-activation to provide novel regeneration therapy.

Key words

・To provide novel paradigm of transcriptional regulation
・To understand role of transcription factor in cell fate determination

Research 1 : Transcription

Transcription proceeds from initiation,via elongation to termination, and eventually PolII is recycled for next rounds of transcription when gene expression is activated. Among many protein factors that regulate transcription cycle, TFIIF and Elongin A function during elongation phase and the dysregulation of Elongin A may cause cancer such as von Hippel-Lindau disease. FCP1, a TFIIF-associating CTD phosphatase, dephosphorylates CTD during the transcription cycle, and its deficiency causes a genetic disease CCFDN. We focus on these factors in order to understand the role in transcription cycle and their implication in human disease.

1-1 Elongin A plays dual roles in stress response

Elongin (Elongin ABC complex) is considered to have at least dual functions, one is activation of transcriptional elongation by RNA polymerase II (PolII), and another is the control of protein degradation since Elongin BC is a component of E3 ligase. By scanning stress response genes by chip analysis, Elongin A is recruited onto the HSP70 and ATF3 gene from their promoter through 3'-downstream region, showing it can associate with transcribing PolII. More importantly, Elongin A forms E3-ligase complex that target Pol II into ubiquitin-mediated degradation upon DNA damage. This is novel finding and is the first report to assign Elongin A as DNA-damage-inducible Pol II degradation gene. Elongin A may function as one of safety net mechanisms in mammalian cells.

1-2 Crosstalk between nucleoplasm and nucleolar

The rRNA is transcribed by RNA polymerase I in nucleolar and provides a place for protein synthesis as a center of ribosome activity. Only recently, a couple of transcription factors have been reported as important players for both in Pol I and Pol II transcriptions, suggesting that the information is dynamically shared between nucleoplasm and nucleolar. The aim of this project is to elucidate the detail of this shuttle regulation between Pol I and Pol II transcriptions. We are focusing on NF-kB and FCP1 as key molecules of this crosstalk mechanism.

Research 2: Cell fate determination by activating transcription factor (ATF) 3

Cells determine their life or death in response to environment. Activating transcription factor (ATF) 3 is an early response gene and functions in cell death, survival and proliferation. Our aim of ATF3 research is to understand dual role of ATF3 in oncogenesis, anticancer therapy, and various stress response, and to search for clinical applicability to the control of cell fate.

2-1 Pro-apoptotic role of ATF3 and its implication in anti-cancer therapy

Our efforts of screening ATF3 binding target gene(s) upon DNA damage as in 2-2 revealed its recruitment onto death receptor gene DR5. Using human colorectal cancer cells, we showed the cell death by TRAIL/CPT combination treatment is dependent on ATF3, since ATF3 knockdown or atf3 null MEFs impaired apoptosis of cells. Further study clearly demonstrates ATF3 co-operates with p53 to induce the DR5 transcription upon DNA damage. Novel paradigm of biological role of p53-ATF3 axis is now under investigation.

2-2 Genome-wide screen of the role of ATF3 in stress response and human cancer

ATF3 functions as both oncogene and tumor suppressor. For example, in prostate cancer and Hodgkin disease, ATF3 expression is positively correlated with cell proliferation and also enhanced metastasis. Conversely, ATF3 inhibits p53 degradation and stabilizes its expression level. As a first step, we are trying to decipher genetic pathway of these biological phenomenon using ChIP-chip and expression profile analysis of 1) cells after DNA-damage, 2) Prostate cancer cells, 3) Hodgkin Reed-Sternberg cells. Comparative study would provide a clue to the role of ATF3. In human colorectal cancer cells, ATF3 binds over 6,000 gene promoters in stress response to DNA damage (MMS), while it binds to ~1,300 gene promoters in ATF3-expressing prostate cancer cells. We also performed the genome-wide expression analysis after ATF3 knockdown followed by expression microarray analysis. The results show ATF3 does regulate approximately 40% of p53 target genes, demonstrating that ATF3, a target gene of p53, functions as co-regulator of p53. ATF3 may play diverse regulatory roles in oncogenesis. Further, we generated genetically engineered mouse model of p53 and ATF3 gene knockout to unravel genetic codes of p53-ATF3 axis regulation. The genome-wide analyses of these mice are now revealing intriguing regulatory networks between these two transcription factors in cancer and stress response.

2-3 ATF3 complex; transcriptional repressor or activator

According to our result from ChIP-chip analysis combined with expression array, ATF3 apparently works as not only traditional transcriptional repressor but as activator. In order to reveal the molecular mechanism for dual function of ATF3, we started to purify ATF3 complexes from Hodgkin's cell line, and identify the component of each complex. We anticipate ATF3 constitutes different complex as an oncogenic protein from as a stress-induced repressor.

2-4 ATF3 transcriptionally regulates microRNA.

Recently, microRNA is attracting many scientists because of its diverse biological function and the possibility for the future clinical application. We started to search the microRNAs regulated transcriptionally by ATF3 and found several microRNA promoters associated with ATF3. As expected, the promoters bound to "stress-induced" ATF3 are different from those bound to "oncogenic" ATF3, suggesting that the biological function of ATF3 varies according to the cell conditions. Additionally, microRNA could be one of the execution tools to bring out the intent of ATF3 expression.

Research 3: H3K36-sepcific histone methyltransferase ASH1.

Core histones that constitute nucleosomes together with DNA are reversibly modified by a large number of nuclear enzyemes. Combinations of such modifications generate highly dynamic histone codes and play important roles in regulation of gene activities. In our laboratory, we have cloned one of mammalian histone lysine methyltransferases called ASH1 (absent, small, or homeotic discs-1) and shown that ASH1 specifically methylates histone H3 lysine 36. ASH1 synergizes strongly with MLL (mixed lineage leukaemia) in Hox gene expression and also plays a crucial role in activation of retrogenes in patients with facioscapulohumeral muscular dystrophy. Thus, our studies will help develop novel strategies to fight against human diseases such as leukaemia and myscular dystrophy.

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Division of Medical Genomics
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Introduction of Department of Epigenetics

"Epigenetics" coupled with "Genetics" enables us to elucidate several 'genomic functions' in inheritance, development and evolution of organisms including our human beings. Genomic imprinting is one of the mammalian specific gene regulation mechanisms. It gives rise to functional differences between paternally- and maternally-derived genomes in development, behavior and growth. Somatic cloned animals give us unique chances to examine 'genetically identical but epigenetically diverged animals'. These studies show us how epigenetics is important in mammalian biology. Our department focuses on these mammalian specific genomic functions to elucidate how these genomic functions work and how new genomic functions have been evolved during evolution. Our final goal is to contribute to the establishment of 21th's medicine and human biology by understanding of such genomic functions.

Latest researches

1. Analyses of Mammalian-Specific Genomic Functions

We have been focusing mammalian-specific genes and epigenetic mechanisms to elucidate mammalian-specific functions, such as viviparity and maternal nursing behavior. We previously demonstrated that mammalian-specific retrotransposon- derived genes, *Peg10* and *Peg11/Rtl1*, play essential roles in mammalian development via formation and maintenance of placentas that are unique to mammals. By collaboration with Prof. Kaneko-Ishino at Tokai University, analyses of Other *SIRH* (sushi-ichi retrotransposon homologue) genes, such as *Sirh3* to *Sirh11*, are under investigation. This year, we have reported that another *SIRH* gene specific to marsupial mammals. It is present in an Australian marsupial species, tammar wallaby. However, its orthologue in a South American marsupial species, grey short-tailed opossum, have degenerative protein coding frames, suggesting it is only functional in the tammar wallaby (Ono *et al*. DNA Res 2011). It is now clear that the eutherians and the marsupials have different sets of *SIRH* genes probably contributed to diversification of these two groups of mammals.

2. Improvement of Somatic Cloning Technology

Cloning mammals by means of somatic cell nuclear transfer (SCNT) is highly inefficient because of erroneous reprogramming of the donor genome. Reprogramming errors appear to arise randomly, but the nature of nonrandom, SCNT-specific errors remains elusive. Last year, we reported that *Xist*, a noncoding RNA that inactivates one of the two X chromosomes in females, was ectopically expressed from the active X (Xa) chromosome in cloned mouse embryos of both sexes. Therefore, deletion of *Xist* on Xa showed normal global gene expression and resulted in about an eight- to ninefold increase in cloning efficiency. This year, we also have succeeded the efficient production of somatic cloned mice with *Xist* knockdown method. It is a great step toward the practical use of the SCNT method in many domesticated animals.

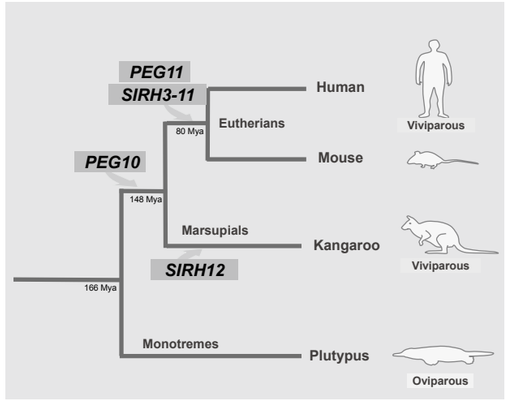

Fig.1. **Acquisition of *SIRH* family genes from an LTR retrotransposon**
Twelve *SIHR* family genes were domesticated in mammals. *PEG10/SIRH1* is conserved in both eutherians and marsupials while *PEG11/SIRH2* and *SIRH3-SIRH11* are present only in the eutherians. We have recently identified *SIRH12* as a domesticated gene derived from a marsupial-specific insertion event. We previously demonstrated that *PEG10* and *PEG11* are essential placental genes in the eutherians, indicating they were deeply involved in the evolution of viviparous reproduction systems in mammals. MYA: million years ago.

