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ANNUAL REPORT 2011

Tokyo Medical and Dental University

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

Annual Report 2011

2011

Annual Report

Medical Research Institute

School of Biomedical Science

Biomedical Science PhD Program

Tokyo Medical and Dental University

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Medical Research Institute

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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 Functional Genomics, 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, Frontier Research Unit, Project Research Unit

School of Biomedical Science

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Department of Molecular Epidemiology, Department of Biodefense Research, Department of Stem Cell Medicine

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
	Project Reserch Unit		
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	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		
Medical Top Track (MTT) Program			
Division of Integrative Research	Department of Pathogenetic Regulation	Visiting Professor	Mitiko Go
	Department of Biosystem Generation		
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
Takeshi Tsubata
Faculty Meeting

Dean of Biomedical Science PhD Program
Hiroshi Tanaka

Research Divisions

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		
Department of Medical Omics Informatics		Professor	Hiroshi Mizushima

Highlight

The 9th Surugadai Symposium / The 1st MRI Joint Usage / Research Symposium

Surugadai International Symposium is annually held by Medical Research Institute and Graduate School of Biomedical Science, to bring together researchers from all over the world who are pursuing cutting edge research in medical and biomedical fields. The purpose of this symposium is to have eminent speakers discuss their exciting achievements and to help participating young researchers and senior scientists exchange knowledge, promote research, and foster collaboration.

In the year 2010, 9th Surugadai Symposium was held at newly opened M&D Tower Lecture Room1 (2nd floor) with joint sponsorship from the International Summer Program 2010 and the Joint Usage/Research Program of Medical Research Institute. The topic of the symposium this year was "Infection and Immunity." We invited 4 speakers from overseas, 1 from Japan, and 3 from TMDU. We had at least 173 participants, and simultaneous audio-visual transmission to M&D Tower Auditorium (2nd floor) was arranged.

<Symposium Program>

Opening Remarks

Dr. Kikuo Ohno (Dean/ Director of Faculty of Medicine, TMDU)

Morning Session

Chair: Toshiaki Ohteki (TMDU)

1. Dr. Masanori Hatakeyama
   University of Tokyo, Graduate School of Medicine, Department of Microbiology
   Helicobacter pylori CagA as a bacterial oncoprotein
2. Dr. Nawarat Wara-aswapati Charoen
   Khon Kaen University, Thailand
   Modulation of Wnt5a in peridontal diseases
3. Dr. Ruslan Medzhitov (canceled due to urgent hospitalization)
   Yale University, School of Medicine/ HHMI, USA
   Host defense: Immunity and Immunopathology

Afternoon Session

Chairs: Takeshi Tsubata (TMDU) & Tetsuya Taga (TMDU)

4. Dr. Toshiaki Ohteki (TMDU)
   Interferons wake up sleeping hematopoietic stem cells
5. Dr. Paola Ricciardi-Castagnoli
   Singapore Immunology Network, SIgN, A*STAR, Singapore
   Immune regulatory role of dendritic cells during sterile and non-sterile inflammation
6. Dr. Takeshi Tsubata (TMDU)
   Membrane-bound lectins and humoral immunity
7. Dr. James W. Kazura
   CaseWestern Reserve University, USA
   Progress and challenges toward malaria vaccine development
8. Dr. Hirokazu Tamamura (TMDU)
   Anti-HIV inhibitors and AIDS vaccines

Closing Remarks

Dr. Shigetaka Kitajima (Director, Medical Research Institute, TMDU)

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】

- We have identified macrophage-inducible C-type lectin (Mincle) as a saturated fatty acid-induced gene in macrophages infiltrated into obese adipose tissue.
- We have demonstrated that activation of RXRγ in the skeletal muscle leads to improvement of systemic glucose metabolism, thereby suggesting the potential usefulness of RXRγ as a therapeutic target of obesity-related diabetes.

【Molecular Pharmacology】

- Nanogel-based scaffold delivery of prostaglandin E(2) receptor (EP4) specific agonist in combination with low dosage of growth factor heals critical size bone defect.
- Per-1 is a specific clock gene regulated by parathyroid hormone (PTH) signaling in osteoblasts and is functional for the transcriptional events induced by PTH.
- Schnurri-2 deficiency counteracts against bone loss induced by ovariectomy.

【Molecular Cell Biology】

- IQGAP1 regulates the canonical Wnt signaling pathway.
- WNK-OSR1 pathway is conserved among many species.

【Molecular Neuroscience】

- ASK1 deficiency attenuates neural cell death in GLAST-deficient mice, a model of normal tension glaucoma.
- Aberrant detergent-insoluble excitatory amino acid transporter 2 accumulates in Alzheimer disease.

【Biodefense Research】

- Elucidation of prominent role for plasmacytoid dendritic cells in mucosal T cell-independent IgA induction.
- Identification of a new mechanism of tolerance induction under infectious and inflammatory conditions.

【Bio-informational Pharmacology】

- We performed genome-wide association study (GWAS) in Japanese population, and identified several atrial fibrillation-associated genes, including Japanese-specific genes.
- We examined the role of inflammation in pathogenesis of atrial fibrillation, and found that a danger signal released from stretched atrial myocytes plays an important role.
- We established human iPS cells from congenital arrhythmia syndrome, LQT, and generated the model to examine diseased cardiomyocytes.

【Stem Cell Regulation】

- We elucidated that neural stem cells maintain the self-renewal status cooperatively by FGF2, Wnt and Notch signaling pathways, in which two components in the growth pathway also lead to inhibition of neuronal and astroglial differentiation.
- We identified that Sprouty family proteins are involved in the developmental stage-dependent fate shift in neural stem cells from neurogenic to astrogliogenic.
- We elucidated a novel role of Sox17 in the maintenance of immature phenotype of hematopoietic progenitors in the aorta-gonad-mesonephros region.

Division of Advanced Molecular Medicine
Department of Molecular Medicine and Metabolism

Professor	Yoshihiro Ogawa, M.D., Ph.D.
Associate Professor	Yasutomi Kamei Ph.D.
Assitant Professor	Takayoshi Suganami M.D., Ph.D.
MTT Associate Professor	Yukio Yamamoto 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., Miyako Tanaka Ph.D., Michiko Itoh M.D., Ph.D., Rumi Hachiya M.D., Ph.D.

A. The metabolic syndrome and chronic 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. Indeed, obese adipose tissue is characterized by adipocyte hypertrophy, followed by increased angiogenesis, immune cell infiltration, extracellular matrix overproduction, and thus increased production of proinflammatory adipocytokines during the progression of chronic inflammation. We demonstrated that a paracrine loop involving saturated fatty acids and TNF α derived from adipocytes and macrophages, respectively, aggravates obesity-induced adipose tissue inflammation. Notably, saturated fatty acids, which are released from hypertrophied adipocytes via the macrophage-induced lipolysis, serve as a naturally occurring ligand for Toll-like receptor 4 (TLR4), thereby activating macrophages. Understanding the molecular mechanism underlying adipose tissue remodeling may lead to the identification of novel therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation (**J. Leukoc. Biol.** 88: 33-39, 2010) (**Figure**).

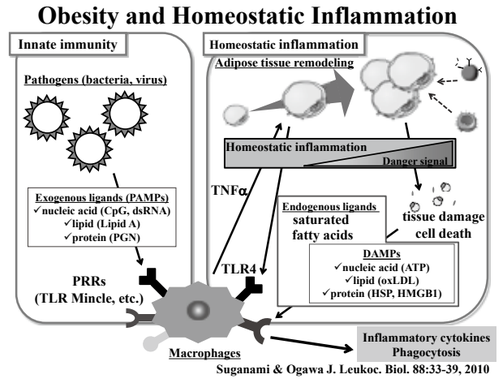
Using cDNA microarray analysis of coculture of 3T3-L1 adipocytes and RAW264 macrophages, we have recently found that macrophage-inducible C-type lectin (Mincle or also called Clec4e and Clecsf9), a type II transmembrane C-type lectin, is induced selectively in macrophages during the interaction between adipocytes and macrophages. Treatment with palmitate, a major saturated fatty acid released from 3T3-L1 adipocytes, induced Mincle mRNA expression in macrophages at least partly through the TLR4/NF-κB pathway. Mincle mRNA expression was increased in parallel with macrophage markers in the adipose tissue of obese mice and humans. The obesity-induced increase in Mincle mRNA expression was markedly attenuated in C3H/HeJ mice with defective TLR4 signaling relative to control C3H/HeN mice. Notably, Mincle mRNA was expressed in bone marrow cell (BMC)-derived proinflammatory M1 macrophages rather than in BMC-derived anti-inflammatory M2 macrophages in vitro. Our data suggest that Mincle is induced in M1 macrophages in obese adipose tissue at least partly through the saturated fatty acids/TLR4/NF-κB pathway, thereby suggesting its pathophysiologic role in obesity-induced adipose tissue inflammation. (**Diabetes** 60 : 819-826, 2011).

B. The skeletal muscle as a target organ in the metabolic syndrome

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 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 the control mice. In this study, we investigated the glucose metabolism of RXR γ mice with induced obesity and impaired glucose metabolism. Glucose tolerance and disposal rate were higher in the 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 KKA^y 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.

C. Role of Ca^{2+} mobilizations in LPS-induced cytokine production in macrophages.

Intracellular Ca^{2+} participates as a second messenger in TLR4-dependent signaling. However, how intracellular free Ca^{2+} concentrations ($[Ca^{2+}]i$) is increased in response to LPS and how they affect cytokine production are poorly understood. Here we examined the role of transient receptor potential (TRP), a major Ca^{2+} permeation pathway in non-excitable cells, in the LPS-induced cytokine production in macrophages. We demonstrated that TRPV2, a TRPV family member, is involved in the LPS-induced TNF α and IL-6 production and IκB α degradation in RAW264 macrophages. Our data also suggest that TRPV2 is involved in the LPS-induced Ca^{2+} mobilization from intracellular Ca^{2+} store and extracellular Ca^{2+}. In addition to Ca^{2+} mobilization through the IP_3-receptor, TRPV2-mediated intracellular Ca^{2+} mobilization is involved in NF-κB-dependent TNF α and IL-6 expression, while extracellular Ca^{2+} entry is involved in NF-κB-independent IL-6 production (**Biochem. Biophys. Res. Commun.** 398: 284-289, 2010).



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4. Y. Yamazaki, Y. Kamei, S. Sugita, F. Akaike, S. Kanai, S. Miura, Y. Hirata, B.R. Troen, T. Kitamura, I. Nishino, T. Suganami, O. Ezaki, and Y. Ogawa. The cathepsin L gene is a direct target of FOXO1 in the skeletal muscle. **Biochem. J.** 427: 171-178, 2010.
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9. A. Sato, H. Kawano, T. Notsu, M. Ohta, M. Nakakuki, K. Mizuguchi, M. Itoh, T. Suganami, and Y. Ogawa. Anti-obesity effect of eicosapentaenoic acid in high-fat/high-sucrose diet-induced obesity: importance of hepatic lipogenesis. **Diabetes** 59: 2495-2504, 2010.

Department of Molecular Pharmacology

Professor	Masaki Noda, M.D., Ph.D.
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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 underlying regulation of the development, differentiation, and function of each group of these cells.

Research Projects

1. Schnurri-2 Deficiency Counteracts Against Bone Loss Induced by Ovariectomy (Nagao M, Hayata T, Ezura Y, Noda M).

Schnurri (Shn)-2 is a transcriptional modulator of bone formation and bone resorption and its deficiency causes low turnover state with higher cancellous bone mass due to the defects in osteoclasts that exceeds the defects in osteoblasts in mice. We addressed whether such low turnover of bone remodeling in Shn2 deficiency may be modulated in the absence of estrogen that induces high turnover state in vivo. Ovariectomy reduced bone mass in wild type compared to sham operated control mice and such reduction in bone mass was also observed in Shn2 deficient mice. However, due to the high levels of basal bone mass in Shn2 deficient mice, the bone mass levels after ovariectomy were still comparable to sham operated wild-type mice. Analysis indicated that estrogen depletion increased bone resorption at similar levels in wild type and Shn2 deficient mice though the basal levels of osteoclast number was slightly lower in Shn2-deficient mice. In contrast, basal levels of bone marrow cell mineralization in cultures were low in Shn2-deficeint mice while estrogen depletion increased the mineralization levels to those that were comparable to sham wild type. This indicates that Shn2-deficient mice maintain bone mass at the levels comparable to wild-type sham mice even after ovariectomy-induced bone loss and this correlates with the high levels of mineralization activity in bone marrow cells after ovariectomy (J Cell Physiol, 2011).

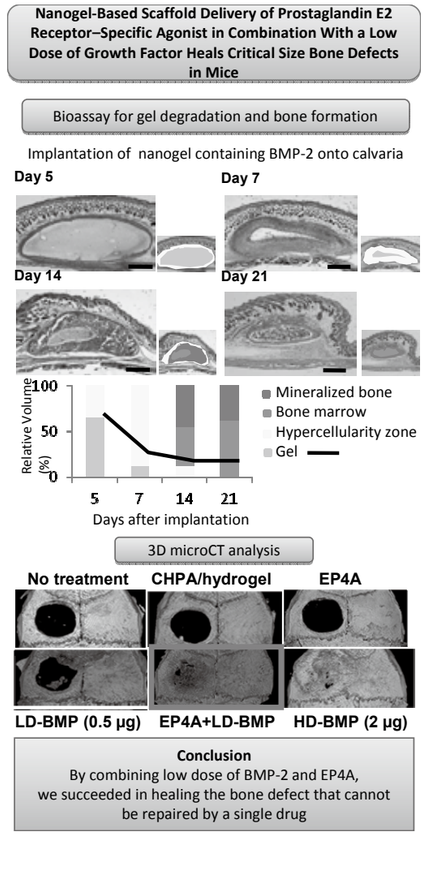
2. Per-1 is a specific Clock gene regulated by parathyroid hormone (PTH) Signaling in osteoblasts and is functional for the transcriptional events induced by PTH. (Hanyu R, Hayata T, Ezura Y, Noda M).

Per-1 is one of the clock genes and is known to regulate various biological events including bone mass determination. Parathyroid hormone is anabolic to bone while the mechanism of its action is not fully understood. Here, we examined the role of PTH on Per-1 gene expression under osteoblast specific PTH signaling. Constitutively active PTH receptor (caPPR) expressed specifically in osteoblasts in transgenic mice activates Per-1 gene expression in bone. This is specific as expression of other clock gene Bmal-1 is not affected by caPPR over-expression. Per-1 is also expressed in osteoblastic cell line. Interestingly, Per-1 expression is required for PTH signaling-induced CRE dependent transcription. This is forming a positive feed back loop in the anabolic action of PTH signaling and Per-1 in bone. These data indicate that PTH singling in osteoblasts activates Per-1 gene expression in vivo in association with its anabolic action in bone at least in part through the regulation of transcriptional events (J Cell Biochem, 2011).

Highlight

Nanogel-based scaffold delivery of prostaglandin E2 receptor–specific agonist in combination with a low dose of growth factor heals critical size bone defects in mice (Kamolratanakul P, Hayata T, Ezura Y, Noda M).

Regeneration of bone requires the combination of appropriate drugs and an appropriate delivery system to control cell behavior. However, the delivery of multiple drugs to heal bone is complicated by the availability of carriers. The aim of this study was to explore a new system for delivery of a selective EP4 receptor agonist (EP4A) in combination with low-dose bone morphogenetic protein 2 (BMP-2). Combined delivery of EP4A and BMP-2 was carried out with a nanogel-based scaffold in the shape of a disc, to repair critical size circle-shaped bone defects in calvariae that otherwise did not heal spontaneously. Combination treatment with EP4A and low-dose BMP-2 in nanogel efficiently activated bone cells to regenerate calvarial bone by forming both outer and inner cortical plates as well as bone marrow tissue to regenerate a structure similar to that of intact calvaria. EP4A enhanced low-dose BMP-2–induced cell differentiation and activation of transcription events in osteoblasts. These data indicate that combined delivery of EP4A and low-dose BMP-2 via nanogel-based hydrogel provides a new system for bone repair (Arthritis Rheum, 2011).



Publications

[Original articles]

1. Kamolratanakul P, Hayata T, Ezura Y, Kawamata A, Hayashi C, Yamamoto Y, Hemmi H, Nagao M, Hanyu R, Notomi T, Nakamoto T, Amagasa T, Akiyoshi K, Noda M. Nanogel-based scaffold delivery of prostaglandin E(2) receptor (EP4) specific agonist in combination with low dosage of growth factor heals critical size bone defect. Arthritis Rheum 63: 1021-33.
2. Hanyu R, Hayata T, Nagao M, Saita Y, Hemmi H, Notomi T, Nakamoto T, Schipani E, Kronenberg H, Kaneko K, Kurosawa H, Ezura Y, Noda M. Per-1 is a specific clock gene regulated by parathyroid hormone (PTH) signaling in osteoblasts and is functional for the transcriptional events induced by PTH. J Cell Biochem 112:433-8, 2011.
3. Nagao M, Saita Y, Hanyu R, Hemmi H, Notomi T, Hayata T, Nakamoto T, Nakashima K, Kaneko K, Kurosawa H, Ishii S, Ezura Y, Noda M. Schnurri-2 deficiency counteracts against bone loss induced by ovariectomy. J Cell Physiol 226:573-8, 2011.
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Division of Advanced Molecular Medicine
Department of Molecular Cell Biology

Professor Hiroshi Shibuya
Associate Professor Toshiyasu Goto
Assistant Professor Atsushi Sato

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.

Roles of IQGAP1 on the canonical Wnt signaling.

We investigated roles of IQGAP and DVL in the canonical Wnt signaling pathway, and we obtained the following results.

1) In vertebrates, three isoforms each of IQGAP and DVL have been identified: IQGAP1, IQGAP2 and IQGAP3, and DVL1, DVL2 and DVL3. We confirmed that IQGAP1 bound to each DVL isoform, and that DVL1 bound to each IQGAP isoform.

2) We found that the domain between the C-terminal IQ repeat domain and the N-terminal Ras GAP-like domain of IQGAP1 (termed DBD; Dishevelled Binding Domain) was responsible for binding to DVL1. The C-terminus of DVL1 (termed IBD; IQGAP Binding Domain) is necessary for binding to IQGAP1.

3) To examine how IQGAP affects DVL localization in Wnt signaling, we investigated the subcellular distribution of DVL fused to green fluorescent protein (GFP) in Xenopus embryonic cells. Depletion of xIQGAP1 by antisense morpholino oligonucleotides (*xIQGAP1*-MO) did not affect the membrane localization of xDVL2-GFP when co-expressed with *Xenopus frizzled 7*. However, depletion of xIQGAP1 decreased the nuclear localization of xDVL2-GFP when co-expressed with *Xwnt-8*. Depletion of xDVL2 also decreased the nuclear localization of xIQGAP1-GFP when co-expressed with *Xwnt-8*.

4) Each fusion protein of GFP to xDVL2-ΔIBD or xIQGAP1ΔDBD was not accumulated in nucleus when co-expressed with *Xwnt-8*.

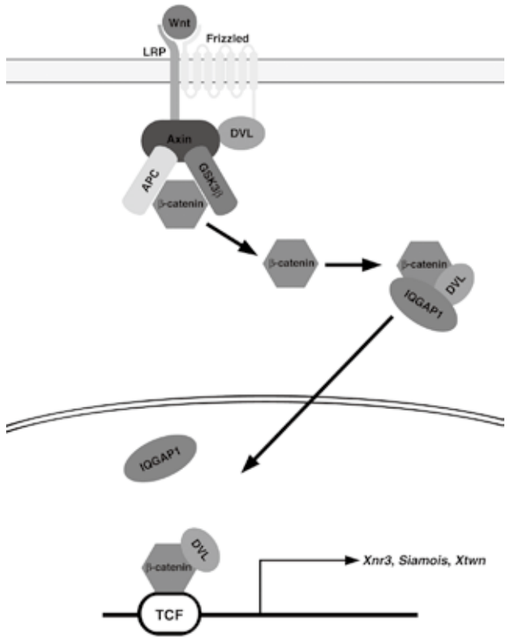
5) xIQGAP1, xDVL2 and β-catenin can form a complex, and each protein contributes to the nuclear localization of each other under the Wnt stimulation.

6) When *xDVL2*, *Xwnt-8* or *β-catenin* mRNA was injected into the ventral sides of four-cell embryos, a secondary axis was formed and Wnt signal target genes were induced. Depletion of IQGAP1 suppressed both induction of secondary axis and Wnt target genes.

7) xIQGAP1 specifically functions in the Wnt signaling pathway, depletion of other isoforms, xIQGAP2 or xIQGAP3 did not have a marked effect on the expression of Wnt target genes. Depletion of xDVL1 or xDVL3 showed redundant effect on the canonical Wnt signal pathway, and depletion of all three xDVLs reduced the expression of the Wnt target genes and the nuclear localization of xIQGAP1 and β-catenin.

8) xIQGAP1 was not recruited to the promoter regions of the Wnt target genes while we observed the recruitment of xDVL2 and β-catenin to the promoter regions of Wnt target genes in *Xenopus*. Moreover, depletion of xIQGAP1 had no effect on the β-catenin stability in the cytoplasm of the dorsal marginal cells in *Xenopus*.

Roles of IQGAP1 on the canonical Wnt signaling



These results demonstrate the existence of a new molecular mechanism regulating the nuclear localization of β-catenin and modulating canonical Wnt signaling.

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

1) Evolutional conservation of WNK signaling pathway

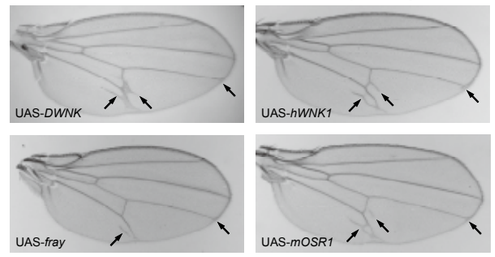
We generated the overexpression system of human WNK, mouse OSR1 and *Drosophila* OSR1 homologue Fray, as well as Drosophila WNK (DWNK). When these were overexpressed at the posterior compartment of wing by *hh-Gal4* driver, all of overexpression caused similar phenotypes such as ectopic wing veins and delta phenotype of the tip of vein 4. These results suggest that WNK pathway is conserved among many species. In addition, since OSR1/Fray are kinases and both overexpression phenotypes are similar, the substrate of the OSR1/Fray, the downstream target, may be also conserved.

2) The downstream transcription factor

The mosaic analysis using *DWNK* mutant showed the defect of abdominal development. The overexpression of the kinase dead form of DWNK, which might work as a dominant negative, also showed the similar phenotype. 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, in DWNK mutant embryos, the expression of the transcription factor X was reduced. These results indicate that the transcription factor X works at the downstream of WNK signaling pathway.

The homologues of gene X are conserved in mammals. In mouse, gene X is known to be involved in the development of the palate and the nervous system. Since the pathological conditions of PHAII showed an intellectual impairment and dental abnormalities, these may suggest that this gene X is involved in the pathogenesis of PHAII. We analyzed the relationship between WNK and the transcription factor X using the cell culture system. WNK is known to be activated by the hypertonic condition. In NIH3T3 cells, the transcription of the transcription factor X was induced by the hypertonic condition. And when we knocked down both *WNK1* and *WNK4* transcripts by siRNA, the transcription of the transcription factor X was not induced under same hypertonic condition. WNK overexpression induced the transcription of the transcription factor X in NIH3T3 cells. In addition, OSR1 oversexpression also induced the expression of the transcription factor X. These results suggest that the transcription factor X is the new downstream target of WNK signaling pathway.

Evolutional conservation of WNK pathway



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Division of advanced Molecular Medicine
Department of Molecular Neuroscience

Professor Kohichi Tanaka
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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.

Apoptosis signal-regulating kinase 1 (ASK1) is an evolutionarily conserved mitogen-activated protein kinase (MAPK) kinase kinase and has an important role in stress-induced retinal ganglion cell (RGC) apoptosis. In the mammalian retina, glutamate/aspartate transporter (GLAST) is a major glutamate transporter, and the loss of GLAST leads to optic nerve degeneration similar to normal tension glaucoma (NTG). In GLAST(/) mice, the glutathione level in the retina is decreased, suggesting the involvement of oxidative stress in NTG pathogenesis. To test this hypothesis, we examined the histology and visual function of GLAST(+/):ASK1(/) and GLAST(/):ASK1(/) mice by multifocal electroretinograms. ASK1 deficiency protected RGCs and decreased the number of degenerating axons in the optic nerve. Consistent with this finding, visual function was significantly improved in GLAST(+/):ASK1(/) and GLAST(/):ASK1(/) mice compared with GLAST(+/) and GLAST(/) mice, respectively. The loss of ASK1 had no effects on the production of glutathione or malondialdehyde in the retina or on the intraocular pressure. Tumor necrosis factor (TNF)-induced activation of p38 MAPK and the production of inducible nitric oxide synthase were suppressed in ASK1-deficient Müller glial cells. In addition, TNF-induced cell death was suppressed in ASK1-deficient RGCs. These results suggest that ASK1 activation is involved in NTG-like pathology in both neural and glial cells and that interrupting ASK1-dependent pathways could be beneficial in the treatment of glaucoma, including NTG.

Alzheimer disease (AD) is characterized by deposition of amyloid-beta, tau, and other specific proteins that accumulate in the brain in detergent-insoluble complexes. Alzheimer disease also involves glutamatergic neurotransmitter system disturbances. Excitatory amino acid transporter 2 (EAAT2) is the dominant glutamate transporter in cerebral cortex and hippocampus. We investigated whether accumulation of detergent-insoluble EAAT2 is related to cognitive impairment and neuropathologic changes in AD by quantifying detergent-insoluble EAAT2 levels in hippocampus and frontal cortex of cognitively normal patients, patients with clinical dementia rating of 0.5 (mildly impaired), and AD patients. Parkinson disease patients served as neurodegenerative disease controls. We found that Triton X-100-insoluble EAAT2 levels were significantly increased in patients with AD compared with controls, whereas Triton X-100-insoluble EAAT2 levels inpatients with clinical dementia rating of 0.5 were intermediately elevated between control and AD subjects. Detergent insolubility of presenilin-1, a structurally similar protein, did not differ among the groups, thus arguing that EAAT2 detergent insolubility was not caused by nonspecific cellular injury. These findings demonstrate that detergent-insoluble EAAT2 accumulation is a progressive biochemical lesion that correlates with cognitive impairment and neuropathologic changes in AD. These findings lend further support to the idea that dysregulation of the glutamatergic system may play a significant role in AD pathogenesis.

2. Role of MYCN in medulloblastoma

Medulloblastoma (MB) is the most common malignant brain tumor of childhood. Sonic Hedgehog (SHH) signaling drives a minority of MB, correlating with desmoplastic pathology and favorable outcome. The majority, however, arises independently of SHH and displays classic or large cell anaplastic (LCA) pathology and poor prognosis. To identify common signaling abnormalities, we profiled mRNA, demonstrating misexpression of MYCN in the majority of human MB and negligible expression in normal cerebella. We clarified a role in pathogenesis by targeting MYCN (and luciferase) to cerebella of transgenic mice. MYCN-driven MB showed either classic or LCA pathologies, with Shh signaling activated in approximately 5% of tumors, demonstrating that MYCN can drive MB independently of Shh. MB arose at high penetrance, consistent with a role for MYCN in initiation. Tumor burden correlated with bioluminescence, with rare metastatic spread to the leptomeninges, suggesting roles for MYCN in both progression and metastasis. Transient pharmacological down-regulation of MYCN led to both clearance and senescence of tumor cells, and improved survival. Targeted expression of MYCN thus contributes to initiation, progression, and maintenance of MB, suggesting a central role for MYCN in pathogenesis.

3. A neural circuit critical for imprinting behavior in chicks

Imprinting behavior in birds is elicited by visual and/or auditory cues. It has been demonstrated previously that visual cues are recognized and processed in the visual Wulst (VW), and imprinting memory is stored in the intermediate medial mesopallium (IMM) of the telencephalon. Alteration of neural responses in these two regions according to imprinting has been reported, yet direct evidence of the neural circuit linking these two regions is lacking. Thus, it remains unclear how memory is formed and expressed in this circuit. Here, we present anatomical as well as physiological evidence of the neural circuit connecting the VW and IMM and show that imprinting training during the critical period strengthens and refines this circuit. A functional connection established by imprint training resulted in an imprinting behavior. After the closure of the critical period, training could not activate this circuit nor induce the imprinting behavior. Glutamatergic neurons in the ventroposterior region of the VW, the core region of the hyperpallium densocellulare (HDCo), sent their axons to the periventricular part of the HD, just dorsal and afferent to the IMM. We found that the HDCo is important in imprinting behavior. The refinement and/or enhancement of this neural circuit are attributed to increased activity of HDCo cells, and the activity depended on NR2B-containing NMDA receptors. These findings show a neural connection in the telencephalon in Aves and demonstrate that NR2B function is indispensable for the plasticity of HDCo cells, which are key mediators of imprinting.

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Department of Biodefense Research

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Our research projects focus on biodefense and maintenance of immunological homeostasis. Our goal is to define the molecular mechanism of immune cell differentiation and activation under healthy conditions as well as conditions of disease. To accomplish this goal, we are trying to clarify the molecular basis of induction and failure of immunological tolerance by focusing on dendritic cells and mucosa-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 (**Immunol Rev** 234, 247-258(2010)).

We have recently found prominent role for pDCs in mucosal T cell-independent (TI) IgA production (**Immunity** 34, 247-257 (2011)). Although both conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs) are present in the gut-associated lymphoid tissues (GALT), the roles of pDCs in the gut remain largely unknown. When pDCs of the mesenteric lymph nodes (MLNs) and Peyer' s patches (PPs) (which are representative GALT) were cultured with naïve B cells to induce TI IgA class switch recombination (CSR), IgA production was substantially higher than in co-cultures of these cells with cDCs. IgA production 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 (Fig. 1). 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.

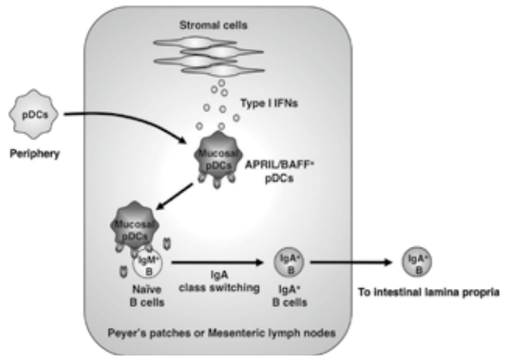

Fig.1 pDC conditioning in the gut-associated lymphoid tissue (GALT)
In the GALT, stromal cells produce type I interferons (IFNs) in response to commensal bacteria, and the stromal cell-derived type I IFNs induce pDC expression of APRIL and BAFF. Such "mucosal-type" pDCs induce effective IgA class switching in B cells.

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. Interestingly, 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. We are currently analyzing the details of this DC progenitor.

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 infectious and inflammatory conditions, and we are currently analyzing the molecular mechanisms and its physiological relevance.

3. Activation of hematopoietic stem cells by interferons and its clinical applications

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 irradiation for the treatment of congenital metabolic disorder.

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4. Asano J, Tada H, Onai N, Sato T, Horie Y, Fujimoto Y, Fukase K, Suzuki A, Mak T.W, and Ohteki T. Nod-like receptor signaling enhances dendritic cell-mediated cross-priming in vivo. **J Immunol** 184,736-745 (2010)

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1. Tezuka H, and Ohteki T. A gas governing mucosal immunity. **Vaccine**, 2 8039-8040 (2010).
2. Tezuka H, and Ohteki T. Regulation of intestinal homeostasis by dendritic cells. **Immunol Rev** 234, 247-258 (2010)

Department of Bio-informational Pharmacology

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Assistant Professor	Yusuke Ebana, M.D., Ph.D.
MTT Assistant Professor	Tetsuo Sasano, M.D., Ph.D.

This laboratory focuses on understanding fundamental pathophysiological roles of ion channels and transporters in cardiovascular system. We employ multidisciplinary approach (patch-clamp, cell biology, optical imaging, genetic analysis and computational analysis) in order to seek novel regulatory mechanisms and modulatory molecules/compounds of ion channels and transporters in cardiac myocytes, vascular smooth muscle and endothelial cells, and circulating cells in vessels (monocytes and macrophages). Our ultimate goal is to establish novel diagnostic and therapeutic strategy for intractable and common cardiovascular diseases.

1. Atrial fibrillation

Atrial fibrillation (AF) is the most frequent persistent arrhythmias. Cardiogenic cerebral embolism, a frequent complication of AF, significantly affects the quality of life (QOL), and is one of the most frequent causes of bedridden old persons. The incidence of AF increases rapidly with age, and the age greater than 70 is the AF risk age. Since Japanese have the longest life-expectancy in the world, AF becomes the serious problem, and the establishment of strategy to prevent and treat AF is urgent task.

a. AF associated gene polymorphism

Recent clinical data indicate the presence of genetic background for development of AF. In collaboration with RIKEN, we performed GWAS (genome-wide association study) to search for SNPs associated with AF. In the 1st project performing typing of 230,000 Tag SNPs, and in the 2nd project performing typing of 610,000 Tag SNPs, we found one genetic risk (Lab. name AF#1) with very high association ($p<10^{-30}$), and several SNPs with borderline significance (p-value 10^{-5} – 10^{-8}). For the borderline SNPs, we further increased the number of samples. As a result, the p-value reached less than 10^{-8} in one SNP (AF#2), and decreased in 3 SNPs (AF#3-5). Using 2 SNPs with the p-value less than 10^{-8}, we could predict the risk of AF with more than 5-fold difference. Using the most significant SNP (AF#2), the recurrence ratio of AF after pulmonary vein isolation procedure was about 15%. Thus, we found AF genetic risks in Japanese population, which are useful for risk stratification of AF.

b. Inflammatory and immunological mechanism in AF pathogenesis

Inflammatory and immunological mechanism has been implicated in the development of AF. Previously, we found in *in vitro* experiments that stretch of atrial myocyte-derived cell line, HL-1, induced recruitment of macrophages, which was inhibited by a chemical compound in a dose-dependent manner. Wu further tested this mechanism in *in vivo* model of atrial dilatation by trans-aortic constriction (TAC). TAC induced invasion of macrophages, fibrillation, and remodeling in atrium, and AF induction. Pre-treatment with the chemical compound suppressed TAC-induced invasion of macrophages, fibrillation, and remodeling in atrium, and AF induction. Thus, stretch-induced macrophage recruitment appears to be the early pathological signal for AF development.

2. Ventricular tachyarrhythmias and sudden cardiac death (SCD)

Ventricular tachyarrhythmias are the most common cause of SCD; however, clarification of their pathogenesis and establishment of prevention and treatment are the biggest challenge in arrhythmia researches. Our laboratory addressed this issue using 2 genetically engineered mouse model.

a. Analysis of *NOS1AP* KO mice

NOS1AP was found to be the most closely related to the QT interval and SCD in European and American GWAS. In the previous year, we confirmed that *NOS1AP* KO mice had slightly prolonged QT interval compared to WT mice. This year, we compared arrhythmogenicity under various pathophysiological conditions between *NOS1AP* KO mice and WT mice. Application of K^+ channel blockers, pharmacological sympathetic stimulation, ischemia/reperfusion, or myocardial infarction did not change arrhythmogenecity and sudden death ratio between WT and KO mice. In contrast, trans-aortic constriction (TAC) and application of Ca^{2+} channel blockers significantly increased arrhythmogenecity and sudden death ratio in KO mice compared to WT mice. Our data suggest that abnormal cardiac function and Ca^{2+} handling appear to be the cause of increased arrhythmogenicity in *NOS1AP* KO mice.

b. KO mice of a transcription factor present in cardiac conduction system

Purkinje fiber, peripheral conduction system, is suggested to play a critical role in development of ventricular fibrillation (VF) and SCD. However, the animal model to examine the role of Purkinje fiber in VF and SCD is not available. We created mice disrupted a transcription factor specifically expressed in peripheral conduction system. This KO mice exhibited serious arrhythmias during nocturnal activity and induced by swimming exercise and pharmacological sympathetic nerve stimulation (Fig. 1). This mouse model provides a novel animal model for study of peripheral conduction system in development of VF and SCD.

3. Use of iPS-derived cardiomyocytes for arrhythmia researches (Fig. 2)

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. Cardiomyocytes differentiated from human iPS cells would bring marked advance in arrhythmias researches. We take following 2 approaches;

a. Establishment of human iPS-derived cardimyocytes (hiPS-CM) from LQT patients

We try to establish and characterize iPS cell-derived cardiomyocytes from human fibroblasts obtained from congenital LQT patients. We have able to established 1 line for type 1 LQT and 2 lines for each of type 2 and 3 LQT. Our data showed that hiPS-CM from LQT patients maintain electrophysiological phenotype found in LQT patients' hearts.

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

New drugs are developed in a research consisted of ①discovery of a lead compound→②pre-clinical study in animals→③clinical study. Because of cardiac toxicity in human, many candidates compounds advanced to clinical trials fail to pass, which is because of the lack of assay system in human tissues in pre-clinical study step. Cardiomyocytes derived from human iPS cells could provide an assay system in human tissues in pre-clinical study step, and facilitate new drug discovery and prevent unexpected drug side effects.

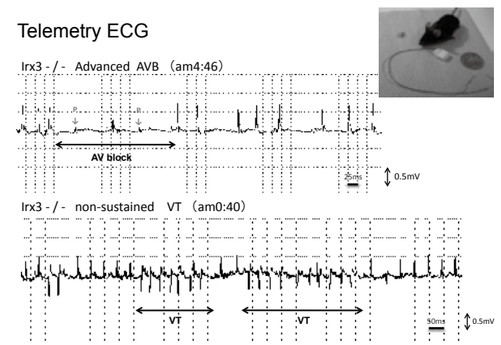

Fig.1 Arrhythmias recorded with telemetry ECG
In a KO mouse, advanced AV block (upper panel) and recurrent non-sustained VT (lower panel) were recorded.

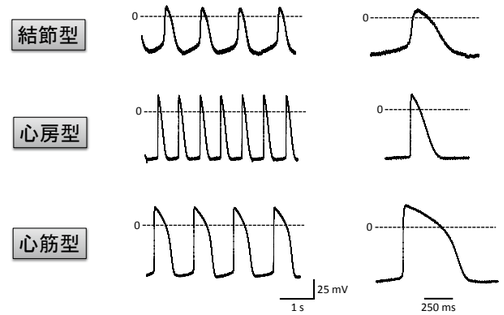

Fig.2 Action potential recorded in iPS-derived cardiomyocytes
Nodal type (upper panel), atrial type (middle panel), and ventricular type (lower panel) action potential were recorded in iPS-derived cardiomyocytes.