3. Epigenetic analysis on ICSI pups

Intracytoplasmic sperm injection (ICSI), one of the human assisted reproductive technologies (ART), has long raised concerns about its influence on development. In this study, the pre- and postnatal effects of ICSI were assessed using comprehensive transcriptome and phenotypic analyses in mice under strict conditions and we demonstrate that, in contrast to *in vitro* fertilization (IVF), ICSI induces distinct long-lasting transcriptome change that remains at the neonatal stage. No remarkable differences were observed in the ICSI adults in either the gene expression or phenotypic profiles, and there was no indication of transmission to the next generation via natural mating. Our results suggest there are no lifelong or transgenerational effects of ICSI, but the ICSI effects during neonatal period remain to be evaluated.

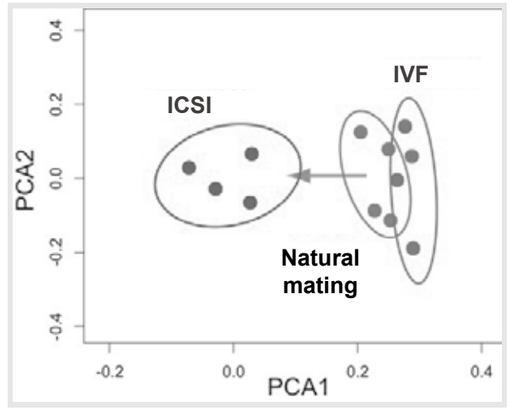

Fig.2. **Epigenetic shift in ICSI pups**
Total gene expression profiles are compared among pups from IVF (in vitro fertilization), ICSI (intracytoplasmis sperm injection) and normal sexual mating. IVF pups exhibit the same profile as that of normal controls, however, 2% genes were over- or underexpressed in ICSI pups. All the ICSI pups exhibit the same epigenetic shifted profiles, indicating that it was caused by the ICSI technique itself. Result of principal component analysis (PCA) using neonatal brain samples is shown.

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Presentation at International meetings

1. Sawa Iwasaki, Takashi Kohda, Tomoko Kaneko-Ishino and Fumitoshi Ishino. Genomic impirinting status of retrotransposon-derived *PNMA* family genes, ICC on Genomic Imprinting and Beyond 2011, September 21-23, 2011, Barcelona, Spain.
2. Fumitoshi Ishino and Tomoko Kaneko-Ishino. Evolution of genomic imprinting in mammals. Symposium "On the critical issues in EvoDevo", 44th JSDB Annual Meeting, May 21, 2011, Okinawa Convention Center (Okinawa).
3. Fumitoshi Ishino, Ryuichi Ono, Sunsuke Suzuki, Yoichi Sekita, Mie Naruse, Takashi Kohda and Tomoko Kaneko-Ishino. Role of retrotransposons in mammalian evolution -Domesticated genes for mammalian-specific functions-, US-Japan Conference: Inflammation, Diabetes and Cancer, August 4, 2011, Beckman Research Institute of City of Hope, California, USA.
4. Fumitoshi Ishino and Tomoko Kaneko-Ishino. Evolution of viviparity and genomic imprinting in mammals by retrotransposons, 15th Evolutionary Biology Meeting in Marseilles, September 27-30, 2011, Marseilles, France.
5. Fumitoshi Ishino. Retrotransposon-derived genes in mammalian development and evolution. From Early Universe to Evolution of Life, Germany-Japan Round Table, December 1-3, 2011, Heidelberg University, Germany.

Laboratory of Systems Biology, School of Biomedical Sciences
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1. Oxidative stress pathways in noncancerous human liver tissue to predict hepatocellular carcinoma recurrence: a prospective, multicenter study

In order to identify predictive factors for recurrence in patients with early hepatocellular carcinoma (HCC), we analyzed gene expression profiles of cancer and non-cancerous tissues in 78 patients. Downregulation of cytochrome P450 1A2 (CYP1A2) in non-cancerous tissues was the only factor that was significantly associated with HCC recurrence. We verified that lower protein expression of CYP1A2 in non-cancerous tissue was significantly associated with HCC recurrence by a multicenter study. The gene expression pattern of CYP1A2 was strongly correlated with gene sets associated with peroxisome function, suggesting that recurrence in early HCC patients is mainly due to oxidative stress in non-cancerous tissue, rather than molecular or clinicopathological characteristics of cancerous tissue.

2. i2b2: a new platform for clinical databases as an infrastructure of translational informatics

Translational informatics is an emerging field for facilitating patient-centered translational research analyzing personal omics data on the basis of mathematical models for diseases. Among the ongoing projects, the i2b2 provides an ontology-based object-oriented database system for integration of clinical information dispersed in different laboratories and different hospitals as well as different scenes in medication. We constructed i2b2 database with 392 clinical patients' data collected from the university hospital of TMDU and recorded in 'Integrated Clinical Omics Database' (iCOD). Japanese NLP technologies were employed to extract clinical terms from doctors' comments. We built a pipeline for extraction of disease names and translation of them into English computationally.

3. Analysis of protein-interaction networks and their applications to drug-target discovery

Since proteins exert their functions though interaction to other proteins, understanding topological and statistical features of the human PIN is of use to discover novel drug-target genes. In this study, to uncover how target genes are distributed over the human PIN, we developed a novel algorithm to decompose the PIN into several small simple sub-networks. We then mapped drug-target genes on to the sub-networks and found that a small sub-network contains majority of the targets. For example, the sub-network contains almost 60% of target-genes of small molecule drugs for cancer cure (*e.g.*, kinase inhibitor). The listing of proteins and interactions in the sub-networks may help drug companies to search more efficiently for novel drug-target genes.

4. Systems pathology analyses on disease progression of cancer, metastasis (EMT), and Alzheimer's disease

Our mission is systems pathology studies on cancer, metastasis (epithelial-mesenchymal transition: EMT), and neurodegenerative disease (Alzheimer's disease) using large-scale molecular biology data, so-called omics data. We inferred transcriptional, gene regulatory and protein interaction networks of disease progression, and then explored master regulator, that is key molecule in their networks. We then estimated an attractor for each cellular state based on gene regulatory network for disease progression, cellular transformation (EMT), and cellular differentiation (iPSC/ESC) processes, showing transition of attractors along with these processes. For omics data analyses, data integration is necessary. We worked on integration of incurable diseases data using Linked Data technology.

5. Change of Positive Selection Pressure on HIV-1 Envelope Gene Inferred by Early and Recent Samples

HIV-1 infection has been on the rise in Japan recently, and the main transmission route has changed from blood transmission in the 1980s to homo- and/or hetero-sexual transmission in the 2000s. We sequenced 142 full-length env genes collected from 16 Japanese subjects infected with HIV-1 in the 1980s and in the 2000s. We examined the diversity change in sequences and potential adaptive evolution of the virus to the host population. The result showed that the selection pressure was weaker in the 2000s than in the 1980s, indicating that it might have become easier for the HIV to infect a new host and to develop into AIDS now than 20 years ago and that the HIV may be becoming more virulent in the Japanese population.

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Frontier Research Unit
Redox Response Cell Biology

Associate Professor　　Shun-Ichi Kurata

Since the living thing on the earth lives under oxygen existence, then they are put to a strong oxidative stress. The major cause of cellular oxidative stress is ROS produced by the mitochondrial electron-transfer system, and therefore, redox regulation and oxidative stress responses are essential for cell survival and homeostasis. Our research deals with molecular mechanisms of redox responses, focusing on mitochondrial biochemical reactions directly linked to 1)cellular signaling pathways to transcriptional control and 2) apoptosis induction. We also investigate p63, a member of the p53 family, for stress-response ability and pathophysiological significance of its high-level expression in squamous cell carcinomas.

1. Reguration of β-catenin-by p63 through nuclear GSK-3β signaling in squamous cell carcinoma

We have recently reported that p63 cooperates with the keratinocyte-specific TGF-β signaling pathway (Fukunishi N. *Neoplasia*. 2010 12:969) to maintain the non-malignant, differentiation phenotypes of squamous cell carcinoma (SCC). Apart from the direct induction of keratinocyte-specific gene expression by p63, modification of β-catenin signaling by ΔNp63α, th e most abundant isoform of p63, has been proposed (Patturajan, M. *Cancer Cell*, 2002, 1:365; Drewelus I. *Cell Cycle*, 2010, 9:580). Our gene expression profiling of p63-knockdown SCC indeed showed significant alterations in transcription of various β-catenin/LEF(TCF)-controlled genes including MMP7, MITF and TGFB2. However, the amount of nuclear β-catenin was not altered by p63 silencing. Interestingly, glycogen synthase kinase-3β (GSK-3β) Ser9 phosphorylation was increased in the nucleus of p63-knockdown cells, but not in the cytosol. Furthermore, ΔNp63α was associated with protein phosphatase 2A (PP2A) whose activity was decreased by p63 knockdown. Thus, DeltaNp63α may control β-catenin-mediated gene expression through the nuclear PP2A-GSK-3β signaling.

2. Activation of the long terminal repeat of human endogenous retrovirus K by melanoma-specific transcription factor MITF-M

Human endogenous retrovirus (HERV)-K with 5'LTR-gag-pro-pol-env-rec/np9-3'LTR sequences represents the newest retrovirus family that integrated into the human genome 1 to 5 million years ago. Although a high-level expression of HERV-K in melanomas, breast cancers, and teratocarcinomas has been demonstrated, the mechanism of the lineage-specific activation of the long terminal repeat (LTR) remains obscure. We studied chromosomal HERV-K expression in MeWo melanoma cells in comparison with the basal expression in human embryonic kidney 293 (HEK293) cells. Cloned LTR of HERV-K (HML-2. HOM) was also characterized by mutation and transactivation experiments. We detected multiple transcriptional initiator (Inr) sites in the LTR by rapid amplification of complementary DNA ends (5' RACE). HEK293 and MeWo showed different Inr usage. The most potent Inr was associated with a TATA box and three binding motifs of microphthalmia-associated transcription factor (MITF). Both chromosomal HERV-K expression and the cloned LTR function were strongly activated in HEK293 by transfection with MITF-M, a melanocyte/melanoma-specific isoform of MITF. Coexpression of MITF and the HERV-K core antigen was detected in retinal pigmented epithelium by an immunofluorescence analysis. Thus, MITF-M may be a prerequisite for the pigmented cell lineage-specific function of HERV-K LTR, leading to the high-level expression in malignant melanomas.