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Department of Stem Cell Regulation

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Research Student	Yin WU

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
5) Epigenetic regulation of neural development

1. Regulation of neural stem cell self renewal

Self-renewing proliferation of neural stem/progenitor cells (NSCs/NPCs) is intimately linked to the inhibition of their neuronal and glial differentiation. However, their molecular linkage has been poorly understood. We recently reported that bFGF and Wnt signals cooperate to promote NSC/NPC proliferation via GSK3beta inactivation, beta-catenin accumulation and cyclin D1 expression. We have also shown that this beta-catenin accumulation results in potentiation of Notch signaling that leads to inhibition of neuronal differentiation. To fully understand the mechanisms underlying self-renewal, it remained unclear how these growth factor signals inhibit glial differentiation as well. In the recent study in our department, we first found that forced expression of cyclin D1, a component of the NSC/NPC proliferative signaling pathway, inhibited GFAP-positive astroglial differentiation of NSCs/NPCs induced by LIF and BMP2. Importantly, CDK4 inhibitor, which disturbs the cyclin D1 function as a cell cycle progression factor, hardly counteracted the inhibitory effect of cyclin D1 on GFAP expression, indicating that cyclin D1 inhibited astroglial differentiation in a manner independent of cell cycle regulation. Furthermore, inhibition of astrocyte differentiation by a GSK3beta inhibitor was relieved by knockdown of cyclin D1 in NSCs/NPCs. We also found that cyclin D1 physically interacted with STAT3 and p300, and inhibited the LIF-mediated formation of the STAT3-p300 transcriptional complex. These data suggested that a proliferative signaling component, cyclin D1, contributes to inhibition of astroglial differentiation by disturbing an astrogliogenic transcriptional complex.

2. Fate regulation of neural stem cells

The three major cell types in the central nervous system, i.e., neurons, astrocytes and oligodendrocytes, are generated from common precursor cells called neural stem cells (NSCs). bFGF is crucial for self-renewal and maintenance of undifferentiated state of NSCs. To analyze gene expression in NSCs cultured with or without bFGF, microarray analysis was performed. Among the genes whose expression was noticeably difference, we focused on sprouty gene family. Protein product of the sprouty gene family is a negative regulator of receptor tyrosine kinase pathways involved in cell differentiation, proliferation and survival. Though expression of sprouty genes is detected in the central nerves system in mouse embryo, the role of Sprouty in neural development remained unclear. Among four sprouty genes found in mammals, sprouty4 was expressed in the ventricular zone of the developing brain where NSCs abundantly exist. We further investigated the function of Sprouty4 on neural development. Sprouty4 was introduced into NSCs prepared from E14.5 mouse telencephalons by using retrovirus vector. Sprouty4 overexpression significantly suppressed NSC proliferation and neurosphere formation. Moreover, sprouty4 induced neuronal differentiation even in the presence of bFGF. Consistent with these observations, the number of neurospheres increased in Sprouty4 KO NSC cultures and neuronal differentiation delayed in Sprouty4 KO cerebral cortex at early developmental stage. Taken together, Sprouty4 is involved in the coordination of NSC proliferation and differentiation, which is important in brain development.

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

Glial cells exhibit various functions to support neural activities: In addition to their classical roles in the delivery of nutrition to neurons, recycling of neurotransmitters and formation of blood-brain barrier, recent studies have demonstrated that glial cells are widely involved in the regulation of synaptogenesis and synaptic activities. When and how gilal cells are specified to exhibit such diverged functions has been poorly understood. We hypothesized that the distinct neuroepithelial domains may contribute to glial 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 (GFP). This will be a useful tool to answer the above question.

4. Characterization of tumor stem cells

Tumor stem cells (TSCs) are primarily responsible for tumor maintenance and relapse, and thus considered as a potential target to eradicate tumors. C6 rat glioma cell line contains a sub-population 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. Recently, it has been proposed that disrupting "tumor stem cell niche" could impair TSC self-renewal and thereby significantly inhibit the tumor growth. We found that MP cells could function as a microenvironment that supports SP cells. Consistent with previous studies, cultures initiated with only SP cells exhibited more than 80% of MP frequency after 1 week, but thereafter the proportion was retained for at least 3 weeks, suggesting that the existence of MP cells was helpful to the maintenance of SP cells. To confirm the positive effect of MP cells on SP maintenance, we co-cultured SP and MP at the constant total number of cells with different SP/MP ratio, and observed better maintenance of the SP cells in a MP-to-SP ratio-dependent manner. To further determine whether the direct interaction with MP cells is required for SP cell maintenance, we co-cultured SP cells with prefixed MP cells, and found efficient maintenance of SP cells, indicating that SP maintenance is mediated by close contact with MP cells. cDNA microarray analysis identified upregulated expression, in MP cells, of molecules known to function in cell communication. Our findings suggest that TSC-derived non-tumorigenic progeny could be a specific candidate for TSC-targeting therapy.

5. Characterization of hematopoietic stem cells in yolk sac

Recent studies have shown that the yolk sac (YS) harbors definitive, in addition to primitive, hematopoietic activity. However, the population of YS cells contributing to definitive hematopoiesis has not been identified. We characterized the YS cell populations in mouse embryos from E9.5 to E14.5 in view of the CD45/c-Kit expression profiles and hematopoietic activity. YS cells from E9.5 to E11.5 could be divided into six populations: CD45 negative/ c-Kit negative, CD45 negative/ c-Kit low, CD45 negative/ c-Kit high, CD45 low/ c-Kit high, CD45 high/ c-Kit high, and CD45 high/ c-Kit very low. Among these populations, CD45 low/ c-Kit high cells showed the highest multilineage hematopoietic colony-forming activity and the ability to differentiate into myeloid and B lymphoid cells when cultured with stromal cells. This cell population disappears from the YS at around E12.5, when the site of hematopoiesis has already shifted.

Publications

[Original Article]

1. Inoue T, Kagawa T, Inoue-Mochita M, Isono K, Ohtsu N, Nobuhisa I, Fukushima M, Tanihara H, and Taga T: Involvement of the HIPK family in regulation of eyeball size, lens formation and retinal morphogenesis. FEBS Lett. 584:3233-3238, 2010.
2. Yoshinaga Y, Kagawa T, Shimizu T, Inoue T, Takada S, Kuratsu J, and Taga T: Wnt3a promotes hippocampal neurogenesis by shortening cell cycle duration of neural progenitor cells. Cell. Mol. Neurobiol. 30:1049-1058, 2010.
3. Ramadan A, Nobuhisa I, Yamasaki S, Nakagata N, and Taga T: Cells with hematopoietic activity in the mouse placenta reside in side population. Genes Cells 15:983-994, 2010.
4. Tabu K, Kimura T, Sasai K, Wang L, Bizen N, Nishihara H, Taga T, and Tanaka S: Analysis of an alternative human CD133 promoter reveals the implication of Ras/ERK pathway in tumor stem-like hallmarks. Mol. Cancer 9:39, 2010.

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 molecular mechanism of learning disturbance in PQBP1-mutation
- Discovery of a novel molecule Maxer regulating non-cell autonomous neurodegeneration

【Pathological Cell Biology】

- Identification of a novel candidate drug for cancer treatment
- Analysis of pathogenesis of Parkinson Disease caused by the loss of Omi/HtRA2

【Developmental and Regenerative Biology】

- Discovery of a molecular mechanism of p38 MAPK-dependent cell differentiation of murine ES cells into cardiomyocytes and neurons.
- Elucidation of physiological roles of JNK signaling pathway in zebrafish embryogenesis.

【Stem Cell Bilogy】

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

【Immunology】

- Demonstration of differentiation-inducing capacity of CD40 signaling in germinal center B cells.
- Proposal of new model for augmented B cell activation by signaling through IgG-BCR (B cell receptor).

【Molecular Pathogenesis】

- Calcium sensitizer delayed the development of disease in a mouse model for hereditary dilated cardiomyopathy.
- A TIM1 haplotype, D3-A, which is related to lower expression was significantly associated with better prognosis HIV-1 infection.

【Virus Research Unit】

- Development of a chronic active EBV infection model animal by transplanting patient’s PBMC to severely immunedeficient mice NOG.
- 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**	**Yasushi Enokido**
Project Lecturer	**Kazuhiko Tagawa**	**Adjunct Lecturer**	**Masaki Sone**
Assistant Professor	**Takuya Tamura**		
Project Assistant Professor	**Hikaru Ito, Akihiko Komuro**		
Research Scientist	**Shigeki Marubuchi**		
Technicians	**Tayoko Tajima, Chie Iinuma, Chiharu Mizoi**		
Secretary	**Reiko Kikuchi, Atsuko Isobe**		
Graduate Students	**Yoko Nakamura, Min Xu, Chan Li, Sainawer Maimaiti, Risa Shiraishi, Yoshie Yuki, Zi-Hyang Chin, Tomomi Imamura, Keisuke Kurosu**		
Research Trainees	**Chaomaolige, Hong Zhang**		

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 these aims, 1) and 2) in this year will be described in the following.

Research Projects

1) Impairment of DNA double strand break repair in polyglutamine diseases

Polyglutamine (polyQ) diseases compose a group hereditary of neurodegenerative disorders whose population is the 3rd largest among various neurodegenerations. Essentially, all polyQ diseases share the features of abnormal aggregation of polyQ proteins, dysfunction of neurons, and neuronal cell death.

We have investigated neuronal dysfunction caused by nuclear translocation of mutant polyQ proteins by using omics approach over the past decade. From the proteome analysis of soluble nuclear protein fraction, we previously reported a reduction of high-mobility-group B (HMGB) proteins in both Huntington's disease and spinocerebellar ataxia type 1 that leads to transcriptional dysfunction and DNA repair impairment (Qi et al., Nat. Cell Biol., 2007).

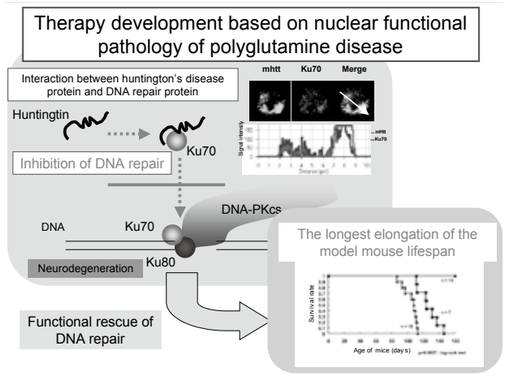


Fig.1 Mutant htt interacts with Ku70 and impairs the DNA double strand break repair. It leads to the accumulation of DNA damage and induces neurodegenerations. Rescue of Ku70 elongate the life span of the R6/2 Huntington's disease model mice.

This year, from the interactome analysis data, we identified Ku70, which plays an essential role in non-homologous end joining, as a novel interacting protein to mutant huntingtin and reported that dysfunction of Ku70 leads to accumulation of DNA damage by using histochemical and cell biological experiments. Furthermore, the rescue of Ku70 function in vivo by using mutant htt/Ku70 double transgenic mice showed a remarkable improvement of their lifespan (Enokido et al., JCB 2010).

2) Molecular mechanism of learning defect by PQBP1 gene mutation

We previously found a novel gene, PQBP1 (Waragai et al., Hum Mol Genet 1999) whose mutations cause MR syndromes (Kalscheuer et al., Nature Genet 2003). Most of the reported mutations in MR families cause the frame shift of amino acid codon and the reduction of PQBP1 mRNA due to non-sense RNA decay (Kalscheuer et al., Nature Genet 2003). Therefore, hypofunction of PQBP1 is suspected to cause MR-phenotype. To investigate the pathology of PQBP1-defect, we developed PQBP1-knock out mouse, PQBP1-knock down mouse and PQBP1-mutant fly model.

We reported a fly model in which the Drosophila homolog of PQBP1 (dPQBP1) is repressed by insertion of *piggyBac*. In classical odor conditioning, learning acquisition was significantly impaired in homozygous *piggyBac*-inserted flies, whereas the following memory retention was completely normal. Mushroom bodies (MBs) were morphologically normal and projection neurons (PNs) were not reduced in number in dPQBP1-mutant flies. However, gene expressions including NMDA receptor subunit 1 (NR1) were decreased in PNs. Targeted double-stranded RNA mediated silencing of *dPQBP1* in PNs, but not in MBs, similarly disrupted learning acquisition. NR1 overexpression in PNs rescued the learning disturbance of dPQBP1 mutants. Collectively, these findings identified PQBP1 as a novel gene regulating learning acquisition at PNs.

In this study, HDAC (histone deacetylase) inhibitor, PBA, that upregulated NR1 partially rescued the learning disturbance. It may be possible that PBA is a lead compound for the effective therapeutic medicine.

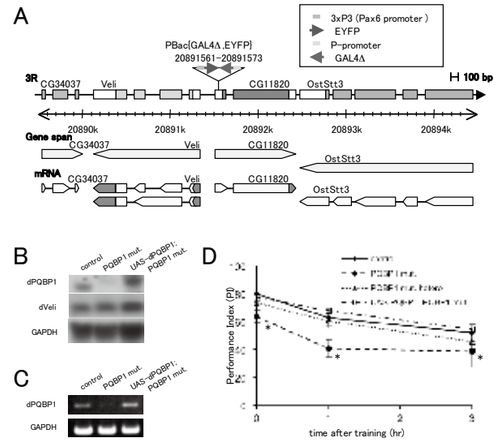
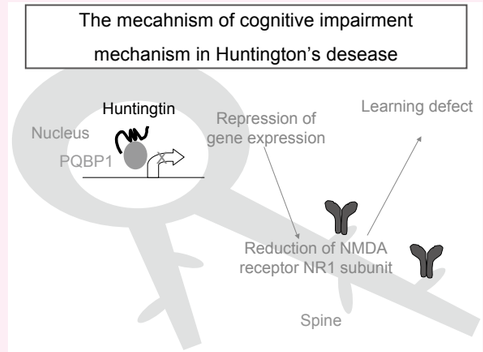


Fig.2. A) Genome structure of PQBP1-mutant fly. B) Northern blot analysis of dPQBP1. C) semi-quantitative RT-PCR analysis of dPQBP1. D) Leaning and memory retention in mutant flies using olfactory conditioning.

Highlight

Mutant htt interaction with PQBP1 results in down-regulation of NR1 subunit, and leads to cognitive impairment.



Publications

1. Enokido, Y., Tamura, T., Ito, H., Arumughan, A., Komuro, A., Shiwaku, H., Sone, M., Foulle, R., Sawada, H., Ishiguro, H., Ono, T., Murata, M., Kanazawa, I., Tomilin, N., Tagawa, K., Wanker, E.E., and Okazawa, H. (2010). Mutant huntingtin impairs Ku70-mediated DNA repair. ***J Cell Biol.*** 189, 425-443.
2. Takahashi, M., Mizuguchi, M., Shinoda, H., Aizawa, T., Demura, M., Okazawa, H., and Kawano, K. (2010). Polyglutamine tract-binding protein-1 binds to U5-15kD via a continuous 23-residue segment of the C-terminal domain. ***Biochim Biophys Acta.*** 1804, 1500-1507.
3. Shiwaku, H., Yoshimura, N., Tamura, T., Sone, M., Ogishima, S., Watase, K., Tagawa, K., and Okazawa, H. (2010). Suppression of the novel ER protein Maxer by mutant ataxin-1 in Bergman glia contributes to non-cell-autonomous toxicity. ***EMBO J.*** 29, 2446-2460.
4. Honda, S., Hayashi, S., Imoto, I., Toyama, J., Okazawa, H., Nakagawa, E., Goto, Y., and Inazawa, J. (2010). Copy-number variations on the X chromosome in Japanese patients with mental retardation detected by array-based comparative genomic hybridization analysis. ***J Hum Genet.*** 55, 590-599.
5. Konno, M., Hamazaki, T. S., Fukuda, S., Tokuhara, M., Uchiyama, H., Okazawa, H., Okochi, H., and Asashima, M. (2010). Efficiently differentiating vascular endothelial cells from adipose tissue-derived mesenchymal stem cells in serum-free culture. ***Biochem Biophys Res Commun.*** 400, 461-465.
6. Tamura, T., Horiuchi, D., Chen, Y. C., Sone, M., Miyashita, T., Saitoe, M., Yoshimura, N., Chiang, A. S., and Okazawa, H. (2010). Drosophila PQBP1 regulates learning acquisition at projection neurons in aversive olfactory conditioning. ***J Neurosci.*** 30, 14091-14101.
7. Aoki, Y., Nakamura, A., Yokota, T., Saito, T., Okazawa, H., Nagata, T., and Takeda, S. (2010). In-frame Dystrophin Following Exon 51-Skipping Improves Muscle Pathology and Function in the Exon 52-Deficient mdx Mouse. ***Mol Ther.*** 18, 1995-2005.

Department of Pathological Cell Biology

Professor Shigeomi Shimizu
Assistant professor Satoko Arakawa, Tatsushi Yoshida
Post-doctoral fellows Michiko Murohashi , Leishuku Li, 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 provision of nutrients when cells face starvation, as well as contributing to the turnover of cytoplasmic components. Studies of yeasts have identified a number of genes, designated ATG, that are required for the formation of autophagosomes. Many mammalian homologues of the yeast ATG genes have also been identified, and studies of mice lacking certain ATG genes, including ATG5, ATG6 (also called Beclin-1), and ATG7, have confirmed that these genes are essential for induction of macroautophagy. However, recently we found that cells lacking ATG5 or ATG7 can still form autophagosomes/autolysosomes and perform autophagy-mediated protein degradation 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 process of macroautophagy. We also found that this alternative process of macroautophagy was regulated by several autophagic proteins, including ULK1 and Beclin-1. In vivo, ATG5-independent alternate macroautophagy was detected in several embryonic tissues. It was also found to play a role even in the clearance of mitochondria during erythroid maturation. 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.

2, 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

Recently, we found that Bcl-2 independent alternative apoptotic pore is present in mitochondria. We are currently investigating its molecular mechanisms.

B, Analysis of autophagic cell death

Bax/Bak-deficient mouse embryonic fibroblasts (MEFs) are resistant to apoptosis induced by various stimuli. Instead, we found that these cells still die by autophagy in response to various death stimuli.

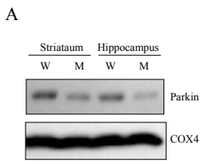
Since the molecular machinery of autophagic cell death is different from that of apoptosis and since most chemotherapeutic agents act primarily to induce apoptosis, autophagic cell death is considered as a useful chemotherapeutic target. Toward this end, we have developed a rapid semi-automated high-throughput method to screen libraries for novel compounds that induce autophagic cell death. Candidate compounds can then be further validated for reduction of *ex vivo* mouse tumor. Using these assays, we screened a library of 20,000 bioactive compounds and identified 4 lead compounds (Fig. 1).

C, Physiological role of cell death in mammals

We are analyzing physiological role of cell death using apoptosis-resistant mice (Bax/Bak doble KO mice, Bcl-2 Tg mice), autophagy-deficient mice, PT-deficient mice (CyPD KO mice).

3, 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. During analyzing these mice, we found that parkin protein, a crucial protein for PD, was decreased in the mnd2 mouse brain. We therefore examined whether parkin-transgenic mice could rescue the phenotype of mnd2 mice. But, parkin-Tg mnd2 mice showed similar phenotype with mnd2 mice (Fig. 2).



B

Survival of mnd2 and Parkin-Tg mnd2

	Survival days (Ave+/-SD)	Maximum	Minimum
Mnd2	30.5+/-9.14	49	21
Parkin-Tg mnd2	27.8+/-8.16	46	21

Fig.2 Neurodegeneration in mnd2 mutant mice is not prevented by parkin transgene.
(A) Down-regulation of parkin in mnd2 mice. Lysates of the striatum and hippocampus were prepared from brains of wild type (W) and mnd2 (M) mice at 4 weeks of age, and subjected to Western blotting for detection of parkin. Parkin protein was reduced in the stritum and hippocampus of mnd2 mice compared with those of wild type mice. COX4 was used as loading control.
(B) No effect of parkin transgene on survival of mnd2 mice. Survival time of mnd2 mice and parkin-Tg mnd2 mice is shown

Highlight

Cancer cells often exhibit mutations in critical molecules of the apoptotic machinery, resulting in resistance to common anticancer therapies. In the absence of apoptosis, autophagic cell death can be an alternative form of cell death by excessive self-digestion. Therefore, autophagic cell death can be considered as a backup cell death mechanism when apoptotic cell death mechanisms fail. Therefore, activation of autophagic cell death in tumors is a potential therapeutic strategy for treatment of cancer.

We have developed a rapid semi-automated high-throughput method to screen libraries for novel compounds that induce autophagic cell death. Candidate compounds can then be further validated for reduction of *ex vivo* mouse tumor. Using these assays, we screened a library of 20,000 bioactive compounds and identified 4 lead compounds. Addition of these compounds preferentially induced autophagic cell death in cancer cells (Fig. 1A), and efficiently reduced cancer volumes (Fig. 1B).

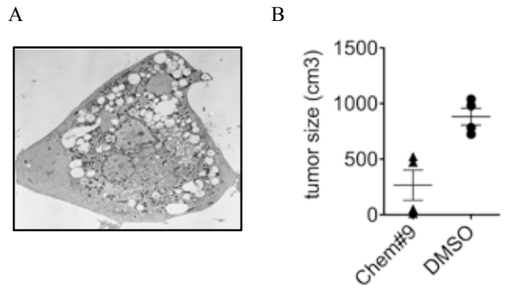


Fig.1 Discovery of novel anti-cancer compound targeting the autophagic cell death.
(A) Induction of autophagic cell death in MEFs by exposure to chemical#9. MEFs were treated by chemical#9 for 12 hours and were analysed by electron microscope.
(B) Reduction of cancer growth by chemical#9. P53-deficient mouse cancer cells were injected subcutaneously into the B6 mice. Then, chemical#9 or DMSO was daily injected intraperitoneally for 7 days. After 14 days, tumors were excised and the tumor volume was measured (n = 4).

List of Publications

[Original paper]

1. Mouri A, Noda Y, Shimizu S, Tsujimoto Y, Nabeshima T. The role of cyclophilin D in learning and memory. **Hippocampus** 20, 293-304, 2010
2. Shimizu S, Konishi A, Nishida Y, Mizuta T, Nishina H, Yamamoto A, Tsujimoto Y. Involvement of JNK in the regulation of autophagic cell death. **Oncogene** 29, 2070-2082, 2010
3. Nabeyama A, Kurita A, Asano K, Miyake Y, Yasuda T, Miura I, Nishitai G, Arakawa S, Shimizu S, Wakana S, Yoshida H, Tanaka M. xCT deficiency accelerates chemically induced tumorigenesis. **Proc. Natl. Acad. Sci. USA** 107, 6436-6441, 2010
4. Ideguchi K, Shimizu S, Okumura M, Tsujimoto Y. Cyclophilin D-dependent mitochondrial permeability transition is not involved in neurodegeneration in mnd2 mice. **Biochem. Biophys. Res. Commun.** 393, 264-267, 2010.
5. Kamiya K, Tsumoto K, Arakawa S, Shimizu S, Morita I, Yoshimura T, Akiyoshi K. Preparation of connexin43-integrated giant Liposomes by a baculovirus expression-liposome fusion method. **Biotechnol. Bioeng.** 107, 836-843, 2010
6. Yoshida T, Mizuta T, Shimizu S. Neurodegeneration in mnd2 mutant mice is not prevented by parkin transgene. **Biochem. Biophys. Res. Commun.** 402, 676-679, 2010.
7. 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

[Review paper]

1. Shimizu S, Arakawa S. and Nishida Y. Autophagy takes an alternative pathway. Autophagy 6.2, 290-291, 2010

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.

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 the precise molecular mechanisms that underlie organ failures found in human disease and will lead to he 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* exhibit a profound (but transient) defect in liver specification that resembles the liver formation defect found in the zebrafish *prometheus (prt)* mutants, 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.

Highlight

We use morpholino-mediated knockdown of the zebrafish orthologs of the Jnk activators Mkk4 and Mkk7 to examine the effect of Jnk signaling abrogation on early vertebrate embryogenesis. Our work demonstrates that Jnk activation is indispensable for multiple steps during vertebrate body plan formation. Furthermore, non-canonical Wnt signaling may coordinate vertebrate CE movements by triggering Jnk activation that represses the expression of the CE-triggering ligand *wnt11*.

Many studies have shown that it is possible to use culture conditions to direct the differentiation of murine embryonic stem (mES) cells into a variety of cell types, including cardiomyocytes and neurons. We show that the p38 MAPK-specific inhibitor SB203580 blocks the spontaneous differentiation of ES cells into cardiomyocytes, and instead induces the differentiation of these ES cells into neurons. At the molecular level, inhibition of p38 MAPK activity suppresses the expression of *bmp-2* mRNA, whereas treatment of ES cells with BMP-2 protein inhibits the neurogenesis induced by SB203580. Our findings reveal the molecular mechanism by which p38 MAPK activity in ES cells drives their commitment to differentiate preferentially into cardiomyocytes, and the conditions under which these same cells might develop into neurons (Fig. 1).

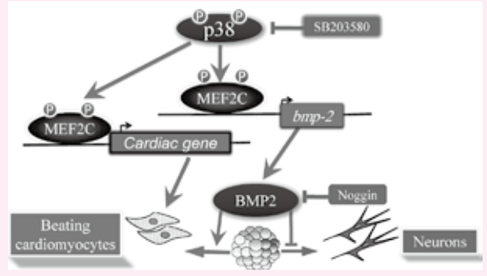


Fig.1. A proposed model for a switch between cardiomyocyte and neuronal commitment of murine embryonic stem cells.

Publications

1. Takahiro Negishi, Yoko Nagai, Yoichi Asaoka, Mami Ohno, Misako Namae, Hiroshi Mitani, Takashi Sasaki, Nobuyoshi Shimizu, Shuji Terai, Isao Sakaida, Hisato Kondoh, Toshiaki Katada, Makoto Furutani-Seiki*, and Hiroshi Nishina* (2010) Retinoic acid signaling positively regulates liver specification by inducing wnt2bb gene expression in medaka. ***Hepatology*** 51, 1037-1045. (*Corresponding authors) Press release

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Department of Stem Cell Medicine

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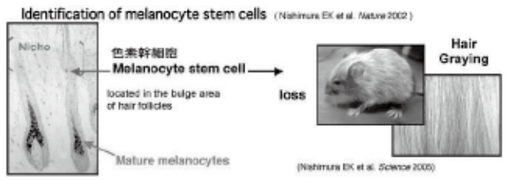
Assistant Professor **Takahiro Aoto, D.V.M., Ph.D.**
Assistant Professor **Hiroyuki Matsumura, Ph. D.**

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 iPS (induced pluripotent stem) cells, to 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" (MelSC), 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.



2) Mechanisms of stem cell maintenance

To understand the mechanisms underlying MelSC maintenance, we hypothesized that the hair graying phenotype is caused by incomplete maintenance of MelCSs. 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 MelSCs underlie the hair graying phenotype in both *Bcl2* deficient and *Mitf-vit* mutant mice. In other words, *Mitf* and *Bcl2* are essential intrinsic genes involved in MelSC maintenance to prevent hair graying (Nishimura EK et al. Science 2005). While we previously found that the niche microenvironment plays a dominant role in MSC fate determination (Nishimura EK et al. 2002), despite the identity of the niche cells and the underlying molecular mechanisms remaining largely unknown. Analysis of MelSC behavior during hair regeneration cycles also indicated that MelSCs are activated at the beginning of each hair cycle (early anagen stage) to self-renew but are then inactivated again by the mid-anagen stage (Nishimura E et al. 2002). Thus, we hypothesized that MelSCs enter the quiescent (non-cycling) state in response to external cues from the stem cell niche at that particular stage. We have searched for the identity of these functional niche cells and the responsible niche-derived factors. This year, we reported that hair follicle stem cells (HFSCs), which directly adhere to MelSCs in the hair follicle bulge, provide a functional niche for MelSCs through transforming growth factor β (TGF-β) signaling (See Highlight below) (Nishimura EK et al. Cell Stem Cell, 2010) (Tanimura S et al. Cell Stem Cell 2011). Through those approaches, we aim to understand the underlying mechanisms for tissue homeostasis, ageing and cancer development.

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

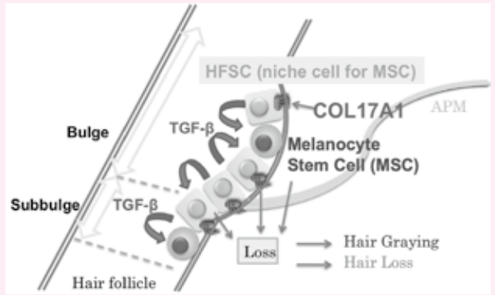
Physiological hair graying is the most obvious outward sign of aging. We previously demonstrated that physiological hair graying is caused by incomplete self-renewal/maintenance of MSCs (Nishimura EK et al. 2005). However, it was still not known what causes the self-renewal of MSCs to become defective 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).

Highlight

Hair follicle Stem Cell Provide a Functional Niche for Melanocyte Stem Cells

Tanimura S et al. Cell Stem Cell, 8, 177-187, 2011

In most stem cell systems, the organization of the stem cell niche is still largely unknown. Melanocyte stem cells (MelSC) 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. While our previous studies indicated that the niche plays a dominant role in MelSC fate determination, the underlying mechanisms and the niche cells for MelSCs are still unclear. Our recent study published in the February issue of *Cell Stem Cell* revealed that HFSCs provide a functional niche for MelSCs through transforming growth facto β (TGF-β) signaling to prevent premature hair graying. To explore the 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 MelSC maintenance, we analyzed deficient mice of *Col17a1* gene and *Tgfbr2* gene that encodes collagen XII and TGF β type II receptor, respectively. *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 MelSCs of the *Col17a1* null mice showed that *Col17a1* is critical for maintenance not only of HFSCs but also of MelSCs, which do not express *Col17a1* but directly adhere to HFSCs through maintaining their quiescence and immaturity. This potentially explains the mechanism underlying hair loss in human *COL17A1* deficiency. Also, we found that transforming growth factor β (TGF-β) signaling is activated in MelSCs when they reenter the quiescent non-cycling state during hair cycles. Therefore we analyzed conditional *Tgfbr2* deficient mice in which *Tgfbr2* is knocked out specifically in the melanocyte lineage and found that *Tgfbr2* is essential for the maintenance of MelSC immaturity and quiescence to prevent hair graying. Interestingly, *Col17a1* deficient mice show defective TGF-β production by HFSCs. These data indicate that the TGF-β signaling pathway is the critical niche factor that regulates MSC immaturity and quiescence. Finally, forced expression of *COL17A1* in basal keratinocytes, including HFSCs, in *Col17a1* null mice rescues MelSCs 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.



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Tanimura S, Tadokoro Y, Inomata K, Binh NT, Nishie W, Yamazaki S, Nakauchi H, Tanaka Y, McMillan JR, Sawamura D, Yancey K, Shimizu H, Nishimura EK. Hair follicle stem cells provide a functional niche for melanocyte stem cells. **Cell Stem Cell**, 8, 177-187, 2011

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Presentation at international meetings

1) Emi K. Nishimura: Genotoxic Stress Abrogates Renewal of Melanocyte Stem Cells by Triggering Their Differentiation in Mice : ISSCR 8th Annual Meeting : (San Fancisco, CA USA) June 19th, 2010
2) Emi K. Nishimura: Melanocyte Stem Cell Maintenance and aging : Cold Spring Harbor AsiaConferences : (Suzhou Dhshu Lake, China) Sept. 24th, 2010
3) Emi K. Nishimura: "Why does our hair turn gray? : JSPS/JHU/NIA-sponsored symposium : (Baltimore,U.S.A) Ageing vs .Regenerative Medicine: How Much Can Stem Cell Do? (NIA, Baltimore, USA) Feb. 19th 2010
4) Emi K. Nishimura : Stem cells in the hair follicle bulge sustain pigmented hair growth through their niche function: 3rd Symposium of the IMSUT & RCAST Global COE, (Auditorium of The Institute of Medical Science, The University of Tokyo, Tokyo) March 4th, 2011

Laboratory of Immunology School of Biomedical Sciences
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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) Elucidation of stress responses of B cells.
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. Immunological defects induced by excess CD40L

CD40L is overexpressed in patients with SLE and its animal models, and treatment of these patients and animals with antagonistic antibodies to CD40L ameliorate the disease, suggesting excess CD40L plays a role in development of SLE. Previously, we established CD40L transgenic mice in which CD40L is ectopically expressed in B cells, and demonstrated that the CD40L transgenic mice spontaneously develop a lupus-like disease (Higuchi et al. 2002), suggesting that excess CD40L alone is sufficient for inducing SLE. We are currently addressing how excess CD40L modulates the immune responses by analyzing CD40L transgenic mice.

During immune responses, B cells form germinal centers in which rapidly dividing B cells undergo somatic hypermutation of the immunoglobulin genes, followed by selective survival of B cells expressing high affinity antibodies. These B cells, then, differentiate to long-lived plasma cells and memory B cells, thereby contributing immunological memory. CD40 and CD40L are required for germinal center reaction. In germinal center B cells, follicular T helper (TFH) cells express a high level of CD40L. Initially,

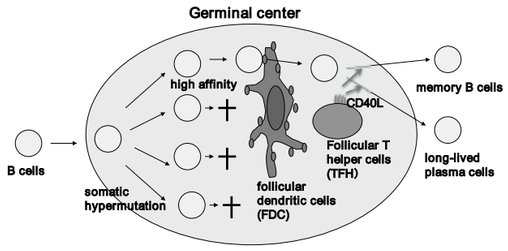

Fig.1. Germinal center reaction and CD40L
In germinal centers, B cells rapid proliferate and undergo somatic hypermutation of immunoglobulin genes followed by selective survival of B cells expressing high affinity antibody. These B cells then differentiate to long-lived plasma cells and memory B cells. CD40L highly expressed on follicular T helper cells induces differentiation of germinal center B cells.

CD40L was proposed to induce survival of germinal center B cells. However, we obtained an unexpected result that germinal centers are prematurely regressed in CD40L transgenic mice after immunization. Further analysis revealed that excess CD40L induces early maturation of germinal center B cells to plasma cells and memory B cells (Fig. 1). Thus, premature regress of germinal centers may be a consequence of augmented maturation of germinal center B cells. Excess CD40L may be involved in augmented antibody production in autoimmune diseases through enhanced maturation of germinal center B cells.

2. Study on the mechanisms for rapid antibody production during memory responses

During memory responses, rapid immune responses are induced as a consequence of rapid activation of memory lymphocytes, and contributing to host defense by rapid elimination of pathogens. Memory B cells mostly express membrane form of IgG as B a cell antigen receptor (BCR), whereas naïve B cells express both membrane form of IgM and IgD as BCR. Previously, it was demonstrate that expression of IgG instead of IgM or IgD on naïve B cells induces rapid and robust antibody production by the analysis of IgG transgenic mice. We independently established IgG-trasngenic mice, and found that augmented tonic BCR signaling plays a key role in rapid antibody response in these mice. In the absence of antigens, IgM and IgD-BCR generates a low level signaling called tonic signaling required for B cell survival. In contrast, our finding suggests that IgG-BCR generates a stronger tonic signaling sufficient for B cell activation probably due to its higher signaling capacity. Based on our findings, we propose the "idling model" in which IgG-producing B cells rapidly produce antibodies by T cell help as a consequence of continuous activating singling through IgG-BCR (Man et al. PLoS ONE, 2010).

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 two membrane-bound lectins CD22/Siglec2 and CD72. These membrane-bound lectins are primarily expressed on B cells and negatively regulate signal transduction through B cell antigen receptor (BCR), which plays a crucial role in B cell response to antigen stimulation. By stimulating B cells in vitro, we previously demonstrated that CD22 and CD72 serve, probably through their capacity to regulate BCR signaling, as a molecular switch determining whether antigen-stimulated B cells undergo activation or cell death, leading to B cell response or tolerance, respectively. CD22 also regulates time course of antibody responses (Onodera et al. J. Immunol. 2008), which is crucial for infection immunity. To regulate CD22, we synthesized a modified glycan ligand of CD22 that binds to mouse CD22 5000 folds more strongly than the natural ligand. Recently we successfully synthesized a compound that bind to human CD22 with high affinity (Abdu-Allah et al. submitted). Such compounds may be useful for drug development for immune regulation.

Highlight

CD40 transmits a differentiation signal in germinal center B cells.

CD40 and CD40L are highly expressed on follicular T helper cells located in germinal centers, and are required for germinal center formation. To address how CD40L contributes to germinal center reactions, we analyzed germinal center responses in CD40L transgenic mice in which CD40L is constitutively expressed in B cells. Although CD40 signaling has been shown to augment survival of B cells in vitro, CD40L transgenic mice show premature regress of germinal centers after immunization. In CD40L transgenic mice, germinal centers are generated as well as in wild type mice up to 5 days, but then regress whereas germinal centers in wild type mice further develop after 5 days of immunization. In contrast, CD40L transgenic mice produced memory B cells almost as efficiently as wild type mice did. Plasma cell generation in CD40L transgenic mice was rather enhanced. These results strongly suggest that excess CD40L augments differentiation of germinal center B cells and their egress, leading to early termination of germinal center responses. Thus, CD40L functions as a differentiation signal rather than a survival signal in germinal center B cells.

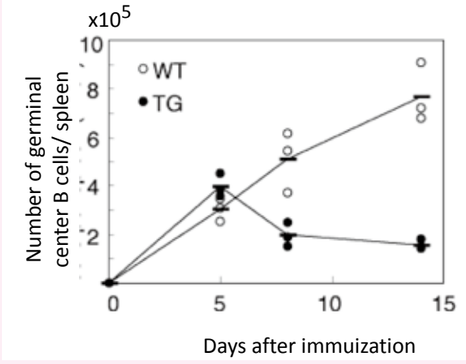

Fig.2. Premature regression of germinal centers in CD40L transgenic mice
Number of germinal center B cells are examined by flow cytometry in wild type (WT) and CD40L-trangenic (TG) mice after immunization.

Publications

[original papers]
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4. Bolduc, A., Long, E., Stapler, D., Cascalho, M., Tsubata, T., Koni, P. A. and Shimoda, M. (2010): Constitutive CD40L expression on B cells prematurely terminates germinal center response and leads to augmented plasma cell production in T cell areas. *J. Immunol.* 185: 220-230.

Division of Pathophysiology
Department of Molecular Pathogenesis
(Laboratory of Genome Diversity)

Professor	Akinori Kimura, M.D., Ph.D.
Associate Professor	Toshiaki Nakajima, M.D., Ph.D.
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 more or 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 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 investigated the phenotype and genotype correlation in long-term follow-up study of HCM. In addition, we revealed that Nebulette gene mutations cause DCM. We also revealed that calcium sensitizer SCH00013 could prevent the development of DCM in the animal model of hereditary DCM, LMNA mutation knock-in mice model (see Highlight). On the other hand, we deciphered that the heart-specific M21 induced a phosphorylation of M110 to prevent function of PP1M via binding and activation of ROCK. (see Highlight)

2. Molecular mechanisms for atherosclerosis

A genome-wide screening of loci for myocardial infarction identified a promoter SNP of MKL1 as the disease gene for coronary artery disease. We demonstrated a higher expression of MKL1 in smooth muscle cells and activated macrophages in the neointima of atherosclerotic lesion. On the other hand, we revealed that the anti-inflammatory effect of Losartan was depending on the activation of PPAR γ . In addition, susceptibility to chronic thromboembolic pulmonary hypertension was conferred by a polymorphism in the 3'-untranslated region of fibrinogen α gene via interaction with miR-759.