Publications

Activation of the long terminal repeat of human endogenous retrovirus K by melanoma-specific transcription factor MITF-M Iyoko Katoh, Anna Mirova, Shun-ichi Kurata, Yasushi Murakami, Kenji Horikawa, Natsuko Nakakuki, Takunobu Sakai, Kunihiko Hashimoto, Ayako Maruyama, Takaaki Yonaga, Nahoko Fukunishi, Kohji Moriishi and Hirohisa Hirai
Neoplasia 2011 13(11):1081-1092

Project Research Unit

Project Research Unit

(Associate Professor; Tokio Yamaguchi)

Summary : Bilirubin, an efficient antioxidant, is shown to scavenge reactive oxygen species (ROS) produced by oxidative stress in vivo. We ndicated that psychological stress contributed to the oxidative conditions, and the oxidative conditions, and the subsequent increase of the urinary concentration of biopyrrins provoked by the reaction of bilirubin with ROS, and that biopyrrins could be useful marker of psychologicalstress.

Research projects

1. Induction of heme metabolic enzyme-systems and production of reactive oxygen species provoked by oxidative stress (pathophysiological significance of bilirubin as an antioxidant)
2. Development of the stress-checker using biopyrrins (oxidative metabolites of bilirubin) as a stress marker by the immuno-chromato assay.

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Pathophysiology

(Associate Professor Saburo Horikawa)

Ischemia/reperfusion (I/R) injury can occur in several pathophysiological situations and is a major cause of tissue injury during transplantation and ablative surgery. I/R is an unavoidable process in these surgical operations. I/R injury is considered to be related to the generation of reactive oxygen species. The aim of our study is to understand the molecular mechanisms underlying I/R injury. Our research projects are: 1) acute lung injury induced by intestinal I/R; 2) hepatic I/R injury; 3) liver regeneration after partial hepatectomy; 4) portal vein stenosis; 5) aquaporin-2 trafficking.

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Medical Genomics

(Assistant Professor Shuji Sassa)

It participated in two students to do the graduation research from the Department of Clinical Laboratory Medicine, Faculty of Health Science Technology, Bunkyo Gakuin University. Amyloid-β deposition in the brain is a hallmark of Alzheimer's disease, and has been shown to induce apoptosis and disrupt cellular ion homeostasis. Ferulic acid, a phenolic compound and major constituent of plants, is well known as an antioxidant. It has been reported that long-time administration of ferulic acid can protect mice from amyroid β-peptide induced learning and memory deficit. As previously reported, extracts from corn germ induced a positive response in the pigeon crop sack test, which was used for the detection of prolactin-related substances. A substance of extracts was ferilic acid , which slightly influenced ganadotropin levels in male rats. In the present sturdy, we investigated the effects of ferulic acid on plasma levels of ovarian hormones and bone mineral density in ovariectomized rats. At 9 weeks of age, Sprague-Dawley female rats were divided into 3 groups of 8 rats each, and the animals of 2 groups underwent ovariectomy. Beginning at 44 weeks of age, the ovariectomized rats in 2 groups were given subcutaneous injections of 0.9% NaCl solution and ferulic acid 1.0 μg once a day for 8 weeks, respectively. Long-term administration of ferulic acid enhanced the reduced levels of plasma estradiol, progesterone and alkaline phosphatase activity, and elevated the decreased bone mineral density of tibia in ovariectomized animals.

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Medical Genomics

(Associate Professor Michinori Kubota)

Functional differences between the right and the left auditory cortex of guinea pigs were investigated using optical imaging with voltage-sensitive dye. Tone bursts (2, 4, 8, 16 kHz) were applied at different sound pressure levels (55, 65, 75 dB SPL). Neural response to the tone burst at the sound pressure level of 55 dB SPL first appeared ventrally in the isofrequency band of the stimulus frequency and expanded along the band. When the sound pressure levels were higher, responses first appeared more dorsally and expanded along the isofrequency bands. These spatial shifts in responses in the isofrequency bands with sound pressure levels were often observed in the left auditory cortex.

International Meeting

Hosokawa Y, Kubota M, Sugimoto S, Horikawa J. Sound pressure sensitivities along the frequency band in the primary auditory cortex of guinea pigs observed by optical recording. J Physiol Sci, Vol. 61, Suppl. 1, S179 (2011).

Affiliated Institutes

Systems Biology for Intractable Diseases

Affiliate Professor **Satoru Miyano Ph.D.**
Affiliate Associated Professor **Seiya Imoto Ph.D.**

Research Summary

It is getting clearer that pathogenesis of intractable disease is a state that deviates from an integrated systems control in the abnormal situation where multiple genes are affecting one another intricately. The recent advances in biomedical research have been producing large-scale, ultra-high dimensional, ultra-heterogeneous omics data through the advanced technologies such as genome sequencing and proteome analysis. The aim of this section is to clarify the biological mechanisms and their failures in the system by applying computational strategy for systems biology and by analyzing these omics data using supercomputer. It is expected that key molecules of the diseases will be searched by the systems biology analysis of molecular pathways and networks related to the diseases which could not be analyzed in the traditional approaches.

This section is collaborating with various laboratories in Medical Research Institute for understanding the pathogenesis of the diseases toward drug discovery and new therapy development.

In 2011, we analyzed microarray gene expression data from 762 cancer cell lines and computed gene networks by using the supercomputer system of Human Genome Center, Institute of Medical Science, University of Tokyo (Fig. 1). We succeeded in extracting the system changes that were related to the epithelial-mesenchymal transition (EMT) (Fig. 2). As a result, a new gene causing the EMT, such as KLF5, is discovered.

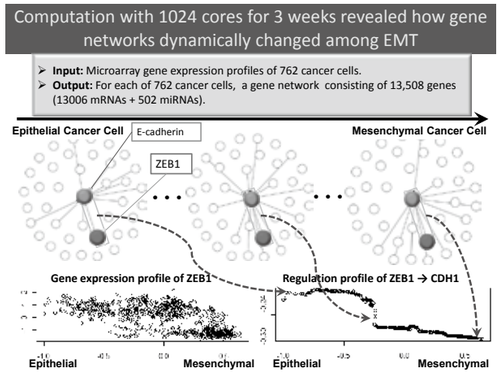

Fig. 1

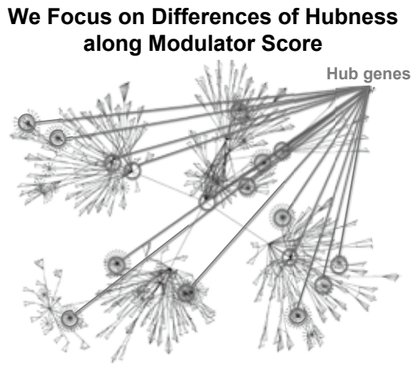

Fig. 2

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Department of Organ Network and Metabolism

Tokunin Professor **Yasutomi Kamei Ph.D.**
Tokunin Assistant Professor **Miyako Tanaka Ph.D.,**
Michiko Itoh M.D., Ph.D

To maintain proper metabolic homeostasis within the human body, metabolic organs, such as adipose tissues, skeletal muscles, liver, kidney and brain communicate with each other via humoral factors and neuronal networks. Dysregulation of these inter-organ networks may lead to metabolic syndrome, i.e., a multi-factorial pathological condition that arises from complex interactions between genetic and environmental factors.

In April 2011, we set-up a new laboratory at the Department of Organ Network and Metabolism with a mission to elucidate the molecular basis of metabolic syndrome and to identify therapeutic targets that may ameliorate the symptoms associated with it. Our studies have focused on the pathophysiological and therapeutic implications of inter-organ networks with regard to the regulation of nutritional adaptation, energy metabolism, and inflammation. Our aims are to better understand the molecular mechanisms underlying metabolic syndrome and its associated diseases, and to establish novel strategies for the prevention, diagnosis, and therapy of metabolic syndrome.

1. Role of FOXO1 in the adaptation of skeletal muscles to starvation

The human body adapts to nutritional changes that occur in different conditions, such as fasting, intense exercise, and metabolic diseases, by controlling the energy metabolism of various organs. The skeletal muscle system, which is the largest organ in the human body, stores energy in the form of proteins. During long-term starvation, these skeletal muscle proteins are degraded to release amino acids into the blood stream. These amino acids are used in the liver as substrates for gluconeogenesis. However, the mechanisms underlying the regulation of protein and amino acid metabolism in skeletal muscles, as well as in other tissues, are largely unknown.

In this study, we are focusing on the transcriptional factor Forkhead box protein O1 (FOXO1), which is a negative regulator of insulin signaling. We have previously showed that FOXO1 is a negative regulator of skeletal muscle mass (**J. Biol. Chem.** 279:41114-41123, 2004; **Biochem. J.** 427:171-178, 2010). Our aims are to identify new target genes for FOXO1 and to clarify mechanisms underlying the adaptation of skeletal muscles to starvation.

2. Role of central leptin signaling in the adaptation of immune system to starvation

Nutritional deprivation or malnutrition suppresses immune function in humans and animals, which results in increased susceptibility to infectious diseases. Indeed, nutritional deprivation induces atrophy of lymphoid tissues, such as the thymus and spleen, and decreases the number of circulating lymphocytes. Leptin, a major adipocytokine, is exclusively produced in adipose tissues in response to the nutritional status and it acts on the hypothalamus, thereby regulating energy homeostasis.

Although it has been reported that leptin plays a critical role in starvation-induced T cell-mediated immunosuppression, little is known about its role in B cell homeostasis under starvation conditions. In this study, we observed alterations in B cell development in the bone marrow of fasted mice. These alternations were characterized by a decrease in the numbers of pro-B, pre-B, and immature B cells and an increase in the number of mature B cells. Interestingly, intracerebroventricular leptin injection could prevent these alterations in B cell development. Our data also suggested the role of serum corticosterone concentration and neuropeptide Y pathway of the hypothalamus in leptin-mediated regulation of B cell development in the bone marrow.

Our study provides the first in vivo evidence for the role of central leptin signaling in starvation-induced alteration in B cell development. Furthermore, our results suggest that the central nervous system, which integrates information from throughout the organism, can control immune function. (**J. Neurosci.** 31: 8373-8380, 2011)

3. Establishing a non-alcoholic steatohepatitis (NASH) mouse model.

Adipose tissue is an energy reservoir that secretes many biologically active substances and hormones. Over-accumulation of fat in adipose tissues results in ectopic lipid accumulation in non-adipose tissues, such as the liver and skeletal muscles where lipotoxicity impairs their metabolic functions. Ectopic lipid accumulation is considered to be involved in the pathogenesis of metabolic syndrome such as non-alcoholic steatohepatitis (NASH).

NASH is a severe form of non-alcoholic fatty liver disease (NAFLD), which could progress to cirrhosis and hepatocellular carcinoma. The “two-hit” hypothesis proposes the involvement of excessive hepatic lipid accumulation (first hit) and chronic inflammation (second hit), mediated by factors such as adipocytokines, oxidative stress, and endotoxins. Since two-thirds of hepatic lipid influx derives from adipose tissues, dysfunction of adipose tissue might play a role in the development of NASH as both the “first” and “second” hits. However, there is no appropriate animal model for NASH with both liver fibrosis and obesity-related dysfunction of glucose-lipid metabolism: thus, the molecular mechanisms underlying the development of NASH remain unclear.

Recently, we demonstrated that melanocortin-4 receptor knockout (MC4R-KO) mice developed a liver condition similar to human NASH during a HFD, which was associated with obesity, insulin resistance, and dyslipidemia. Notably, all of the MC4R-KO mice developed well-differentiated hepatocellular carcinoma after being fed a high-fat diet for one year. They also exhibited increased adipose tissue inflammation (e.g., increased macrophage infiltration and fibrotic changes), which might contribute to excessive lipid accumulation and enhanced fibrosis in the liver.

Our study suggests that MC4R-KO mice would provide a novel mouse model for NASH with which to investigate the sequence of events that comprise diet-induced hepatic steatosis, liver fibrosis, and hepatocellular carcinoma. Furthermore, these findings will help us to understand the inter-organ networks in the pathogenesis of NASH, investigate the specific biomarkers, and evaluate the potential therapeutic strategies (**Am. J. Pathol.** 179: 2454-2463, 2011).

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Medical Research Institute
Advanced Technology Laboratories

Genome Laboratory

This laboratory is established to support the education and research in the Medical Research Institute and Graduate School of Biomedical Science. Sequencing analyses requested by researchers belonging to the Medical Research Institute and those to the other Departments of Tokyo Medical and Dental University are done in this laboratory. In addition, several equipment including Flow Cytometer and Plate Reader are under management of the laboratory. Followings are the achievements in 2011.

1. Sequencing analyses

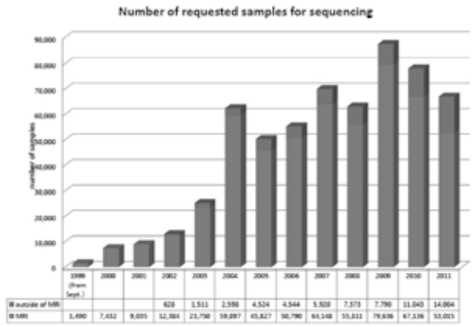
A total of 67,019 samples from 3,633 researchers were sequenced in the year of 2011. Among them 14,004 (21%) samples were requested by researchers outside the medical Research Institute (see below).

2. Equipment under the management of the Genome Laboratory.

DNA sequencer (ABI3130xl) × 2, PCR machine (ABI7900) × 5, Flow Cytometer, Luminescent Plate Reader, Ice Machine, Centrifuge, and others.

3. Introductory seminars

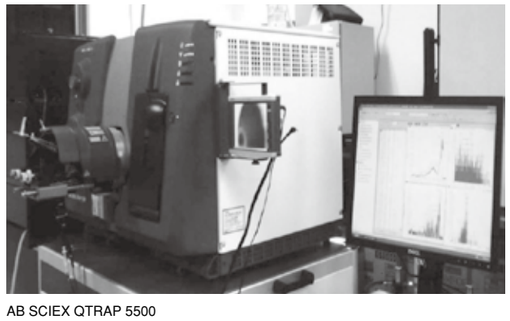
Introductory seminars were done for next generation sequencing method (3 times) and for manipulation of flow cytometer (3 times).



Laboratory of Cytometry and Proteome Research

This laboratory will help researchers to separate specific cells and analyze the proteome expressed in the cells. we set up equipments written below.

For proteome analysis, we have a 2D-electrophoretic system, LC-MSMS system and Biacore system in this laboratory. We can accept the consignment analysis of proteins with the two dimension electrophoresis and the mass spectrometry by request of researchers of this university. In addition, we can give technical advices on cytometry and proteome researches to young scientists who wish to start own research.



AB SCIEX QTRAP 5500

Q-Tofmicro

Publication

Proteome Analysis Of Bronchoalveolar Lavage Fluid in Chronic Hypersensitivity Pneumonitis.

Tsukasa Okamoto,Yasunari Miyazaki,Ryutaro Shirahama,Meiyo Tamaoka and Naohiko Inase. Allergology International. 2011 Oct 25;0(0).

Laboratory of Anatomy and Cell Function

Laboratory of anatomy and cell function (LACF) has a support system for histological technologies at Medical Research Institute (MRI). We equip basic technical instruments and materials for analyzing research of cells and tissue available for common use. LACF provides technological assist for all research laboratories at MRI as shown in the following list and facilities for study.

Common equipment

Confocal laser microscope
Fluorescence microscope
Cryostat
Rotary microtome
Spin-tissue-processor
Tissue-embedding-station
Real-time PCR
Laser microdisection

Laboratory of Bioresource

Bioresource Lab. of Medical Research Institute was established in 2004, to support researchers and help postgraduates in cell culture. The center safely supply domestically or internationally well-recognized cultured cell lines as research materials. All of the cell lines handled are collected after exchanging MTA with original developers. EB-virus transformed cell lines are also established with B-lymphocytes from patients with intractable diseases including genomic disorders after written informed consent from each of the patients or their parents and also with approval of the Internal Review Board on ethical issues.

Laboratory for Stem Cell Research

In order to assist researchers working on stem cell-related subjects across Medical Research Institute, the Laboratory for Stem Cell Research was established as of December, 2009, and thereafter expanded in the year of 2010 and 2011. This Laboratory has two rooms in Surugadai Area (1st floor) and M&D Tower (21st floor), each equipped with basic and state-of-the-art research facilities. For instance, we have high-speed cell sorter MoFlo Legacy, flow cytometer FACSCalibur, rotary tissue processor, and rotary microtome in Surugadai Area (1st floor), and high-speed cell sorter MoFlo XDP and confocal laser scanning microscope in M&D Tower (21st floor). Equipments in these two rooms are supposed to be moved to a new room on the 24th floor in M&D Tower by March, 2012.

This Laboratory is managed by the Operating Committee composed of five Professors and three Associate Professors in the Institute, and the services are provided by one Technical Staff who was stationed on April 1, 2010. A technical assistant was appointed to this Laboratory as of June 1, 2011. The Operating Committee was held 10 times in the fiscal year of 2011 to discuss the way to smoothly set up the Laboratory and efficiently provide the services. In 2011, as part of activities of this Laboratory, we held technical courses for the equipment: e.g. twice for MoFlo XDP, and five times for confocal laser scanning microscope.

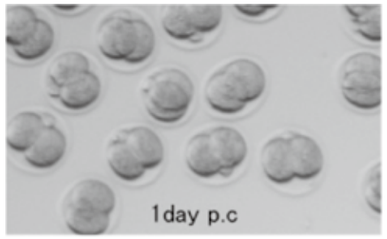
Laboratory for Structure Analysis

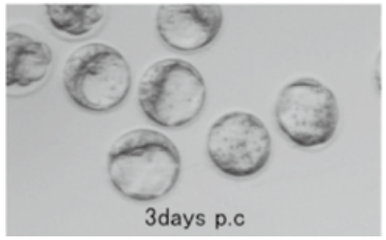
The Laboratory for Structure Analysis is the latest member of the Facilities and equipped with a high-brilliancy X-ray generator and an image plate X-ray detector. The Laboratory acquired a dynamic light scattering (DLS) instrument this year, enabling the measurements of particle size (thus oligomeric state and aggregation) of protein molecules. An introductory practical course was held for students in Graduate School.

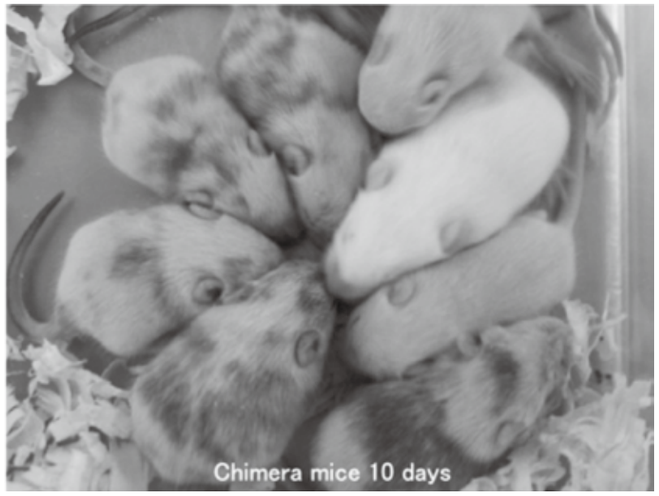
Recombinant Animal Laboratory

Recombinant animals including transgenic, knock-out and knock-in mice are now essential for the medical and biological studies and education in graduate schools. Those animals are also essential for the research on the pathogenesis and pathophysiology of intractable diseases, and development of new treatments for these diseases. In the Recombinant Animal Laboratory, excellent technical staffs generate, clean up, maintain and store recombinant mice to facilitate the biomedical research in Medical Research Institute.