3. Molecular mechanisms for arrhythmia

We revealed that ZASP/Cypher regulated the activity of cardiac Na channel and that a DCM-associated mutation induced cardiac arrhythmia via dysregulation of the Na channel. On the other hand, NOT3 was found to play a crucial role in the cardiogenesis and maintenance of cardiac function. In addition, NOT3 polymorphism was correlated with the QT interval in electrocardiogram.

4. Analysis of MHC in human and old world monkeys

We revealed the interaction of HLA-A*02, -DPB1*0501 and CTLA4-CT60-G in controlling the susceptibility to Graves disease. In addition, HLA-A*02 and -DRB1*0901 was associated with the susceptibility to uveitis accompanied by juvenile arthritis. On the other hand, polymorphisms of MHC class I genes in rhesus macaques of Burmese origin, Mamu-A and Mamu-B, were investigated in detail and many new alleles were identified. As well, MHC class I genes of crab-eating macaques, Mafa-A and Mafa-B, were also investigated for their polymorphisms and many new alleles were found. In addition, live vaccine could be effectively used in developing neutralizing antibodies against SIV via MHC-independent mechanism.

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. We found that a TIM1 gene haplotype D3-A was associated with better survival prognosis of AIDS. In addition, a comparative genomic analysis of immunoglobulin super family genes from human, chimpanzee, orangutan, rhesus macaque, and marmoset revealed that a significant selection pressure had operated in 11 genes during the primate evolution.

Highlight

By administration of a calcium sensitizer SCH00013, we could delayed the development of dilated cardiomyopathy (DCM), prolonged the survival prognosis (Fig. 1), and suppress the cardiac remodeling (Fig. 2) in a mouse model for DCM, lamin A/C mutation knock-in (LMNA-KI) mouse.

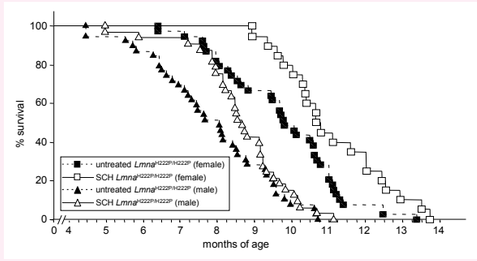

Fig.1. Survival prognosis of LMNA-KI mice with or without treatment by SCH00013
SCH00013 prolonged the survival of LMNA-KI mice in both male and female.

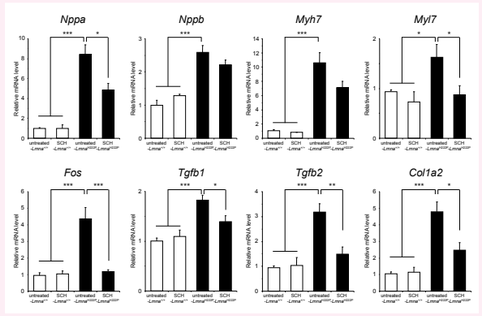

Fig.2. Suppression of cardiac remodeling in LMNA-KI via administration of SCH00013
Remodeling associated gene expression was induced in the LMNA-KI mice, which was suppressed by the treatment with SCH00013.

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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. We show that these humanized mice can recapitulate key main aspects of EBV infection in humans. 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. This is the first animal model of CAEBV and suggests that EBV-infected cells in CAEBV lack the capability of autonomous proliferation and hence are not truly malignant.

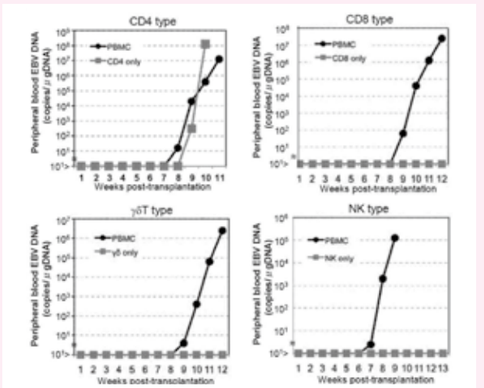
2. Development of an exhaustive pathogenic microbe screening system

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 in collaboration with the Center of Cell Therapy (Tokyo Medical and Dental University Hospital Faculty of Medicine).

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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3. Zhang Y et al. Aberrant expression of NK cell receptors in Epstein-Barr virus-positive gammadelta T-cell lymphoproliferative disorders. ***Hematology.*** **15**(1):43-47, 2010.
4. Kariya Y et al. Dominant-negative derivative of EBNA1 represses EBNA1-mediated transforming gene expression of Epstein-Barr virus infection independent of rapid loss of viral genome. ***Cancer Sci.*** **101**(4):876-881, 2010.
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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 2010 we contributed as follows;

1. Identification of novel genes responsible for cancer and unknown genetic diseases.
2. Development of high-resolution in-house CGH arrays and established their applications for detection of cryptic genomic and epigenetic aberrations in cancer and genomic disorders.
3. Establishment of practical tools for diagnosis in personalized medicine of cancer and genomic disorders in the clinical setting.

【Biochemical Genetics】

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

1. Through the genome-wide ChIP-chip analysis, we found that death receptor 5 is one of p53-ATF3 targets upon DNA damage, suggesting a co-operative role of ATF3 as tumor suppressor with p53.
2. Elongin A has dual activity toward PolII, transcriptional elongation and PolII degrading activity. Transcription activity but not E3 ligase activity was required in response of several stress genes, suggesting two activities are separated in inducing some of immediate response genes.

【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. We have demonstrated that BRCA2 complexed with plectin is required for centrosome positioning.
2. We have identified that Protein kinase C delta activates RelA/p65 and NF-kappaB signalling in response to TNF-alpha.
3. Analyses of molecular domains of translesion DNA polymerases by introducing a point mutation by homologous recombination in vertebrates.

【Molecular Epidemiology】

The aim of our lab is to decipher variations in the human genome, which by itself or together with environment factors affect the risks of developing common chronic diseases.

1. We found that CYP3A5 polymorphism affects the blood pressure level by interacting with daily salt intake.
2. We found that SERIPNE2 polymorphism affects the risk of pulmonary emphysema, which is a component of COPD.

【Functional Genomics】

Our department "Functional Genomics" seeks to resolve how gene expression process is regulated in an individual, and made following progress this year.

1. We found chemical compounds such as SRPIN340 and TG003, which affect mRNA splicing patters and have potentials to be clinically applicable to some incurable diseases including retinopathy of prematurity and viral diseases
2. We established the transgenic reporter worm system to monitor alternative splicing patterns in vivo and identified regulatory factors of tissue-specific alternative mRNA splicing in *C.elegans*.

【Epigenetics】

1. We have been carring our systematic analysis of the sushi-ichi retrotransposon-derived genes (*Sirh* genes) that are unique to mammals using these knockout mice to understand the mammalian-specific genomic functions, such as placentation and nursing behavior.
2. We have analyzed genomic imprinting status of spermatogonia cells in a mice with male infertility produced by ENU-mutagenesis.
3. Assisted reproductive technologies, such as *in vitro* fertilization and intracytoplasmic sperm injection, become very popular, however, their effects on human development remain unclear. We have extensively examined pre-and postnatal epigenetic effects caused by such technologies.

【Bioinformatics】

1. We developed a new mathematical method to analyze topological and statistical properties of complex networks. By the method, we revealed that proteins with intermediate connectivities form a backbone of protein-protein interaction networks. Proteins in the backbone tend to be drug targets, while almost no drug targets were found among hub proteins.
2. We conducted collaborative works with several research laboratories including following topics based on bioinformatics analysis: (1) identification of gene sets and their interaction networks associated with phenotypes and prognosis of hepatocellular carcinoma (HCC), (2) expression analysis of Aurora kinase B and alternative variant forms in HCC, (3) identification of IQGAP1 as a key regulator genes in naturally occurring hepatotumorigenesis induced by oxidative stress, and (4) identification of MUC12 as a prognosis marker in colorectal cancer.
3. We developed a new computational algorithm for inferring the dynamics of within-patient HIV evolution under anti-HIV therapy.
4. By conducting in silico and in vivo analyses, we revealed Hes1 is a master regulator to keep the stem cell undifferentiated state in the developmental process of taste receptor cells.

Division of Medical Genomics
Department of Molecular Cytogenetics

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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 chromosome aberration syndromes. 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 tools for diagnosis in Personalized Medicine of cancer and 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 CGH

Comparative genomic hybridization (CGH) was developed as a molecular cytogenetic technique to compensate for difficulties presented by conventional methods. CGH analyses of solid tumors have revealed a number of recurrent copy-number aberrations including amplifications that had not been detected previously by any other technique. For the last decade we performed CGH analysis in over 1700 cases of various types of cancer, and we constructed a 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. For example, we identified *GASC1* (Gene Amplified in Squamous Cell Carcinoma 1) and *cIAP1*, as targets for the 9p22-23 amplification or 11q22 amplification in esophageal squamous cell carcinomas (ESCs), respectively. The former is a demethylase for tri- or dimetylated lysine 9 on histone H3, and the latter is a member of the *IAP* (anti-apoptotic) gene family. We also detected frequent amplifications at 5p12-13 in small and non-small cell lung cancers and at 17q23 in neuroblastoma (NB). Consequently we identified novel target genes of *SKP2* or *PPM1D*, respectively. Some of them are being now focused as the targets for the molecular target therapy in collaboration with the Research Institute of Pharmaceutical Companies.

2. Development of innovative techniques for genomics and/or epigenomics in medical science

Standard CGH to metaphase chromosomes can provide only limited resolution: 5-10 Mb for detection of copy-number losses and gains, and 2Mb for amplifications. To circumvent this limitation, we have also constructed 8 different types of BAC-based CGH-arrays (so-called MCG array series). Among those, MCG Genome Disorder (GD) Array, which harbors BACs involved in loci responsible for congenital chromosomal aberrations for their diagnosis. Recently, we have constructed the MCG CNV Database, which provides copy number variants (CNVs) detected in healthy Japanese by our MCG arrays. The MCG CNV Database 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. URL: http://www.cghtmd.jp/CNVDatabase/top!changeEngLocale

Fig.1. The banner of MCG CNV database

3. Identification of cancer-related genes within genomic aberrations detected by array-CGH

We recently identified cancer-related genes upregulated by a gene amplification mechanism as follows, *CDK6* in gastric cancer, *CCND3* in liver metastatic colon cancer, *DUSP26* and *ITCH* in anaplastic thyroid carcinoma, *KLK5* in bladder cancer, *BCL2L2* in lung cancer, *POU2AF1* in multiple myeloma, and *YAP1* in esophageal cancer within novel amplifications detected by in-house BAC-array platform. Moreover, we recently identified novel candidate TSGs including *LRP1B* in ESCC, *DBC1* and *PCDH20* in NSCLC, *ADAM23* and *VLDLR* in gastric cancer, *RGC32* in malignant glioma, *PRTFDC1* and *MTNR1A* in OSCC, *CTGF* and *ANGPTL2* in ovarian cancer. Some of those genes are also epigenetically silenced due to the promoter CpG island methylation in some GCs. In the last few years, microRNAs (miRNAs) have started a revolution in molecular biology and emerged as key players in the cancer process. Recently, we successfully identified novel tumor-suppressor miRNAs, which are epigenetically silenced in various types of cancer.

4. Applications of array-CGH

To accomplish genome-wide screening for methylated sites in the whole genome, we have combined array-CGH with MCA, and our preliminary data show that this "BAC array-based MCA (BAMCA)" can discriminate BAC clones that harbor methylated CpGs on an array platform. Our BAMCA method not only allows us to explore methylated CpG sites within spotted BACs, for if we apply BAMCA to a tiling-resolution CGH-array, methylated CpG sites emerge on an array platform throughout the entire genome. Recently, by using this BAMCA method, we identified candidate tumor suppressors, *NR1I2* and *PTGER2* in the progressive type of neuroblastomas, and *CRABP1* in ESCC. In addition, a combination of chromatin immuno-precipitation (ChIP) assays with hybridization to DNA arrays, the so-called "ChIP-on-chips" technique, has proven to be a powerful way to explore sites of interaction among DNA-binding proteins across the entire genome.

5. Molecular cytogenetic investigation of genomic disorders

Array CGH is also one of the most powerful tools to detect cryptic chromosome aberrations in genomic disorders including multiple congenital anomalies and mental retardation (MCA/MR). To apply the aCGH technique to the diagnosis as well as investigation of multiple congenital anomalies and mental retardation (MCA/MR), we constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 patients with clinically uncharacterized MCA/MR, whose karyotypes were 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 54 of 536 cases (10.1%), whereas the second screening of the 349 cases negative in the first screening using a genome-wide high-density array at intervals of approximately 0.7 Mb detected pCNVs in 48 cases (13.8%), indicating that. The results show the efficient application of aCGH in the clinical setting.

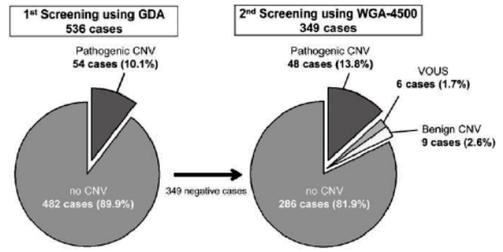

Fig.2. The two-stage screening of CNVs in 536 patients with MCA/MR by MCG BAC-arrays.

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Division of Medical Genomics
Department of Molecular Genetics

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Associate Professor	Akira Nakanishi, Ph.D.
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.

BRCA2 protein interacts via the BRC (breast cancer) domain with RAD51, an essential component of the cellular machinery for the maintenance of genome stability and double strand-breaks repair. Moreover, BRCA2 is localized, surrounding around centrosome from the G1/S transition to the early M phase of the cell cycle. When the cell cycle progresses further, and cytokinesis begins, BRCA2 is localized in the mid-body.

(1) BRCA2 binds to motor domain of myosin IIC

We reported that human non-muscle myosin heavy chain (NMHC) IIC and phosphorylated BRCA2 by Plk1 was recruited between both of the plus-ends of microtubules of the midbody. Indeed, we found that the HA-NMHC IIC can be coimmunoprecipitated with BRCA2-FLAG. Next, we divided the NMHC IIC into two fragments (IIC-N: motor domain (1-1000 a.a.) and IIC-C: coild-coil domain (887-2003 a.a.)) (Figure 1). Furthermore,

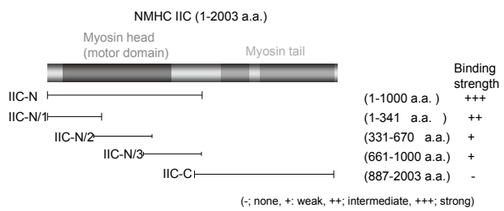

Fig.1. BRCA2 binding region of NMHC IIC

we divided the IIC-N region into three fragments (IIC-N/1: 1-341 a.a., N/2: 331-670 a.a., N/3: 661-1000 a.a.). As a result, the interaction with BRCA2 was observed only in the IIC-N/1 region. Conversely, we tried to narrow down the NMHC IIC-binding region of BRCA2. A series of overlapping FLAG-fused deletion mutants of BRCA2 (R1: 1-157 a.a., R2: 113-685 a.a., R3: 639-1508 a.a., R4: 1475-1620 a.a., R5: 1596-2280 a.a., R6: 2241-2940 a.a., R7: 2611-3318 a.a., R8: 3119-3418 a.a.) were incubated with COS-7 cell lysates transfected with HA-NMHC IIC. HA-NMHC IIC specifically associated with R3, R5, R6 and R7 (Figure 2). We demonstrated that there was strong (R5 and R6), intermediate (R3) and weak (R7) binding to NMHC IIC. The results suggest that the residues in the 1596-2940 a. a. regions of BRCA2 appear to be required for its interaction with NMHC IIC.

(2) Identification of a cleavage product of BRCA2 in cancer cell lines

We performed immunoblot for detecting BRCA2 of breast cancer cell line MCF7 using two kinds of antibodies with different epitopes (Central part; 1651-1821 a.a. and C terminus; 2959-3418 a.a.). As a result, the specific bands of 250k and wild type BRCA2 protein (molecular mass 380k) were detected by the antibody that recognizes the central part. The 250k band was not detected by the antibody that recognizes C terminus. However, this band was not detected in the normal human mammary epithelial cell (HMEC) and normal human breast epithelial cell (MCF10A). As a result, we confirmed that this was a cleavage product of BRCA2.

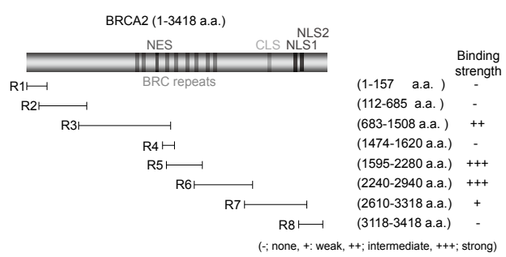

Fig.2. NMHC IIC binding region of BRCA2

(3) Identification of novel BRCA2-associated proteins and analyses of functions of their association in numerical integrity of centrosomes.

We identified nucleophosmin (NPM) and Rho-associated coiled-coil containing protein kinase 2 (ROCK2) as novel BRCA2-associated proteins by mass spectrometry. Because it is known that ROCK2 binds to NPM at centrosomes, these 3 proteins may form a complex. NPM-binding region in BRCA2 was determined to be within amino acids 639-1,000. Exogenous expression of this BRCA2 region resulted in aberrant centrosome amplification and a high frequency of multinucleated cells. Our results suggested that a complex consisting of BRCA2, NPM, and ROCK2 maintains the numerical integrity of centrosomes and accurate cell division and that dysfunction of this regulation might be involved in the tumorigenesis of breast cancer.

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

(1) Pim-1 activates RelA/p65 and NF-kappaB signaling in response to TNF-alpha.

The NF-kappaB signaling pathway is controlled by the ubiquitin-mediated proteolysis. Whereas a main subunit of NF-kappaB, RelA/p65 is ubiquitinated for degradation by SOCS-1, the functional mechanism of its ubiquitination remains poorly understood. Here we show that phosphorylation of RelA/p65 at Ser276 prevents its degradation by ubiquitin-mediated proteolysis. In contrast, impairment of Ser276 phosphorylation affects constitutive degradation of RelA/p65. Importantly, we identify Pim-1 as a further kinase responsible for the phosphorylation of RelA/p65 at Ser276. Depletion of Pim-1 hinders not only Ser276 phosphorylation but also transactivation of RelA/p65 target genes. We also demonstrate that Pim-1 contributes to recruitment of RelA/p65 to kappaB-elements to activate NF-kappaB signaling following TNFalpha stimulation. In concert with these results, knock down of Pim-1 impairs IL-6 production and augments apoptosis by interfering RelA/p65 activation. These findings provide a model in which Pim-1 phosphorylation of RelA/p65 at Ser276 allows defense against ubiquitin-mediated degradation and whereby exerts activation of kappaB signaling.

(2) ATM augments nuclear stabilization of DYRK2 by inhibiting MDM2 in the apoptotic response to DNA damage.