Medical Research Institute runs this laboratory as one of the eight core facilities. The committee of this laboratory composed of faculty members of Medical Research Institute regulates the laboratory by educating new users, and controling generation and introduction of new recombinant mice.







Chemical Biology Screening Center

Professor **Hiroyuki Kagechika**
Assistant Professor **Mari Yuasa**

"Chemical Biology" is a scientific interdisciplinary field, and approaches biological problems by using chemical knowledge and techniques. In Tokyo Medical and Dental University (TMDU), Chemical Biology Screening Center (CBSC) was established in 2006, in order to promote chemical biological research. The center supports the researchers in medical, dental and biological fields who want to use chemicals or chemical techniques in their researches, or chemists who want to collaborate with the researchers in medical, dental and biological fields.

The center keeps the chemical library with about 20,000 compounds (Fig. 1). The compound library consists of the functionally known and unknown compounds, including the original compounds, synthesized or isolated by the chemists in TMDU. Each compound was stocked as DMSO solution, and was supplied to the researchers as the 96-well plates with the compounds (10 μl/well).

The center also promotes the chemical screening, and set up high content screening facilities, including plate reader (ARVO MX, ARVO X5), high content cellular Imager (Array Scan VTI), sample preparation (384 well format) (Table 1). The center holds the explanatory meetings several times per year.

The center constructs the TMDU Chemical Biology Database (CBDB, http://bsmdb.tmd.ac.jp/) which supply the information about the chemicals in CBSC and their biological properties, including the data obtained in CBSC.

The center will be reorganized in April 2012, in order to promote chemical biology researches and the related collaboration further in TMDU.

Information about CBSC is available at the web site (http://www.tmd.ac.jp/mri/SBS/cbsc/index.html).

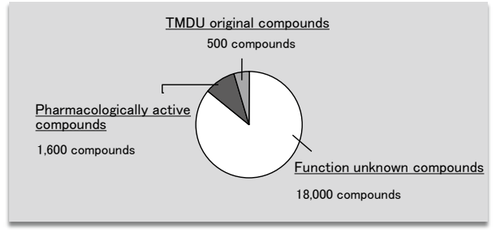


Fig. 1 Chemical library of TMDU

Equipment for screening	Characteristics
Multi-Label plate reader ・ARVO X5 : Perkin Elmer ・Wallac 1420 ARVO MX : Perkin Elmer 	Luminescence, Fluorescence Intensity, UV-, Time-Resolved Fluorescence and Fluorescence Polarization technologies (Example) ・Cellular Assays including GPCR and kinase ・Binding Assays including DELFIA and LANCE TRF assays etc..
High-Contents Image Analyzing System Cellomics ArrayScan VTI :ThermoFisher Scientific 	Flexible imaging platform, designed for high-capacity, automated, quantitative cellular and sub-cellular analysis in fixed and live cell formats (Example) ・Virus infection cell counting by immunohistochemical staining ・Determination of expression level (ratio) using GFP/RFP fusion protein ・Quantification of cellular protein translation by fluorescence substrate uptake assay etc..
Clean room ・Clean bench : SANYO ・CO_2 Incubator : SANYO	Biosafety level 2 laboratory space
Multi Dispenser ・EDR-384SII : Bio Tec ・Multi-CHOT : NICHIRYO	Plate re-format dispensing from compound dilution to assay plate
Auto Sera Washer ・AMM-96SII : Bio Tec	Automatic successive washing equipment for 96 well for ELISA

Table 1 Features of Chemical Biology Screening Center

Publications

A cell-based assay to screen stimulators of the Hippo pathway reveals the inhibitory effect of dobutamine on the YAP-dependent gene transcription. Bao Y, Nakagawa K, Yang Z, Ikeda M, Withanage K, Ishigami-Yuasa M, Okuno Y, Hata S, Nishina H, Hata Y. *J Biochem*. 2011 150, 199-208.

School of Biomedical Science

Laboratory of Structural Biology

Professor	Nobutoshi Ito
Associate Professor	Teikichi Ikura
Adjunct Assistant Professor	Makoto Nakabayashi
Adjunct Assistant Professor	Minako Abe
Technical Assistant	Michiko Hattori
Postgraduate Student	Kenrou Shinagawa

The advance of genome science and proteomic analysis has produced a large amount of information about the primary structure of proteins and their spatial and temporal distributions. On the other hand, most of the proteins only function when they take certain three dimensional structures. As obviously seen in so-called prion diseases, proteins which are chemically correct but structurally incorrect not only fail to function properly but also can harm cells. Our laboratory aims to understand the function of biological macromolecules at atomic level through structure analysis and other methods of physical chemistry, in the hope that accumulation of such knowledge will eventually lead to development of drugs. We are also involved in providing database of such structural data to scientists through the activities of Protein Data Bank Japan.

1. Structural analyses of potential drug targets

Collaborations have been set up with other laboratories within and without the School for structural analyses of potential drug targets and their interaction with various compounds. Among them are the dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (DYRK1A) and the vitamin D receptor (VDR). The latter is described below as examples.

Nuclear receptors (NRs) are a series of receptors that play physiological actions caused by steroid hormones, lipophilic vitamin A or D, and they act as ligand-dependent transcriptional factors to regulate specific gene expressions. Vitamin D receptor, one of the NRs utilizes 1 α , 25-dihydroxy vitamin D_3 (1,25-D_3) as an endogenous agonist, which relates to various actions, such as cell differentiation, inhibition of proliferation, immune regulation as well as calcium homeostasis. Development of therapeutic compounds that operate the NRs and studies of chemical biology dealing with such compounds are expected for application to drug discovery, so it is being done actively because the NRs' functions are closely engaged in various diseases and their therapies.

Isoleucine 268 of the ligand-binding domain of human vitamin D receptor (VDR-LBD) is located in the ligand-binding pocket, and adjacent to the 22nd carbon atom of a natural ligand 1,25-D_3 in the complex. A missense mutation of the isoleucine to threonine I268T is known to affect an interaction between VDR and another NR (RXR), causing hereditary vitamin D resistant rickets.

Here we succeeded crystallization and structure determination of a ternary complex with I264T mutant of rat VDR-LBD corresponding to human I268T mutant, the natural ligand and a 13mer-peptide derived from coactivator DRIP205's sequence. The I264T mutant was crystallized as a canonical active conformation that was common to other VDR-LBDs reported previously. We observed several structural differences between this mutant and wild-type VDR-LBD near the mutational region, although their overall structures were almost the same. A side chain's hydroxyl group of Thr264 in the mutant was more close to a side chain's carbonyl group of Asn390 on helix 10 than in wild-type protein, causing structural deformation at helix 4/5 and loop 8-9. (Fig.1)

We scarcely detect structural change of Arg387 on helix 10 which was interacted with RXR. This result, however, does not mean that this residue was not affected by the mutation. Structure of Arg387 might be constrained by a crystallographic packing effect because the helix 10 is located at the interface between the proteins in this crystal. Thus, further analysis on structure and dynamics of this mutant VDR is necessary to elucidate whether or not Arg387 is affected by this mutation.

2. Analysis of interactions between tau protein and Pin1

Tau protein is essential to assembly of microtubule, which mainly consists of two types of tublin. Hyperphosphorylation of tau protein abolishes its ability to bind tublin and promote microtubule assembly. When released from tublin, phosphorylated tau protein aggregates into paired helical filaments (PHF), which are characterized as the neuropathological hallmarks of Alzheimer's disease. Recently, it was revealed that a peptidyl-prolyl isomerase Pin1 restored the ability of phosphorylated tau protein to bind tublin and promote microtubule assembly. Pin1 specifically isomerizes phosphorylated serine or threonine that precedes proline (pS/T-P) and regulates the function of the phosphoproteins. The mechanism of regulation of tau protein by Pin1, however, is still unsolved. Here we investigated interactions between tau protein and Pin1. Our exhaustive search showed that Pin1 interacted only with the pS/T-P sites of tau protein, but their interactions were too weak and transient to form a stable complex. This suggests that Pin1 prevents formation of PHF by means of some methods except segregation or equilibration-shift.

3. Improvement in Protein Data Bank

The advance of structural biology with X-ray crystallography and NMR has generated a huge amount of data such as atomic coordinates. The increase is expected to continue with even more rapidly by the advent of structure genomics. The vast information is now a precious resource of wide range of science such as bioinformatics. Ever since 1970's, Protein Data Bank (PDB) has been acting as the main and single international archive of the structural data of biological macromolecules. Currently wwPDB, a consortium of Research Collaboratory of Structural Bioinformatics (RCSB), European Bioinformatics Institute (EBI) and Protein Data Bank Japan (PDBj) maintains it. We, as a member of PDBj, contribute to the continuous effort by wwPDB to improve PDB.

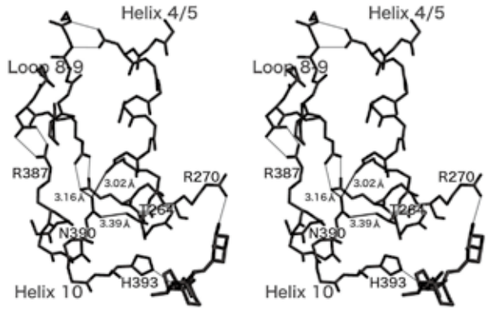


Fig.1a

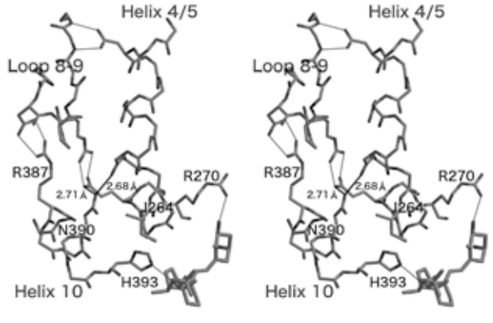


Fig.1b

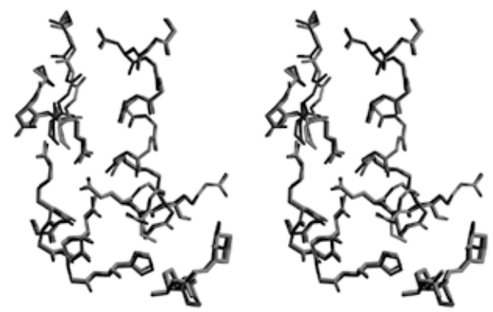


Fig.1c

Fig.1. Asn390 indirectly interact with Arg387 via helix 4/5 and loop 8-9. (Stereo diagram)
Asn390 is located adjacent to Ser261 and Glu265 on helix 4/5. Arg387 directly interacts with RXR. Wild-type rVDR-LBD (**1b** light grey) shows that a side chain's amide group of Asn390 is close to a main chain's carbonyl group of Ser261 and a side chain's carboxyl group of Glu265. On the contrary, I264T mutant (**1a** dark grey) shows that the side chain's amide group of Asn390 is slightly away from Ser261 and Glu265, presumably because a hydroxyl group of Thr264 is located adjacent to Asn390. Superimposition of both figures 1a and 1b clearly shows the difference between wild-type and mutant proteins (**1c**).

Research Papers

1. Nomura W, Ohashi N, Okuda Y, Narumi T, Ikura T, Ito N, Tamamura H (2011). Fluorescence-quenching screening of protein kinase C ligands with an environmentally sensitive fluorophore. *Bioconjug. Chem.* **22**, 923-930.
2. Fujii S, Masuno H, Taoda Y, Kano A, Wongmayura A, Nakabayashi M, Ito N, Shimizu M, Kawachi E, Hirano T, Endo Y, Tanatani A, Kagechika H; Boron cluster-based development of potent nonsecosteroidal vitamin D receptor ligands: direct observation of hydrophobic interaction between protein surface and carborane. J. Am. Chem. Soc., 133, 20933-20941 (2011).
3. Tamashiro T, Tanabe Y, Ikura T, Ito N and Oda M: Critical roles of Asp270 and Trp273 in the α-repeat of the carbohydrate-binding module of endo-1,3-β-glucanase for laminarin-binding avidity. *Glycoconj. J.*, **29**, 77–85 (2012).

Domestic conferences

1. Ito N; OIST/CCP4 school 2011: From data processing to structure refinement and beyond, Okinawa, 2011.

Laboratory of Gene Expression, School of Biomedical Science

Associate Professor	Hidehito KUROYANAGI
Project Assistant Professors	Takako IDEUE (-Mar, 2011), Mariko KIMURA (Dec-, 2011)
Technicians	Hiroshi KUROKAWA, Saaimatul HUQ (-Mar, 2011), Yohei WATANABE (Apr-, 2011), Marina TOGO (Apr-, 2011)

Research Interests

Recent whole genome sequence analyses revealed that a high degree of proteomic complexity is achieved with a limited number of genes. This surprising finding underscores the importance of alternative splicing, through which a single gene can generate structurally and functionally distinct protein isoforms. Based on transcriptome analyses, >90% of human multi-exon genes encode more than one alternatively spliced isoforms. The regulation of splice site usage, so called "splicing code" provides a versatile mechanism for controlling gene expression and for the generation of proteome diversity. Thus splicing code may play essential roles in many biological processes, such as embryonic development, cell growth, and apoptosis.

A Transgenic Reporter Worm System Offers a Path to Alternative Splicing Codes *in vivo*.

We have recently developed a transgenic alternative splicing reporter system that visualizes expression profiles of mutually exclusive alternative exons of a nematode *C. elegans* at a single cell level *in vivo* (Nat Meth, 2006; Nat Protoc, 2010). With this reporter system, we have visualized spatiotemporal profiles of tissue-specific and developmentally regulated alternative splicing events in living worms (Fig.1). By isolating and analyzing mutant worms defective in the color profiles, we have identified *trans*-acting factors and *cis*-elements involved in the splicing regulation (Mol Cell Biol, 2007; Genes Dev, 2008). Through these studies, we are coming to realize that molecular mechanisms of the alternative splicing regulation are conserved throughout metazoan evolution. Our reporter system will further elucidate expression profiles and regulation mechanisms of alternative splicing *in vivo*.

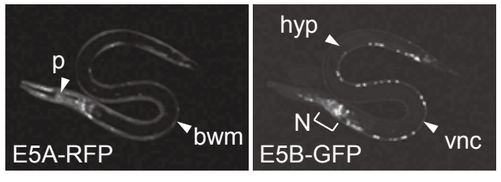


Fig1. An *egl-15* alternative splicing reporter worm with tissue-specific expression of exon 5A-RFP and exon 5B-GFP.

Global search for target genes of tissue-specific splicing factors *in vivo* by transcriptome analyses

Through genetic screening for mutant worms defective in alternative splicing regulation of specific model genes, we obtained mutants of a variety of splicing regulators. To further decipher splicing codes *in vivo*, we are globally searching for alternative splicing events that are affected in the splicing factor mutants by transcriptome analyses utilizing a next generation sequencer. We found tens of new target genes and further bioinformatics analyses predicted candidate *cis*-elements involved in the splicing regulation. We are currently analyzing tissue-specific splicing patterns of the new target genes *in vivo*, and investigating whether the predicted *cis*-elements are really involved in the tissue-specific regulation. These systematic analyses will lead to understanding of combinatorial regulation of alternative splicing events by multiple factors *in vivo*.

Regulation mechanisms of cardiac muscle-specific and developmentally regulated splicing of the *Ttn* gene in vertebrates

Dilated cardiomyopathy (DCM) is caused by mutations in sarcomere protein genes including *TTN*. Titin protein, encoded by the *TTN* gene, is a huge protein and passive tension of the myofibers is mainly attributed to titin protein. The *TTN* gene consists of 363 exons and pre-mRNA splicing patterns and apparent molecular weight of titin protein are developmentally regulated and vary between cardiac muscles and skeletal muscles. In DCM models, titin protein tends to be larger, sarcomere length increases and passive tension decreases. It is therefore considered that passive tension of the DCM heart can be restored by manipulating the titin isoforms. To this end, we are trying to analyze regulation mechanisms of the cardiac muscle-specific alternative splicing of the *TTN* gene in vertebrates.

Research Grants

1. Hidehito KUROYANAGI. Grant-in-Aid for Scientific Research on Innovative Areas. Ministry of Education, Culture, Sports, Science and Technology (MEXT).
2. Hidehito KUROYANAGI. Precursory Research for Embryonic Science and Technology (PRESTO), "RNA and Biofunctions". Japan Science and Technology Agency (JST).
3. Hidehito KUROYANAGI. JSPS Bilateral Joint Projects with France (SAKURA). Japan Society for the Promotion of Science (JSPS).

Laboratory of Chemical Bioscience

Professor Takamitsu Hosoya
Assistant Professor Suguru Yoshida
Assistant Professor Yuto Sumida

Enhanced clickability of doubly sterically-hindered aryl azides

Click reaction, epitomized by copper(I)-catalyzed azide–alkyne cycloaddition, has become one of the most reliable methods to connect molecules covalently in broad disciplines including materials chemistry and chemical biology. In particular, strain-promoted click reaction, a copper-free variant exploiting a cyclooctyne derivative that reacts spontaneously with an azide, has realized harmless chemical modification of biomolecules in cultured cells and in living animals. Recently, we have developed the "double-click" reaction to conjugate conveniently an azido-biomolecule with a small azido compound using Sondheimer diyne (**1**) as a bis-dipolarophile (Fig. 1a). In this reaction, an efficient assembly of two azides takes place by virtue of the two highly strained triple bonds of **1** providing bis-cycloadduct in high yield. The practical utility of the double-click strategy has been demonstrated by efficient labeling of azido-glycoconjugates on the cell surface as well as an azido-installed recombinant protein with a fluorescein-conjugated azide. While the double-click technique has been shown comparable in labeling efficiency to the single-click procedure, experimental and computational studies presented that the monoyne intermediate, considered as the initial cycloadduct of this reaction, is remarkably reactive than the starting diyne **1**, which might pose an insufficient conjugation. To make the double-click conjugation system more efficient, we have conceived the idea of performing a sequential double-click reaction using diazidobenzene derivative **2**, which bears two sterically-differentiated azido groups, with an anticipation to connect first alkyne at the less hindered side and then the second alkyne at the remaining sterically-hindered side (Fig. 1b). Contrary to our expectations, however, the click reaction of diazide **2** with strained alkyne **3a** proceeded predominantly at the sterically-hindered side furnishing **4b** in high yield (Fig. 1c). This unexpected but very intriguing result prompted us to elucidate the origin of enhanced reactivity of sterically-hindered azido group.