A previous study has shown that, upon exposure to genotoxic stress, DYRK2 translocates into the nucleus and phosphorylates p53 at Ser46, thereby inducing apoptosis. However, less is known about mechanisms responsible for intracellular control of DYRK2. Here we show the functional nuclear localization signal at amino-terminal domain of DYRK2. Under normal conditions, nuclear and not cytoplasmic DYRK2 is ubiquitinated by MDM2, resulting in its constitutive degradation. In the presence of proteasome inhibitors, we detected a stable complex of DYRK2 with MDM2 at the nucleus. Upon exposure to genotoxic stress, ATM phosphorylates DYRK2 at Thr33 and Ser369, which enables DYRK2 to escape from degradation by dissociation from MDM2 and to induce the kinase activity toward p53 at Ser46 in the nucleus. These findings indicate that ATM controls stability and pro-apoptotic function of DYRK2 in response to DNA damage.

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Many common chronic diseases 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 as well as their interaction with environmental factors. We also pay attention in epigenetic alteration that may contribute to the development of the diseases.

1. CYP3A5 polymorphism and sensitivity of blood pressure to dietary salt.

The drug metabolizing enzyme, cytochrome P-450 3A5 (CYP3A5) has recently been implicated in blood pressure regulation through metabolism of endogenous steroids. The genetic effect of CYP3A5*1 (expressor) and *3 (non-expressor) variants on blood pressure have been studied in African American and Caucasian but the effect on Asian population has not been addressed. The potential interaction with sodium intake needs to be taken into account. A total of 238 unrelated apparently healthy Japanese male workers (20-64 year) were included in the study. The A6986G polymorphism (rs776746) in intron 3 was determined by melting curve analysis. Sodium intake level was inferred from spot urine specimen by calculating 24-h urinary sodium excretion (24HUNaClV). CYP3A5 *1/*1 genotype group had higher diastolic BP but not systolic BP than *3/*3 genotype group (p=0.038), which remained significant after adjustment with age, BMI and sodium intake. When sodium intake was considered, CYP3A5*1/*3 had a significant interactive effect on both SBP (p=0.046) and DBP (p=0.003). These results suggests that the functional polymorphism of CYP3A5 may have an effect on blood pressure by interacting with sodium intake in Japanese men.

2. Smoking confers a MTHFR 677C>T genotype-dependent risk for systemic atherosclerosis

We explored the interactions between smoking and genetic polymorphism of 24 atherosclerosis-related candidate genes in systemic atherosclerosis. The study comprised 1,503 consecutive autopsy cases. The men-to-women ratio was 1.16 and the average age at death was 80.3 years. 70.3% of men and 21.6% of women were current or past smokers. The degree of atherosclerosis in 10 arteries was semi-quantitatively assessed. Thirty-four single nucleotide polymorphisms (SNPs) of 24 genes were analyzed by melting curve analysis. Twenty-four SNPs did not interact with smoking on atherosclerosis, while 7 SNPs interacted with smoking in one artery and 2 SNPs in two arteries. The genotypes of MTHFR 677C>T and smoking significantly interacted in four arteries: the common carotid artery, common and external iliac arteries, and femoral artery. The odds ratios of smoking on atherosclerosis were high (3.03-4.63) in MTHFR TT homozygotes, intermediate (1.75-5.24) in heterozygotes, and low (1.75-2.63) in CC homozygotes in systemic arteries except for the cerebral and coronary arteries. In conclusion, MTHFR 677 TT homozygotes are more likely to develop atherosclerosis than heterozygotes or CC homozygotes, if they smoke. Thus, smoking cessation may be more important in the prevention of atherosclerosis in the MTHFR 677 TT homozygotes.

3. Computational gene knockout reveals trans-disease-transgene association structure.

Genome-wide association studies for a variety of diseases are identifying increasing numbers of candidate genes. For so-called life-style diseases (e.g., metabolic syndrome) polygenetic changes that are caused by environmental factors lead to disease susceptibility. Now we have been confronted with the fact that some genes are common candidates across diseases. Thus there is a strong need to develop a hypothesis formulation methodology for comprehending multifaceted associations between genes and diseases. By introducing the basic rationale underlying the gene knockout approach as an information processing procedure to a network constructed on the basis of hyperlinks between disease and gene pages listed in the Online Mendelian Inheritance in Man (OMIM) database, relations of genes with diseases are computationally quantified. We did successively 'knockout' gene s expected to contribute to metabolic syndrome, and catalogued each gene's association with various diseases. We thereby apply a co-clustering method to the gene-disease relations for obtaining an association structure by classifying diseases and genes simultaneously. Observing an association structure between over 100 diseases and their related genes, we then found the structure revealed gene classes that were commonly associated with diseases as well as gene classes that were selectively associated with a specific disease class. In conclusion, we have shown a computational methodology for building transdisease-transgene association structure. Such structuralization may provide novel relations between genes and diseases, which might shed light on the etiology of diseases.

4. Genome-wide DNA methylation analysis reveals phytoestrogen modification of promoter methylation patterns during embryonic stem cell differentiation

Environmental challenges during development affect the fetal epigenome, but the period(s) vulnerable to epigenetic dysregulation are not clear. By employing a soy phytoestrogen, genistein, that is known to alter the epigenetic states of the Avy allele during embryogenesis, we have explored the sensitive period for epigenetic regulation. The post-implantation period, when de novo DNA methylation actively proceeds, is amenable to in vitro analysis using a mouse embryonic stem (ES) cell differentiation system. Mouse ES cells were differentiated in the presence or absence of genistein, and DNA methylation patterns on day 10 were compared by microarray-based promoter methylation analysis coupled with a methylation-sensitive endonuclease (HpaII/McrBC)-dependent enrichment procedure. Moderate changes in methylation levels were observed in a subset of promoters following genistein treatment. Detailed investigation of the Ucp1 and Sytl1 promoters further revealed that genistein does not affect de novo methylation occurring between day 0 and day 4, but interferes with subsequent regulatory processes and leads to a decrease in methylation level for both promoters. Genistein perturbed the methylation pattern of differentiated ES cells after de novo methylation. Our observations suggest that, for a subset of genes, regulation after de novo DNA methylation in the early embryo may be sensitive to genistein.

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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 c 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 trasncriptional 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 to 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 and 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 mechanism 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 show 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 funstions 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 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 role in oncogenesis.

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.

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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" rovides 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*.

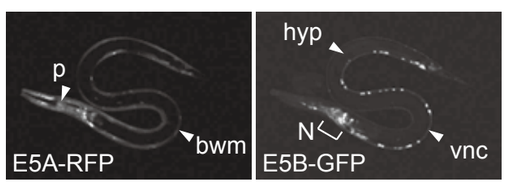


Fig1. An *egl-15* alternative splicing reporter worm with tissue-specific expression of exon 5A-RFP and exon 5B-GFP.

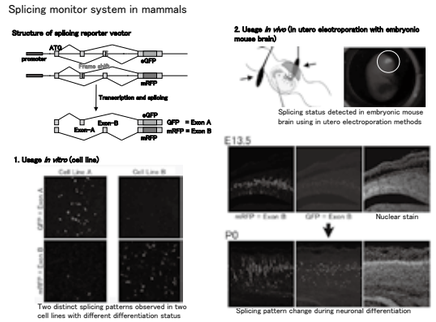
Regulating mechanism of alternative splicing and its physiological function during the development of mouse brain

In the brain, many important molecules are regulated their structures and functions with this system, like signaling receptors, cell adhesion molecules, transcription factors, and so on. This mechanism contributes to the functional complexity and high performance of the mammalian brain through making divergent protein subsets from small number of genes. Also many neuronal diseases are caused by the mis-regulation of alternative splicing, i.e.; neurodegenerative disease, mental disorder, neuronmuscular dystrophy, or brain tumors. However, the mechanism of gene-, cell-, organ-, developmental stage-, and brain structure-specific regulation of alternative splicing is still mostly unknown.

We are developing a monitoring system of alternative splicing during the development of the brain using a fluorescent-based reporter vector system, and revealing their dynamism in single cell resolution (PLos ONE, 2010). With this system, we screen and select molecules that are essential for regulating alternative splicing, and exam their biological function during development (Fig. 2). This project will reveal the regulating mechanism of alternative splicing and its important roles from *in vitro* to *in vivo*.

mRNA splicing regulation and virus infection.

Although the viral genome is often quite small, it encodes a broad series of proteins. The virus takes advantage of the host-RNA-processing machinery to provide the alternative splicing capability necessary for the expression of this proteomic diversity. Serine-arginine-rich (SR) proteins and the kinases that activate them are central to this alternative splicing machinery. We originally developed SR protein phosphorylation inhibitor 340 (SRPIN340), which preferentially inhibits SRPK1 and SRPK2.



SRPIN340 suppressed propagation of HIV, herpes simplex virus (HSV) type 1 and 2, Sindbis virus, SARS virus, and cytomegalovirus. These observations have led us to apply SRPIN340 to an antiviral drug. We are also going forward synthesis of series of SRPIN derivatives and testing the effect of these compounds on viral replication. Some SRPIN compounds dramatically inhibit the replication of hepatitis C virus, influenza virus, and Dengue virus.

Furthermore, we showed herpesvirus protein ICP27 changes the alternative splicing of *promyelocytic leukemia protein* (*PML*) pre-mRNA to affect virus replication (*NAR*, 2009). Our paper is first report showing the relationship between virus replication and host alternative splicing in detail.

Development of Novel Specific Inhibitors of "PSYCHIK" Family Kinases and their Potentials as Pharmaceutical Drugs

We have previously reported the development of SRPIN340, a specific inhibitor of SR protein kinase (SRPK) family, and, TG003, a specific inhibitor of cdc2-like kinase (Clk) family. To date, they are the only specific inhibitors for SRPK and Clk, respectively. Significantly, we also proved that SRPIN340 is a potent anti-viral agent.

We are further developing novel inhibitors of other protein kinase families that are phylogenetically related to SRPK and Clk. SRPK family and Clk family are closely related protein kinase families, and they constitute a larger family of phylogenetically related protein kinases together with dual-specificity tyrosine-(Y)-phosphorylation regulated kinase (DYRK) family, Homeodomain interacting protein kinase (HIPK) family, and human pre-messenger RNA processing 4 protein kinase (PRP4). We propose to call the entire family as PSYCHIK family (PRP4, SRPK, DYRK, Clk, HIPK Family). These kinases are suggested to play important physiological roles including development and normal functioning of central nervous system, regulation of apoptosis, and pre-mRNA splicing.

We focus on PSYCHIK family members as targets for discovery of specific inhibitors, and indeed some novel specific inhibitors have been obtained.

We have characterized the new compounds through 1) *in vitro* assays, 2) in cell functional assays, 3) X-ray crystallography, and 4) whole embryo development assay. The findings demonstrated that the compounds are not only useful biological tools, but are potential drug seeds for hitherto untreatable diseases (Nat Commun, 2010).

Splicing regulation and stress response

Cells are often exposed to circumstance changes and various kinds of stresses such as canceration, viral infection, hypoxia condition, heat shock and radical toxicity. As reported by many researchers, various kinds of genes are regulated their expressions and functions by stress-responsive change of alternative splicing. However, the mechanisms underlying the stress-dependent splicing regulations are poorly understood. In order to reveal the molecular mechanisms, we utilize our splicing reporter system with model genes, whose splicing patterns are affected by several stress conditions.

Under stress condition, processes of gene expression such as transcription, pre-mRNA processing and translation are suppressed to avoid production of abnormal proteins. We found a novel mode of splicing promoted under stress conditions, when conventional splicing is generally arrested, and that a gene undergoes this stress-induced splicing serves to quick recovery of the cell after stress removal.

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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 that 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. We have recently demonstrated that another sushi-ichi retrotransposon-derived gene, *Sirh7/Ldoc1*, is also an essential gene for formation of three layers in placentas. These results indicate that such mammalian-specific retrotransposon-derived genes are the key genes to understand the mammalian developmental systems at present. Therefore, we are now analyzing *Sirh3*, *Sirh4*, *Sirh5*, *Sirh6* and *Sirh11* knockout mice to elucidate new mammalian-specific genomic functions by collaboration with Prof. Kaneko-Ishino at Tokai University.

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. We found 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 (Figures 1 and 2). Deletion of *Xist* on Xa showed normal global gene expression and resulted in about an eight- to ninefold increase in cloning efficiency. We also identified an *Xist*-independent mechanism that specifically down-regulated a subset of X-linked genes through somatic-type repressive histone blocks. Thus, we have identified nonrandom reprogramming errors in mouse cloning that can be altered to improve the efficiency of SCNT methods.

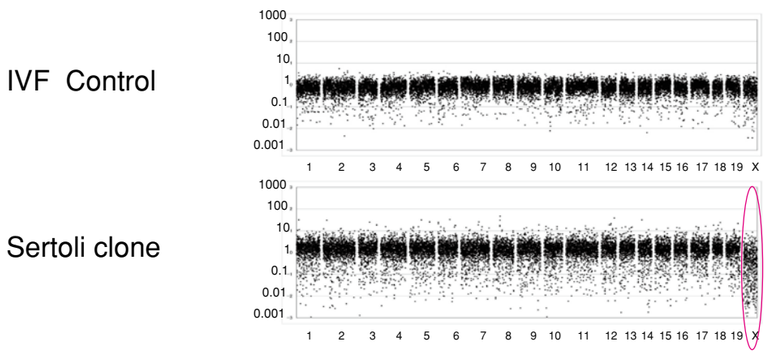

Fig1. **Comprehensive Analysis of Gene Expression in cloned blastocysts**
DNA microarray analysis was carried out using somatic cloned blastocysts and normal fertilized controls. Expression of genes on sex chromosome X are totally reduced in the cloned blastocysts, indicating abnormal X chromosome inactivation in both males and females.

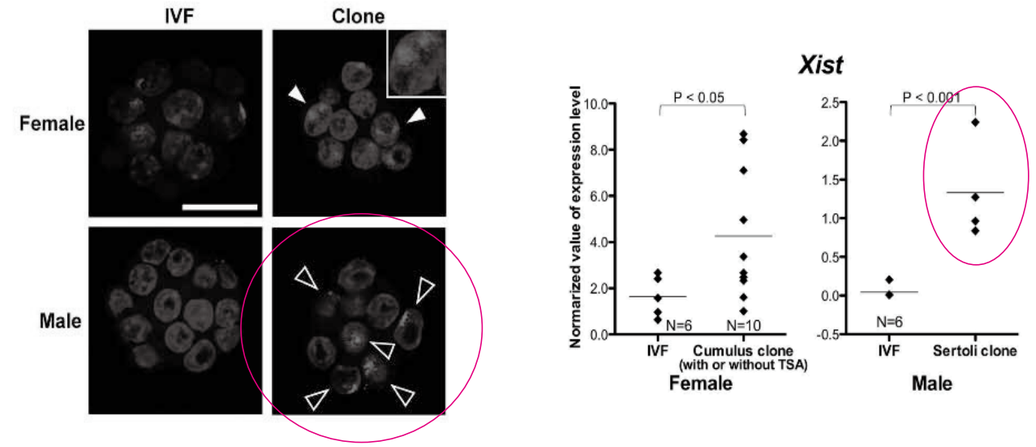

Fig2. **Ectopic expression of Xist gene in cloned blastocysts**
A. Upper column: a female blastocyst, Lower column: a male blastocyst. *Xist* is a major responsible gene for X inactivation. One of two X chromosomes in female cells is usually inactivated associated with expression of *Xist* gene while no *its* expression is observed in male cells. In cloned blastocysts, however, some female cells had two *Xist*-positive signals and even in the male cells *Xist* expression was confirmed.

Publications (Original papers)

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Presentation at International meetings

1. Fumitoshi Ishino, Ryuichi Ono, Shunsuke Suzuki, Yoichi Sekita, Mie Naruse, Takashi Kohda and Tomoko Kaneko-Ishino. Evolution of mammalian viviparity and genomic imprinting by retrotransposons. NAIST Global COE International Symposium 2010 Plasticity in Development and Evolution November 11-12, 2010 (NAIST Millennium Hall).

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Department of Bioinformatics, Medical Research Institute

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Assistant Professor	Soichi Ogishima	Visiting Professor	Hiroshi Mizushima
Visiting Assistant Professor	Yasen Mahmut		
Project Associate Professor	Jun Nakaya, Fengrong Ren, Takako Takai		
Project Lecturer	Kazuro Shimokawa, Kanae Oda		
Project Assistant Professor	Takeshi Hase (-Jan., Dec.-), Naoki Hasegawa, Kaei Hiroi, Keisuke Ido, Kaoru Mogushi, Satoshi Shoji (-March), Masaki Morioka (-May)		

In our laboratory, we conduct biological and medical researches from the viewpoint of Systems Biology.

Biological sciences: Recently, the whole genome sequences of diverse organisms have become available. Moreover, various "omix" information such as a proteome, transcriptome, and metabolome are currently accumulating. Our goal is to establish a grand-theory of biological sciences from the viewpoint of "evolving networks composed of biological molecules" by integrating omix information.

Medical sciences – Genomic and omix data are also utilized in the field of medicine. It has been revealed that most diseases are caused by the interaction among abnormalities of multiple genes, those at the tissue level, and environments. It is therefore possible to consider diseases as a system. From this standpoint, we try to establish the omix-based medicine.

1. Difference in gene duplicability may explain difference in overall structure of protein-protein interaction networks among eukaryotes

To uncover the evolutionary mechanisms of protein-protein interaction networks (PINs), we investigated PINs from several eukaryotes, i.e., yeast, worm, fly, human, and malaria parasite. Our analyses showed that the yeast, worm, fly, and human PINs are disassortative while the malaria parasite PIN is not. By conducting simulation studies on the basis of a duplication-divergence model, we showed that a preferential duplication of low- and high-degree genes can generate networks with disassortative structure and those without disassortative structure, respectively. From this observation, we hypothesized that the difference in degree dependence on gene duplications accounts for the difference in assortativity of PINs among eukaryotes. By comparing 55 proteomes in eukaryotes, we revealed that genes with lower degrees showed higher gene duplicabilities in the yeast, worm, and fly, while high-degree genes tend to have high duplicabilities in the malaria parasite. The observation supports the above hypothesis.

Our results suggest that disassortative structures in PINs among eukaryotes are merely a byproduct of preferential duplications of low-degree genes, which might be caused by a living environment of an organism.

2. Investigation of disease mechanism using omics-based analysis

Recent advances in analysis techniques in molecular biology have led to the investigation of genome-wide data such as genome, transcriptome and proteome. In order to reveal the underlying biological mechanisms from such a large amount of "omics" data, integration of biomedical knowledge with multivariate statistical analysis or machine learning methods is one of the most crucial tasks for bioinformatics research. We have been performing collaborative research with our university hospital and other institutes mainly based on transcriptome analysis using DNA microarray, including the following topics: 1) identification of diagnosis marker for early relapse in hepatocellular carcinoma patients, 2) development of predictive marker for metastatic relapse in colorectal cancer, and 3) analysis of the molecular mechanisms of epithelial-mesenchymal transition in breast cancer cells.

3. Bioinformatics on disease Omics data

The i2b2 (Informatics for Integrating Biology and the Bedside) is a database system developed by Harvard Medical School to facilitate integration of clinical patients data collected in various forms and by various people such as university hospitals, clinics, and organizations of patients. The i2b2 is designed to enable integration of many different data by ontology-based object-oriented database technologies. We constructed the i2b2 database with Japanese clinical patients data from our university hospital. We developed a computational pipeline to extract disease names from doctor's comments in Japanese and translate them into English using Natural Language Processing techniques. Comprehensive information on proteins plays an important role in elucidating molecular progress of a disease. There had been no concise and systematic method to identify terminus of proteins in proteomics. We applied a unique enzyme that digested N- and C- terminus of a Lys residue and developed a new method to identify protein terminus in MASS data. We investigated human plasma by our method and detected un-known protein terminus that may have pathogenic links to a disease.

4. iCOD: an integrated clinical omics database based on the systems-pathology view of disease

In order to provide a basic platform to realize a future medicine based on the integration of molecular and clinico-pathological information of disease, we have developed an integrated clinical omics database (iCOD) in which comprehensive disease information of the patients is collected, including not only molecular omics data such as CGH (Comparative Genomic Hybridization) and gene expression profiles but also comprehensive clinical information such as clinical manifestations, medical images (CT, X-ray, ultrasounds, etc), laboratory tests, drug histories, pathological findings and even life-style/environmental information. Furthermore, we developed several kinds of integrated view maps of disease in the iCOD, which summarize the comprehensive patient data to provide the information for the interrelation between the molecular omics data and clinico-pathological findings as well as estimation for the disease pathways, such as three layer-linked disease map, disease pathway map, and pathome-genome map. With these utilities, our iCOD aims to contribute to provide the omics basis of the disease as well as to promote the pathway-directed disease view. The iCOD database is available online, containing 140 patient cases of hepatocellular carcinoma, with raw data of each case as supplemental data set to download. The iCOD and supplemental data can be accessed at http://omics.tmd.ac.jp/icod_pub_eng.

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6. Ohashi K, Ota S, Ohno-Machado L, Tanaka H: Smart medical environment at the point of care:Auto-tracking clinical interventions at the bed side using RFID technology, Computers in Biology and Medicine, 40:545-54, 2010
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11. Nakaya J, Kimura M, Hiroi K, Ido K, Yang W, Tanaka H: Genomic sequence variation markup language (GSVML), International Journal of Medical Informatics, 79:130-42, 2010
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Frontier Research Unit Redox Response Cell Biology

Associate Professor　　Shun-Ichi Kurata

Induction of ΔNp63 by the newly identified keratinocyte-specific TGF-β signaling pathway with Smad2 and IKKα in squamous cell carcinoma

Expression of *p63* (TP63/p51) occurs in the basal cells of stratified epithelia, and strongly enhanced at early stages of squamous cell carcinomas (SCCs) of head-and-neck, skin, cervix, etc. To gain an insight into the stage-specific p63 promoter activation, we analyzed a promoter/enhancer region (2kΔN) that drives the predominant expression of ΔNp63, for sensitivity to Smad signaling pathways. Reporter assays in HepG2 cells showed a moderate activation of 2kΔN by Smad2 and IκB kinase α (IKKα), partners of the newly identified keratinocyte-specific TGF-β signaling, but not by other Smad molecules. In A431 cells, 2kΔN was activated by Smad2 and IKKα, for which a Smad binding element (SMD2) at –204 was essential. Binding of Smad2 to the chromosomal SMD2 site was detectable. Association of Smad2 with IKKα was evident in the nucleus of A431, accounting for the enhancement of ΔNp63 expression by TGF-β. Moreover, both ΔNp63 and IKKα were necessary to maintain the non-invasive phenotype of this cell line. FaDu, an invasive, Smad4-deficient SCC, also allowed 2kΔN trans-activation by transfected Smad2 in the presence of endogenous IKKα. Reflecting the lack of chromosomal SMD2-Smad2 association and the absence of nuclear IKKα, however, endogenous ΔNp63 was not controlled by TGF-β or IKKα in FaDu. Immunofluorescence analyses with SCC tissue arrays revealed that nuclear accumulation of IKKα and p63 intensification take place at the stage of well-differentiated, non-invasive SCC. This study indicates that p63 is a target gene of the proposed keratinocyte-specific TGF-β signal pathway for suppression of the malignant conversion of SCC.

Lung-lunginteraction in isolated perfused unilateral hyperventilated rat lungs

High tidal volume (TV) ventilation-induced lung inflammation including remote organs has still been open to discussion, and our aim is to determine this issue in isolated ventilated rat lungs perfused with salt solution. Selective right lung (RL) hyperventilation (TV of 15 ml/kg with air containing 5% CO2 on zero or 2.5 cmH20 positive end-expiratory pressure (ZEEP or PEEP)) and left lung (LL) on 2.5 cmH20 continuous positive airway pressure (CPAP) for 60 min was realized after 30-min both lung ventilation by occluding the left main bronchus, and allocated to five groups: hyperventilation under ZEEP and 3 under PEEP with re- or non-recirculation (R-ZEEP or NR-ZEEP and R-PEEP or NR-PEEP), Control; (recirculation means the same perfusate recirculates the system throughout the procedure). Wet dry ratio and protein content of bronchoalveolar lavage fluid (Prot-BALF), cytokine mRNAs, localization of TNF-α immunofluorescence double staining), and TNF-α concentration in the perfusate and BALF in each lung were measured and compared between groups by Kruskal-Wallis test. Lung injury (increased wet dry ratio, Prot-BALF, and TNF-α on endothelial and epithelial cells) was shown in the hyperventilated RLs with ZEEP compared to their corresponding CPAP LLs, and PEEP prevented these injury. Lung injury was also proved in the recirculated LL compared to the non-recirculated LL (Prot-BALF, TNF-α and IL-1β mRNAs: LL of the R-ZEEP > LL of NR-ZEEP by $p<0.01$). Unilateral hyperventilated lungs with ZEEP induced TNF-α, permeability increase, and injured the control lung via perfusion.

Publications (Original papers)

Fukunish N, Katoh I, Tomimori T, Tsukinoki K, Hata RI, Nakao A, Ikawa Y, Kurata S Induction of ΔNp63 by the newly identified keratinocyte-specific TGF-β signaling pathway with Smad2 and IKKα in squamous cell carcinoma
Neoplasia 2010; 12(12):969-979.

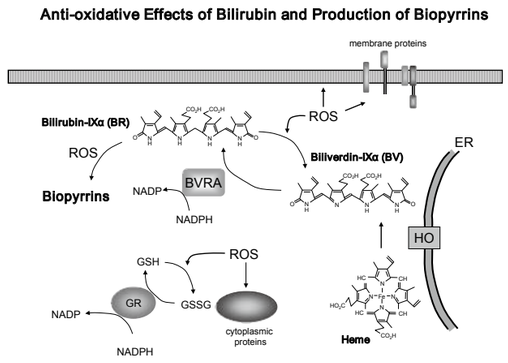
Bilali A, Kurata S, Ikeda S, Georgieva GS, Zhu C, Tomita M, Katoh I, Mitaka C, Eishi Y, Imai T. Lung-lung interaction in isolated perfused unilateral hyperventilated rat lungs. ***Transl Res***. 2010; 155(5):228-237.

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.

Publications

1. Complex of branched cyclodextrin and lidocaine prolonged the duration of peripheral nerve block. **Journal of Anesthesia** 2009. 23, 295-297. Suzuki R., Arai YCP., Hamayasu K., Fujita K., Hara K., Yamaguchi T., Sasaguri S.
2. Monitoring of urinary biopyrrins after rat cardiac transplantation. **Journal of Surgical Research** 2009. 151(2), 266. Maeda H., Yamamoto M., Radhakrishnan G., Nakagawa A., Yamamoto M., Okada H., Yamaguchi T., Sasaguri S.
3. Biphasic elevation of bilirubin oxidation during myocardinal ischemia reperfusion. **Circulation Journal** 2008. 72(9), 1520-1527. Yamamoto. M., Maeda H., Hirose N., Yamamoto M., Nakagawa A., Radhakrishnan G., Nakagawa A., Yamamoto M., Okada H., Yamaguchi T., Sasaguri S

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.

Publications

Eto K, Noda Y, Horikawa S, Uchida S, Sasaki S. Phosphorylation of aquaporin-2 regulates its water permeability. Journal of Biological Chemistry 285:40777-40784, 2010.

Medical Genomics

(Assistant Professor Shuji Sassa)

It participated in three students to do the graduation research from the Department of Clinical Laboratory Medicine, Faculty of Health Science Technology, Bunkyo Gakuin University. In an attempt to define the effects of recombinant human erythropoietin on DNA synthesis in hematopoietic organs, we investigated DNA-synthesizing enzyme activities, i.e., thymidylate synthase and thymidine kinase activities, and bromodeoxyuridine-immunohistochemistry in hematopoietic cells of bone marrow and spleen in rat. Treatment with recombinant human erythropoietin slightly increased enzyme activities, and markedly enhanced cell number of erythroid series in bone marrow cells; it also slightly increased organ weight, and remarkably enhanced S-phase cells in the spleen, followed by an augmentation of the number of erythrocytes and a rise in the hemoglobin and hematocrit levels in peripheral blood.

Publications

1. Sassa S, Nemoto N, Okabe H, Suzuki S, Kudo H, Sakamoto S. Effects of Chinese Herbal medicines on bone loss in castrated female rats. Recent Progress in Medicinal Plants 29 : Drug Plants Ⅲ, 31-40, 2010.
2. Suzuki S, Kudo H, Nakayama A, Sassa S, Kikuchi H, Sakamoto S. Effects of recombinant human erythropoietin on DNA synthesis in rat hematopoietic organs. Bunkyo J Health Sci Technol 3, 41-45, 2010.

Medical Genomics

(Associate Professor Michinori Kubota)

We investigated functional differences between the right and the left auditory cortex of guinea pigs using optical imaging. Harmonic sounds composed of 2, 4, 8, 16 kHz tones were used as stimuli. The amplitude of the 2 kHz-band became larger as the number of higher frequency tone components increased. In contrast, the amplitude of the 16 kHz-band became smaller as the number of lower frequency tone components increased. The early part of the response in 16 kHz-band was reduced in the left auditory cortex while the late part of the response in 16 kHz-band was reduced in the right auditory cortex.

International Meeting

Hosokawa Y, Kubota M, Horikawa J. Optical imaging of neural activities of the right and left guinea pig auditory cortices evoked by the harmonic sound. J Physiol Sci, Vol. 60, Suppl. 1, S139 (2010).

Affiliated Institutes

Department of Pathogenetic Regulation

Visiting Professor Mitiko Go
Visiting Associate Professor Aya Tanatani

1. Improvement of homology modeling required for functional prediction of alternative splicing product

Alternative splicing is often molecular mechanism of diseases. To understand functional change of alternative proteins produced by alternative splicing, we need the three-dimensional (3D) structure by comparative modeling, a one-to-one match of amino acid residues in the template and target sequences. The quality of alignment for comparative modeling still constitutes a major bottleneck to obtain high quality model of protein 3D structures. Substantial efforts have been made for the improvement of alignment quality, however, improvement of gap penalty has not been the focus. We revisited the correlation between protein 3D structure and the gap location in a large protein 3D structure data set, and found that the frequency of the gap location was approximated with the exponential function of the solvent accessibility of the inserted residues. The non-linearity of the gap frequency against the accessibility well corresponded to the relationship between residue mutation pattern and residue accessibility. By introducing this relationship into a gap penalty calculation for the pairwise alignment between a template and a target amino acid sequences, the sequence alignment much closer to the structural alignment was obtained. The quality of the alignments was substantially improved on a pair of sequences with identity in a twilight zone, approximately around 20 to 40%. The relocation of gaps by our new method made a significant change in comparative modeling as exemplified on Bacillus subtilis yitF protein. We contributed to CASP9 in 2010 by our method. The method was implemented in a computer program ALAdeGAP (ALignment with Accessibility dependent GAp Penalty) and is available at http://cib.cf.ocha.ac.jp/target_protein/.

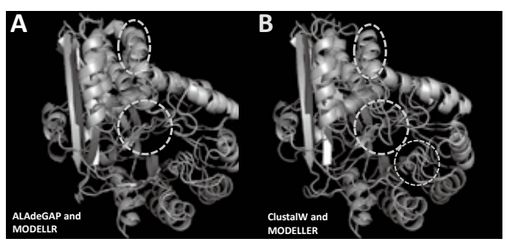


Fig.1 3D structure of Bacillus subtilis yitF based on ALAdeGAP alignment between yitF and *Escherichia* coli GlucD (A) and on ClustalW alignment (B). The colored chain is the modeled structure and white chain is the structure determined by X-ray crystallography. Yellow dotted circles emphasize the difference in both structures. The structure is viewed to the active site and the active site is covered by a miss-modeled loop in (B).

2. Development of Novel Ligands for Nuclear Receptors.

Nuclear receptors are ligand-dependent transcription factors, and regulate various biological phenomena including cell differentiation, proliferation and metabolism. We proposed the hypothesis on the nuclear receptor activation, and applied it to develop novel nuclear receptor ligands. The present major targets are androgen receptor (AR) and vitamin D receptor (VDR).

(1) Development of non-steroidal androgen antagonist:

AR plays critical roles in numerous physiological processes, such as the development and regulation of the male reproductive system, and maintenance of muscle and bone mass. AR is closely related to prostate carcinogenesis, and AR antagonists are used in the treatment of prostate cancer. In order to overcome the anti-androgen withdrawal syndrome, that is significant problem of clinical use of conventional AR antagonists, novel AR antagonists bearing different chemical structures are developed. Some compounds (Fig. 2a) exhibited potent antagonists toward both wild-type and mutated ARs.

(2) Development of non-seco-steroidal vitamin D analogs:

VDR is molecular target for drug discovery in the field of cancer, osteoporosis, and autoimmune diseases. The potent VDR ligands so far developed have seco-steroid structure, like the endogenous VDR ligand. We have developed potent non-seco-steroid type VDR ligands such as the nitrogen-containing aromatic compounds (Fig 2b).

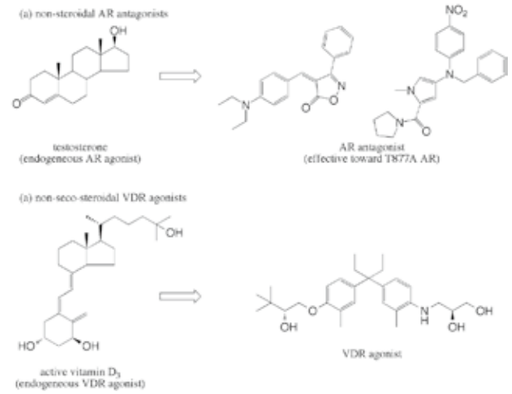


Fig.2 Novel AR antagonists (a) and VDR agonists (b)

3. Development of Functional Aromatic Foldamers Based on the Conformational Properties of Amide Bond

Amide bond is a significant structural factor for the structure and function of proteins and various bioactive molecules. In this study, various functional aromatic molecules were designed and synthesized based on the conformational properties of amide and the related functional groups. For example, the compound shown in Figure 3 changed the conformation depending on the solvent property, which can be detected by the change of the fluorescence spectra. These properties could be applied to the molecular switch or fluorescence sensors towards some external stimulus.

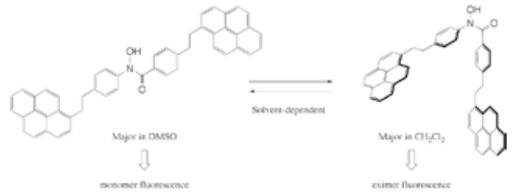


Fig.3 Aromatic amide with unique fluorescence property depending on the solvent

Publications

1. Hijikata, A., Yura, K., Noguti, T. and Go, M., Revisiting gap locations in amino acid sequence alignment and a proposal for a method to improve them by introducing solvent accessibility. PROTEINS: Structure, Function and Bioinformatics 2011 (in press).
2.Kakuta, H., Azumaya, I., Masu, H., Matsumura, M., Yamaguchi, K., Kagechika, H., Tanatani, A. Cyclic-tri(N-methyl-meta-benzamide)s: Substituent Effect on the Bowl-shaped Conformation in the Crystal and Solution States. Tetrahedron 66: 8254-8260, 2010.

Organizer of International Symposium

1.Go, M, and Clutter, M., Connections, Bringing Together the Next Generation of Women Leaders in Science, Technology, Engineering and Mathematics, the National Women's Education Center, Saitama, Japan, July 5-7, 2010.

International Contest

1.Yura, K., Hijikata A., Noguti, T. and Go, M., Application of Computer Program ALAdeGAP for Prediction Contest of Newly Determined Protein Structures, CASP9 (9th community wide experiments on the Critical Assessment of Techniques for Protein Structure Prediction) from April 14 to August 7, 2010.

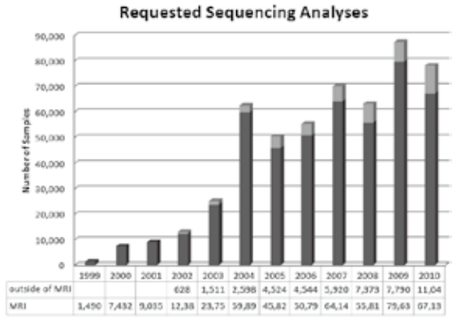
Presentation at International Meetings

1.Yura, K., Hijikata A., Noguti, T. and Go, M., Improvement of the Quality of Model Structures by Improving the Template-Target Alignments, CASP9 (9th community wide experiments on the Critical Assessment of Techniques for Protein Structure Prediction) Meeting, Asilomar, Califrornia, USA , December 5-9, 2010.
2. Tanatani, A, Control of Molecular Structure and Function. Development of Functional Aromatic Molecules in Materials Science and Medicinal Chemistry. Jpn-USA Symposium: Connections-Bringing Together the Next Generation of Woman Leaders in Science, Technology, Engineering and Mathematics, Tokyo, July, 2010.
3.Tanatani, A., Sakai, H., Mori, S., Fujii, S., Hirano, H., Kagechika, H. Development of Novel Progesterone Antagonists Bearing A 6-Arylcoumarin Skeleton. ICE2010 Official Satellite Symposium - Nuclear Receptor and its Frontier-, Kyoto, March, 2010.
4. Matsumura, M., Muranaka, A., Uchiyama, M., Development of Novel Porphyrin Derivatives Based on Steric Properties of Amide Bond. 5th ISMSC 2010, Nara, June 2010.
5. Kudo, M., Hanashima, T., Muranaka, A., Sato, H., Uchiyama, M., Kagechika, H., Tanatani, A. Synthesis and Conformational Analysis of Helical Aromatic Multilayered Ureas. ISCD-22, Sapporo, July. 2010.
6. Matsumura, M., Muranaka, A., Uchiyama, M., Masu, H., Azumaya, I., Kagechika, H., Tanatani, A. Development of Novel Porphyrin Derivatives Based on Steric Properties of Amide Bond. 2010 International Chemical Congress of Pacific Basin Societies, Honolulu, Dec. 2010.
7. Kudo, M., Hanashima, T., Muranaka, A., Sato, H., Uchiyama, M., Azumaya, I., Kagechika, H., Tanatani, A. Synthesis and Conformational Analysis of Helical Aromatic Multilayered Ureas. 2010 International Chemical Congress of Pacific Basin Societies, Honolulu, Dec. 2010.

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 2010.



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
outside of MRI				628	1,511	2,598	4,524	4,544	5,920	7,373	7,790	11,04
MRI	1,450	7,432	9,035	12,38	23,75	39,89	45,82	30,79	64,14	55,81	75,63	67,13

Fig1. Sequencing analyses
A total of 11,043 samples from the total number of 1,248 researchers were sequenced in the year of 2011.Among them about 10% were requested by researchers outside the medical Research Institute (see below).