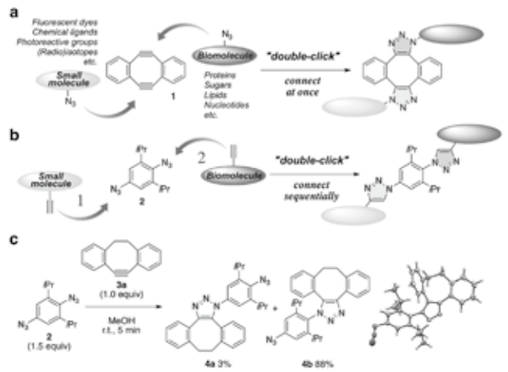
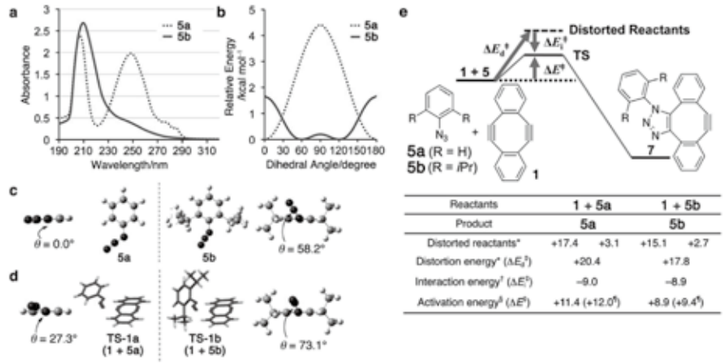


Fig. 1 Double-click reactions using a bis-reactive compound for efficient assembly of molecules.

A valuable hint to understand the role of bulky substituents in enhancing the reactivity of sterically-surrounded azido group was provided from UV absorption spectra of the azides (Fig. 2a). The intensity of the peak at long-wavelength region observed for **5a** (λ_{max} = 248 nm) decreased considerably in **5b**, suggesting that the conjugated state of the azido group with the aromatic ring between these azides differs substantially. The stationary structure of azides at the ground state optimized by a density functional theory (DFT) (B3LYP/6-31G(d)) method supported this implication indicating that the azido group of **5a** lies coplanar with the benzene ring, while that of **5b** is largely twisted out of the plane, forced by the bulky substituents at both ortho-positions (Fig. 2c). The calculation of rotation energy of the azido group also exhibited that **5a** takes predominantly the highly-conjugated structure, showing a sharp contrast with **5b**, which rather prefers the markedly-twisted conformation (Fig. 2b). Interestingly, the rotational barrier of the sterically-hindered azido group of **5b** was significantly lower than that of **5a**. These data have implied that the reactivity enhancement could be attributed to the inhibition of resonance between the aromatic ring and the azido group lowering its motional energy.



Reactants	1 + 5a		1 + 5b	
Product	5a		5b	
Distorted reactants[a]	+17.4	+3.1	+15.1	+2.7
Distortion energy[a] ($\Delta E_d^{\ddagger}$)	+20.4		+17.8	
Interaction energy[b] ($\Delta E_i^{\ddagger}$)	−9.0		−8.9	
Activation energy[a] ($\Delta E^{\ddagger}$)	+11.4 (+12.0[a])		+8.9 (+9.4[a])	

Fig. 2 Twisted-conformation of 2,6-diisopropylphenyl azide (**5b**) enhancing its clickability.
(a) Absorption spectra of phenyl azide (**5a**) and 2,6-diisopropylphenyl azide (**5b**) in MeOH (100 μM). (b) Calculated rotation energy for azido group of **5a** and **5b**. (c) Side and overhead views of the global minima on the potential energy surface obtained for **5a** and **5b**. (d) Calculated transition state (TS) structures for the first cycloaddition of diyne **1** with **5a** and **5b** and the side views of azides at the TS. θ indicates the rotational angle of the azido group from the aromatic plane. (e) Distortion, interaction and activation energies (in kcal mol^{-1}) for the first cycloaddition. All calculations were performed by a density functional theory (DFT) method (B3LYP/6-31G(d)) with a GAMESS suite of program codes on a TSUBAME 2.0 system at Tokyo Institute of Technology.

To gain a mechanistic insight, the distortion/interaction model, a generalized theory for 1,3-dipolar cycloadditions recently proposed by Houk and coworkers, led us to a comprehensive understanding. They elegantly explained the enhanced clickability of strained cycloalkynes by dividing the activation energy into distortion and interaction energies, demonstrating that the energy required to distort the 1,3-dipole and dipolarophile into their transition-state geometries is the crucial factor as well as the frontier molecular orbital interaction energy. To apply this theory, the transition state (TS) structures for the first cycloaddition of **1** with **5a** and **5b**, **TS-1a** and **TS-1b**, were also obtained at the same level of the theory (Fig. 2d). The activation energy for the reaction of **1** with **5b** was estimated to be 2.5 kcal mol^{-1} lower than that with **5a**, providing a good agreement with the experimental result (Fig. 2e). The difference in distortion energies, unexpectedly, was almost equal to that of the activation energies indicating that there is little difference in interaction energies, which must include the factor of steric repulsion arising between the reactants. Considering that the difference in individual distortion energy of diyne **1** between each reaction was comparatively smaller (0.4 kcal mol^{-1}) than that between azides (2.3 kcal mol^{-1}), the enhanced reactivity of **5b** can be mostly attributed to its decreased distortion energy compared with **5a**.

This work indicates, though it may sound paradoxical, a possibility of designing a highly reactive functional group by strategically locating it in an appropriate sterically-congested environment.

Other Research Topics

Development of new methodology, photoreactive functional groups, and molecular probes for radioisotope-free (non-RI) photoaffinity labeling to identify target proteins of bioactive small compounds.

Design and synthesis of efficient substrates for bioluminescence reactions and fluorescent probes for bioimaging and diagnosis of diseases.

Development of new PET (positron emission tomography) probe candidates for in vivo imaging to promote drug discovery.

Publications

1. Yoshida S, Shiraishi A, Kanno K, Matsushita T, Johmoto K, Uekusa H, Hosoya T. Enhanced clickability of doubly sterically-hindered aryl azides. Sci Rep 1:82 doi: 10.1038/srep00082, 2011.
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School of Biomedical Science, Laboratory of Organic and Medicinal Chemistry

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Research Outline

1. Medicinal Chemistry of Nuclear Receptors

Small hydrophobic molecules such as steroid hormones and activated vitamins A/D control various biological phenomena, including growth, development, metabolism, and homeostasis, by binding to and activating specific nuclear receptors. Nuclear receptors are ligand-inducible transcription factors that regulate the expression of their target genes. Nuclear receptors have become one of the most significant molecular targets for drug discovery in the fields of cancer, metabolic syndrome, autoimmune diseases, and so on. We have developed various agonists and antagonists of retinoid nuclear receptors, retinoic acid receptors (RAR α, β, γ) and retinoid X receptors (RXR α, β, γ) (Fig. 1). Among them, Am80 (tamibarotene, RAR α, β agonist) was approved as a drug for relapsed acute promyelocytic leukemia (APL) in Japan (2005).

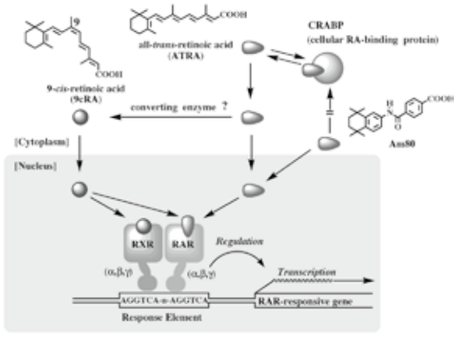

Fig. 1. Action mechanism of retinoic acids and synthetic retinoid, Am80.

We also developed novel fluorescent ligands for nuclear receptors. We found a lead compound **1** as progesterone receptor (PR) antagonists among the fluorescent coumarin compound library. Structural modification of compound **1** yielded highly active PR antagonists **2** and **3**. Among them, compound **2** exhibited potent PR binding affinity and changed its fluorescent properties by binding to PR (Fig. 2)

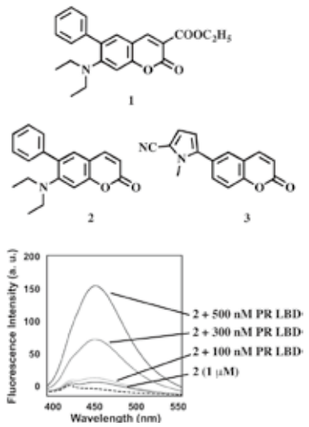

Fig. 2. Development of fluorescent progesterone antagonists.

2. Development of Novel Functional Fluorescent Molecules for Elucidation of Intracellular Signal Transduction Pathways

Functional fluorescent molecules are useful in many fields of scientific research, including analytical chemistry or cell biology. This year, we developed novel coumarin derivatives as sodium ion sensors. Compounds **4** and **5** have crown ether derivative at 6 position of coumarin skeleton, and their substituents at 7 position that would affect 6-substituent are different each other (diethylamino group for **4** and methoxy group for **5**) (Fig. 3). Compounds **4** and **5** showed different fluorescent changes induced by binding sodium ion, that is, increase of the fluorescent intensity for **4**, and shift of fluorescent maximum wavelength for **5**. Thus, various types of fluorescent sensors can be developed based on the coumarin structure.

3. Aromatic Architecture Based on the Steric Properties of *N*-Methylated Amides

The amide bond structure of amide derivatives often plays a key role in functions such as molecular recognition events or biological activities. In contrast to the extended

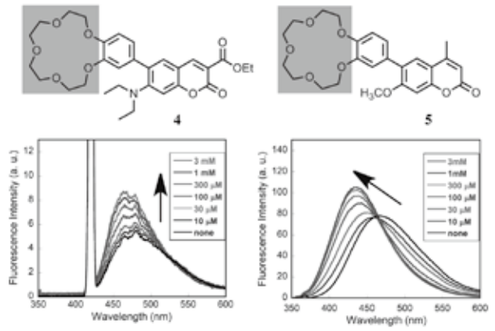

Fig. 3. Development of sodium ion sensors with different fluorescent properties.

trans structures of most secondary amides, the corresponding *N*-methylated compounds exist in cis form in the crystals and predominantly in cis form in various solvents. The cis conformational preference is useful as a building block to construct aromatic molecules with unique crystal or solution structures. Further, we found that cyclic triamide **6** with chiral backbone could be formed by condensation of *m*-(methylamino)benzoic acid, which formed unique capsule-type chiral dimer structure by simple recrystallization (Fig. 4).

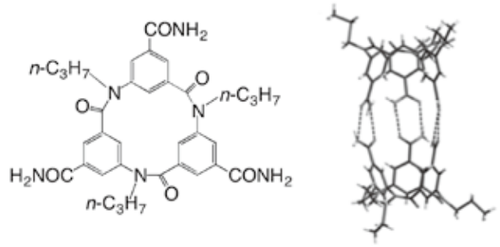

Fig. 4. Chiral dimer structure of cyclic triamide **6**.

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Laboratory of Biosystem Modeling, School of Biomedical Science

Associate Professor Yutaka Fukuoka

1. Bioinformatics

1) Effect of miRNAs on expressions of neighboring genes

MicroRNAs (miRNAs), which are non-protein-coding RNA molecules, are increasingly implicated in tissue-specific transcriptional control. Because there is mounting evidence for the localized component of transcriptional control, we investigated if there is a distance-dependent effect of miRNA. We have shown that expression levels of *C. elegans* genes are lower in the vicinity of 59 of 84 (71%) miRNAs as compared to genes far from such miRNAs and that the lower expression could be, in part, explained by an increased frequency of seed matching near miRNA. Further analyses on mouse and human data revealed that the localized effect in mammalian could be different from that in *C. elegans*.

To investigate the local effect of miRNA in cancer, we analyzed gene and miRNA expressions in hepatocellular carcinoma (HCC) and surrounding non-tumor tissues (N=20). We calculated the Pearson correlation coefficients between miRNAs neighboring genes and compared the coefficients in the tumor and non-tumor tissues. This analysis was repeated for intronic and intergenic miRNAs. Finally, the correlation coefficients between miRNAs and their target genes were compared in the tumor and non-tumor tissues. The results suggested that in HCC, more miRNAs correlated with neighboring genes positively and that the correlation coefficients between the intronic miRNAs and their host genes were higher in HCC. The correlation analysis between miRNAs and their targets suggested that there was no significant difference between the correlation coefficients in the tumor and non-tumor tissues.

2) Systems analysis of Inflammatory Bowel Disease based on comprehensive gene information

The rise of systems biology and availability of highly curated gene and molecular information resources have promoted a comprehensive approach to study disease as the cumulative deleterious function of a collection of individual genes and networks of molecules acting in concert. These human disease networks (HDN) have revealed novel candidate genes and pharmaceutical targets for many diseases and identified fundamental HDN features conserved across diseases. A network-based analysis is particularly vital for a study on polygenic diseases where many interactions between molecules should be simultaneously examined and elucidated. We employ a new knowledge driven HDN gene and molecular database systems approach to analyze Inflammatory Bowel Disease (IBD), whose pathogenesis remains largely unknown.

Based on drug indications for IBD, we determined sibling diseases of mild and severe states of IBD. The sibling disease of mild state was rheumatoid arthritis while those of severe state were psoriasis, ankylosing spondylitis and Bachet disease. Approximately 1,000 genes associated with the sibling diseases were retrieved from four public databases. After ranking the genes by the frequency of records in the databases, we obtained 250 and 253 genes highly associated with the mild and severe IBD states, respectively. We then calculated functional similarities of these genes with known drug targets and examined and presented their interactions as protein-protein interaction (PPI) networks. These PPI networks are the HDNs for the mild and severe states of IBD.

The identified HDNs are shown in Fig. 1. Both networks consisted of similar kinds of functional groups: i.e., inflammation, innate and acquired immune response, apoptosis, tumorigenesis, and tissue remodeling. However, the HDN of severe state included genes of tumorigenesis and apoptosis larger in number than that of mild state. The network of mild state also included some genes of tumorigenesis and apoptosis, but the genes stayed peripherally around a central gene group of inflammation and immunoregulation. In contrast, the HDN of severe state included close interconnection among genes from the tumorigenesis and apoptosis group. This feature was not observed in the HDN of mild state. The results demonstrate that this knowledge-based systems approach, predicated on functionally similar genes important to sibling diseases is an effective method to identify important components of the IBD human disease network. Our approach elucidates a previously unknown biological distinction between mild and severe IBD states.

2. A simulation study of a method for guiding the ablation catheter to the ablation site

Radio-frequency catheter ablation procedures for treating ventricular arrhythmias have evolved significantly over the past several years. In this project, we employ computer simulations to investigate the accuracy of a computer algorithm to guide the tip of an ablation catheter to the site of the origin of arrhythmias. In this process, we model both the electrocardiogram corresponding site of the origin of the arrhythmia and current pulses generated from a pair of electrodes at the tip of the ablation catheter with a single equivalent moving dipole (SEMD). In the forward problem we employ a realistic anatomic geometry torso model and the boundary element method. In the inverse problem we use the SEMD model in an infinite homogeneous volume conductor. Although the bounded, heterogeneous volume conductor in the inverse calculation introduces systematic error in the estimated compared to the true dipole location, we have demonstrated that the systematic error had minor influence in the ability of the algorithm to guide the tip of the ablation catheter to the site of the origin of the arrhythmia.

Currently, we are investigating influences of uncertainty in the electrode location on the accuracy of identifying the position of a SEMD as well as guiding the tip to the origin of arrhythmia. As the uncertainty increased, both the mean and standard deviation of the identification errors became larger. Also, the number of unsuccessful trials, in which the inverse algorithm could not converge, increased as the uncertainty increased. The differences between the results with different uncertainty levels were greater than those with different electrode noise levels, indicating that the influence of the uncertainty in electrode location had a stronger effect on the accuracy in identifying the SEMD location. However, when the uncertainty was small (up to 1 cm), the accuracy in guiding the catheter tip was not deteriorated significantly.

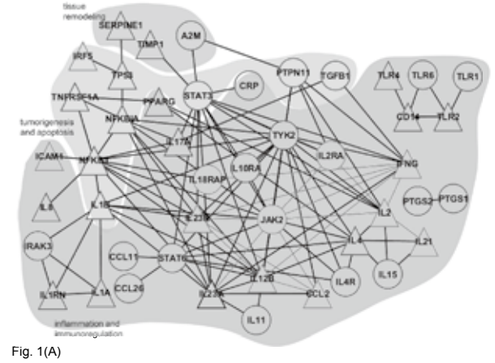

Fig. 1(A)

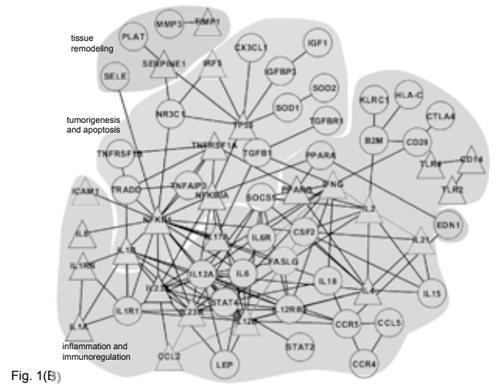

Fig. 1(B)

Fig.1. Human disease networks identified for the mild (A) and severe (B) states of IBD.

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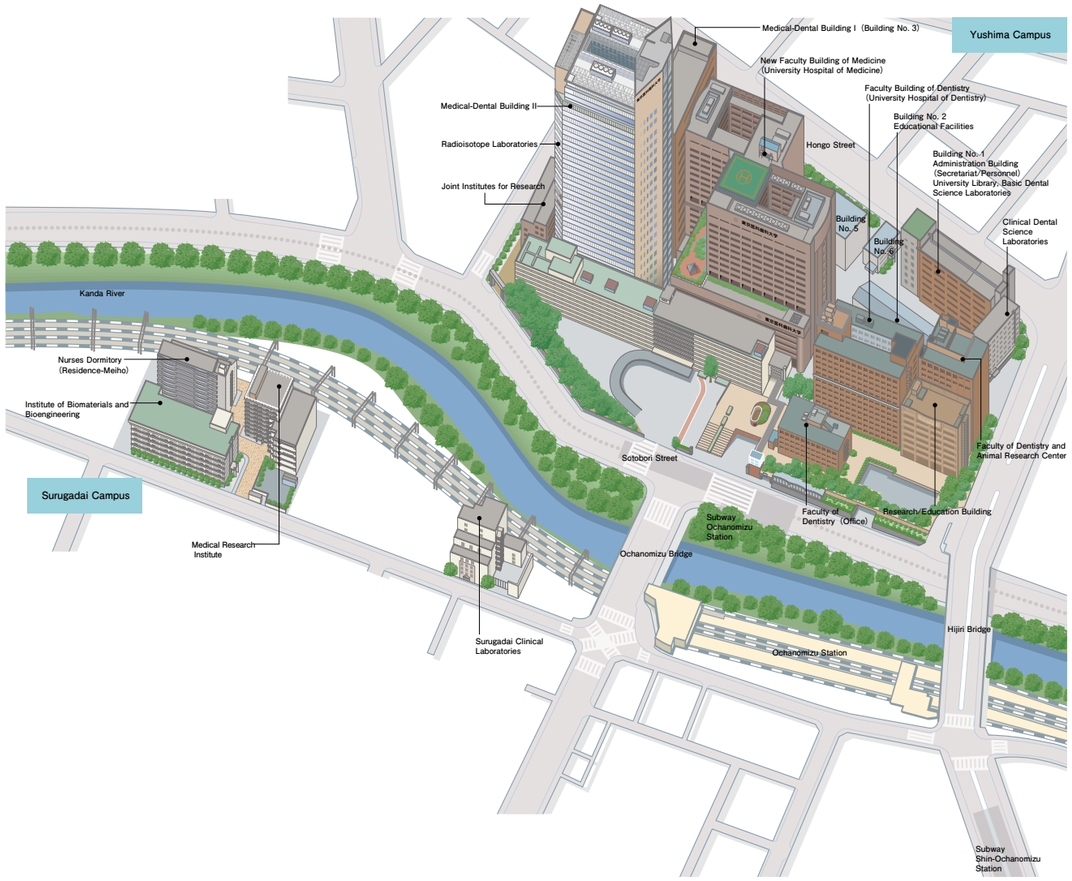

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