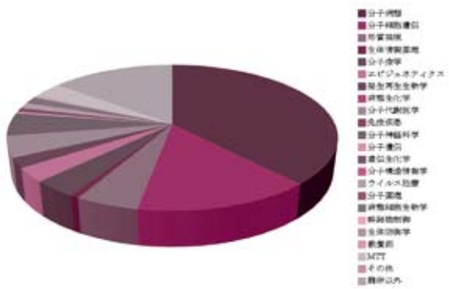
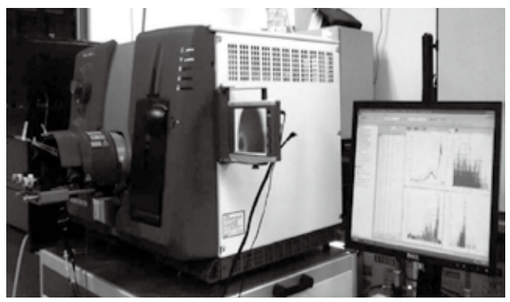


Fig2. Equipment under the management of the Genome Laboratory.
DNA sequencer (ABI3130xl)×2, PCR machine (ABI7900)×5, Light Capture, Flow Cytometer, Luminescent Plate Reader, Ice Machine, Centrifuge, and others.

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.



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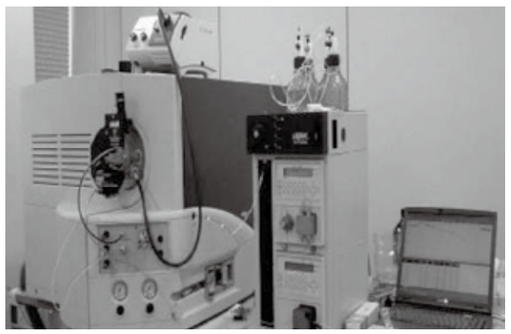


Q-Tofmicro

Publication

1. Proteome Analysis Of Bronchoalveolar Lavage Fluid in Lung Fibrosis Associated with Systemic Sclerosis. Ryutaro Shirahama,Yasunari Miyazaki,Tsukasa Okamoto,Naohiko Inase and Yasuyuki Yoshizawa. Allergology International. 59(4). 409-15(2010)
2. HumanPRP19interacts with prolyl-hydroxylase PHD3 and inhibits cell death in hypoxia Masuhiro Sato,Miki Sakota and Koh Nakayama. Experimental Cell Research. 316(17). 2871-82(2010)

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 and School of Biomedical Science.

Medical Research Institute and School of Biomedical Science collaboratively run this laboratory as a part of the core facility. The committee of this laboratory composed of faculty members of Medical Research Institute and School of Biomedical Science regulates the laboratory by educating new users, and controling generation and introduction of new recombinant mice.

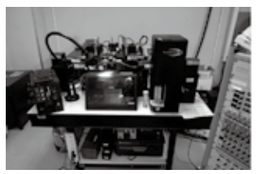
Laboratory for Structure Analysis

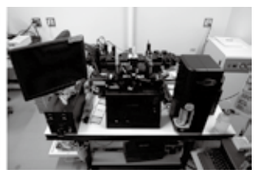
The Laboratory for Structure Analysis is the 2nd latest member of the Facilities and equipped with a high-brilliancy X-ray generator and an image plate X-ray detector. The Laboratory acquired a dynamic light scattering (DLS) instrument this year, enabling the measurements of particle size (thus oligomeric state and aggregation) of protein molecules. An introductory practical course was held for students of Graduate School of Biomedical Science. (The laboratory has moved to the 22nd floor of the M&D Tower in February 2010.)

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 16, 2009, and thereafter expanded in the year of 2010. 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).

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. The Operating Committee was held 22 times in 2011 to discuss the way to smoothly set up the Laboratory and efficiently provide the services. In 2010, as part of activities of this Laboratory, we held technical courses for the equipment: e.g. twice each for MoFlo Legacy, MoFlo XDP, and rotary tissue processor, and six times for confocal laser scanning microscope.





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

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.

Medical Top Track (MTT) Program

MTT Fellow: Yoshiko Iwai

Technical staff: Shoko Kuroda

Research Project

Regulatory mechanism of memory T cell diversity

Memory T cells are an important component of protective immunity against infectious pathogens and tumors. Upon antigen re-encounter, memory T cells expand more rapidly and more vigorously than naïve T cells and differentiate into secondary effector T cells. Following the cell burst, memory T cells are maintained at approximately constant numbers throughout life. This size control of the memory pool is critical for the host defense because a diverse repertoire of memory T cells against various pathogens should be maintained within the limited space of the memory pool. However the mechanisms that maintain memory pool size are largely unknown.

The scientific question being addressed by our study is to determine key factors that maintain the diversity of memory T cell pool. By generating knock-in mice, we have shown that a novel AP-1 transcription factor plays an important role for the maintenance of size and diversity of memory T cell pool by inhibiting secondary clonal expansion of memory T cells (manuscript in preparation). Since its expression is restricted to activated lymphocytes, this transcriptional factor will be a promising therapeutic target to control memory T cell diversity in vaccine strategies and immunotherapies.

Publications

1. Okamoto, K., Iwai, Y., Oh-hora, M., Yamamoto, M., Morio, T., Aoki, K., Ohya, K., Jetten, A.M., Akira, S., Muta, T., and Takayanagi, H. IκBζ regulates TH17 development by cooperating with ROR nuclear receptors. Nature, 2010, 464(7293):1381-5.

MTT fellow: Koh Nakayama

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

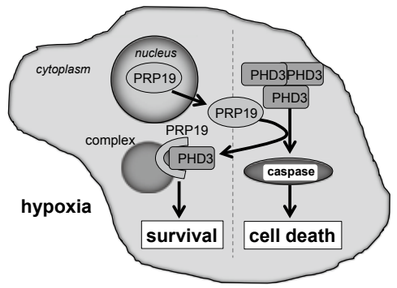
1. Signal transduction of hypoxia response

Hypoxia-inducible factor (HIF)-1α is a transcription factor which plays a central role during hypoxia response by altering multiple cellular functions including metabolism, respiration, and cell growth. HIF-1α 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-1α. We focus on PHD enzymes and study 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 PHD and other unidentified proteins. We have identified spliceosomal protein PRP19, as a component of the complex by proteomics approach. 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. PRP19 appears not to have any effect on HIF pathway. We are further characterizing this HIF-independent cell survival signal during hypoxia response.

Regulation of hypoxic cell death by PHD3-PRP19 interaction



Publications

1. Sato M., Sakota M., and Nakayama K.* Human PRP19 interacts with prolyl-hydroxylase PHD3 and inhibits cell death in hypoxia. ***Exp. Cell Res.*** 318, 2871-2882, (2010)

2. Qi J., Nakayama K., Cardiff R.D., Borowsky A.D., Kaul K., Williams R., Krajewski S., Mercola D., Carpenter P.M., Bowtell D., and Ronai A.Z.* Siah2-depenent concerted activity of HIF&FoxA2 regulates formation of neuroendocrine phenotype & neuroendocrine prostate tumors. ***Cancer Cell*** 18, 23-38, (2010).

3. Nakayama K.* Growth and progression of melanoma and non-melanoma skin cancers regulated by ubiquitination. ***Pigment Cell Melanoma Res.*** 23, 338-351, (2010).

MTT Fellow: Naoyuki Kataoka, Ph.D.

In higher eukaryotes, most genes in the nucleus are separated by introns. Therefore, a step to remove introns from pre-mRNAs, termed splicing, is required for gene expression. Recently it has been shown that many human diseases are caused by errors of RNA processing, which are called as "RNA diseases". Muscular dystrophy caused by mutations in dystrophin gene is one of the major RNA diseases. Recently we found a dystrophinopathy patient who has a point mutation in exon31 of the dystrophin gene. Although the mutation generates a stop codon, a small amount of truncated, but functional, dystrophin protein was produced in the patient cells. An analysis of the mRNA revealed that the mutation promotes exon skipping and restores the open reading frame of dystrophin. Presumably, the mutation disrupts exonic splicing enhancer and creates an exonic splicing silencer. Therefore, we searched for small chemicals that enhance exon skipping, and found that TG003 promoted the skipping of the mutated exon 31 in the endogenous dystrophin gene in a dose-dependent manner and increased production of the dystrophin protein in the patient's cells.

MTT Fellow: Shingo SUZUKI

Research Summary

Brain-derived neurotrophic factor (BDNF) is a molecule which regulates the development and specific functions of CNS neurons. Although BDNF can control transcription and protein synthesis, it still remains unclear whether BDNF controls neuronal lipid homeostasis in brain. Our previous study indicated that BDNF-induced cholesterol biosynthesis in cultured CNS neurons is essential for synapse development. However its effects on cholesterol metabolism in vivo or on other lipid species are not fully investigated. For a better understanding of the effect of BDNF on lipid homeostasis in brain, we investigated the metabolomic analysis of neuronal lipids using Gas Chromatography-Mass Spectrometry (GC/MS), and profiled the brain lipids in BDNF-KO mice. Our results indicated the impairment of cholesterol biosynthesis and metabolism in BDNF-KO mice brain. This suggested that BDNF not only controlled brain cholesterol synthesis, but also regulated cholesterol metabolism in brain. Alterations of cholesterol homeostasis in brain are observed in neurodegenerative disorders. Therefore, we now focused on the role of cholesterol homeostasis in CNS neurons.

Publications

1. Numakawa T, Suzuki S, Kumamaru E, Adachi N, Richards M, Kunugi H. BDNF function and intracellular signaling in neurons. Histol and Histopathol. 25(2):237-58.(2010)

MTT Fellow: Tetsuo Sasano

Research Project

Extracellular ATP released through pannexin mediates infiltration of macrophage and inducibility of atrial fibrillation in stretched atrium.

Atrial fibrillation (AF) is the most common sustained arrhythmia in the world. Since people with AF have a greater risk of stroke and heart failure, it is important to establish the treatment for AF. Recent findings indicate that atrial inflammation plays a critical role in the initiation and progression of AF, also called as atrial remodeling. However the mechanism of inflammation is still not well understood.

We found that mechanical stretch of atrial myocyte induced transient release of ATP to extracellular space through pannexin, a gap junction family channel. The extracellular ATP recruited migration of macrophage, and expression of inflammatory cytokines. These changes were inhibited by gap junction channel blocker. Then we established the atrial pressure overload murine model. Pressure overload induced infiltration of macrophage and deposition of collagen fiber in atria, in addition to increased inducibility of AF by electrical stimulation. Administration of gap junction channel blocker attenuated the macrophage infiltration, fibrosis, and AF inducibility. This novel mechanism via extracellular ATP contributes, at least in part, to the infiltration of macrophage, followed by fibrosis and electrical instability of atria. These results might give us an idea of novel therapeutic target for AF.

Role of NOS1AP in cardiac function and arrhythmogenicity.

Recent genome-wide association study revealed that single nucleotide polymorphism of NOS1AP (Nitric Oxide synthase 1 Adaptor Protein) was highly associated with QT interval and sudden cardiac death. We have reported that NOS1AP regulates Ca channel activity via NO production in guinea pig cardiomyocytes.

We evaluated the role of NOS1AP in heart using knockout (KO) mice. KO mice showed slightly prolonged QT interval, diminished cardiac function, and increased reactive oxygen species (ROS) production than wildtype (WT) mice. In pressure overload model, KO mice showed increased mortality with much higher occurrence of ventricular tachycardia than WT. Acute oxidative stress model by administration of doxorubicin reduced cardiac contractile function resulting in high mortality by heart failure.

Genetic deletion of NOS1AP exhibits increased arrhythmogenicity and/or reduced cardiac function under pathological conditions. These results give us the information for risk stratification of sudden cardiac death.

Publications

1. Yamashiro K, Sasano T, Tojo K, Namekata M, Kurosawa J, Sawada N, Suganami T, Kamei Y, Tanaka H, Tajima N, Utsunomiya K, Ogawa Y, Furukawa T. Role of transient receptor potential vanilloid 2 in LPS-induced cytokine production in macrophages. Biochem Biophys Res Commun. 2010; 398: 284-9.

MTT Fellow: Takeshi Matsui

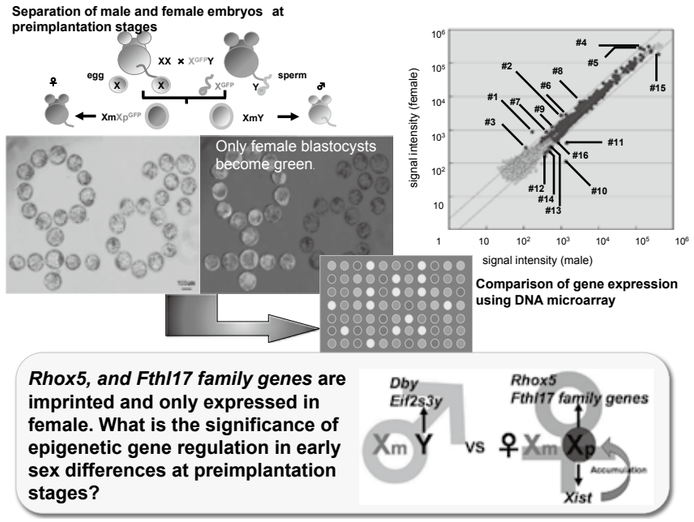
The Adaptive Evolution of Epithelial Cells

About 360 million years ago, the first vertebrate, amphibian emerged from the sea and adapted to life on land. They evolved their surface epithelium into multilayered stratified epithelia. Placing particular emphasis on this epithelium, intends to uncover the mystery of 'epithelial evolution'. We have recently generated skin-specific protease, SASPase-deficient hairless mice. Biochemical and physiological analysis of epidermis of these mice revealed that SASPase activity is indispensable for maintaining the texture and hydration of the skin.

MTT Fellow: Shin Kobayashi

Epigenetic regulation in male and female at preimplantation stages

When do sex differences first appear in mammals? To answer the question, our group tried to compare the gene expression patterns by sex before implantation. However, the sex of pre-implantation embryos is difficult to determine morphologically. So, utilizing the sex-determining method based on EGFP transgenic mouse, we collected more than 1000 sexed blastocyst samples. Furthermore, we compared the gene expression patterns of male and female blastocysts using DNA microarray, and found that nearly 900 genes showed differential expression between sexes at the stage! Among these, *Rhox5*, *Fthl17 family genes, and Xist* showed female specific expression. Using DNA polymorphisms, we successfully verified *Rhox5 and Fthl17 family genes* are imprinted and expressed from paternal X chromosome, like the *Xist* gene (Kobayashi, S. et al. Curr. Biol. 2006; Kobayashi, S. et al., Nucleic Acids Res., 2010). The discovery of imprinted genes only expressed in female suggests that there is epigenetic gene regulation in early sex differences. Now, we are trying to clarify the relationship between epigenetic gene regulation and mammalian development and/or diseases.



Publications

Shin Kobayashi*, Yoshitaka Fujihara, Nathan Mise, Kazuhiro Kaseda, Kuniya Abe, Fumitoshi Ishino, Masaru Okabe (2010) The X-linked Imprinted Gene Family *Fthl17* Shows Predominantly Female Expression Following the 2-cell Stage in Mouse Embryos **Nucleic Acids Res.** 38(11), 3672-81. (*: corresponding author)

Invitation to be plenary speaker

Shin KOBAYASHI
82 Annual Meeting THE GENETICS SOCIETY OF JAPAN (Sapporo, 2010/09/22)
Title: Epigenetic gene regulation in male and female development

List of research grants

Grant-in-Aid for Scientific Research on Innovative Areas (2010-2011) Principal Investigator: Kobayashi Shin.
Sumitomo Foundation Aid for basic scientific research (2010) Principal Investigator: Kobayashi Shin

MTT fellow: Akimitsu Konishi

Research assistant: Kyoko Tsujimura

Subjects of Research

1. Understanding of DNA damage signaling

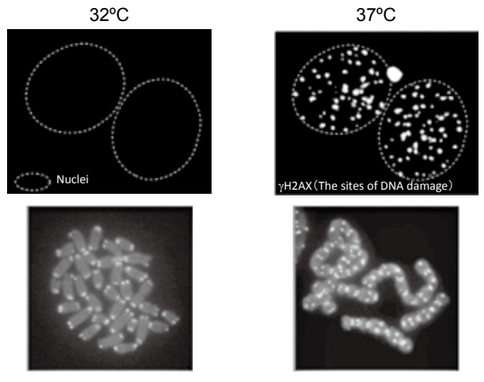
2. Mechanism of the chromosome end protection

Research Summary

The biological response to DNA damage is one of the central issues in modern biology. The continuity of life depends on the ability of cells to protect their DNA from intrinsic and extrinsic damage and deficiencies in the DNA damage response can contribute to tumorigenesis, aging, hereditary disorder, and fertility problems. Eukaryotic chromosomes end in specialized structures, called telomeres, to cap chromosomal ends, preventing induction of DNA damage response. Our group studies DNA damage signaling using the dysfunctional telomere as a tool.

We have developed the system to control telomere function for chromosome ends protection (Figure, Genes Dev 2008).

Now, we are trying to apply this system to understand the general DNA damage responses. We also study the mechanism of telomere length regulation that is deeply involved in tumorigenesis and aging.



Telomere dysfunction induction system
DNA damage response was induced at telomeres by temperature shift (Upper)
Dysfunctional telomeres were fused at G1 cell cycle stage (Lower)

Publications

Shimizu, S., Konishi, A., Nishida, Y., Mizuta, T., Nishina, H., Yamamoto, A., and Tsujimoto, Y. Involvement of JNK in the regulation of autophagic cell death. ***Oncogene*** (2010), 29, 2070-2082.

Chemical Biology Screening Center

Chemical biology is a new study to clarify the mechanism of biological phenomena by chemical method. Chemical Biology Screening Center (CBSC) was set up as the support facility to promote the chemical biology. Main aim of CBSC is 1) storage and distribution of small chemical compounds for screening, 2) support of the screening of chemical compounds and 3) preparing the platform for sharing the information about the chemicals which CBSC hold.

Currently, about 19,000 chemical compounds are available from CBSC. All compounds soluble in DMSO are distributed as the plate format or independently, according to the request. Equipments in CBSC are open to all staff and students in TMDU. Information about CBSC is available at the web site (http://www.tmd.ac.jp/mri/SBS/cbsc/index.html, currently only Japanese version is available). The TMDU Chemical Biology Database (CBDB, http://bsmdb.tmd.ac.jp/) has also been available to share the research information from the chemical compounds provided by CBSC.

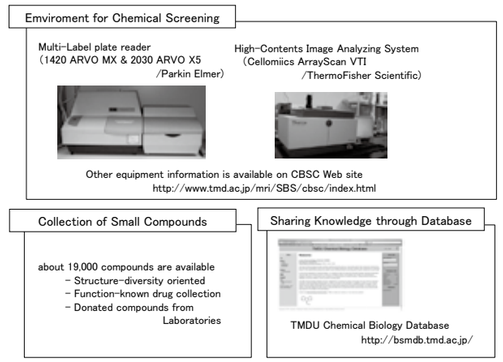


Fig.1: Overview of CBSC

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
Postgraduate Students	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), which are described below as examples.

1-1. Development of a novel selective inhibitor of the Down syndrome-related kinase DYRK1A

DYRK1A is a serine/threonine kinase essential for brain development and function, and its excessive activity is considered as a pathogenic factor in Down syndrome. The development of potent, selective inhibitors of DYRK1A would help to elucidate the molecular mechanisms of normal and diseased brains, and may provide a new lead compound for molecular-targeted drugs. We found a novel DYRK1A inhibitor, INDY, a benzothiazole derivative showing a potent ATP-competitive inhibitory effect with IC_{50} and K_i values of 0.24 and 0.18 μM, respectively. Unlike harmine, another well-known inhibitor of DYRK1A, INDY does not inhibit monoamine oxidase (MAO), implying its high specificity.

Our X-ray crystallographic studies of the DYRK1A/INDY complex and DYRK1A/harmine complex revealed that both inhibitors bind to the enzyme at its ATP pocket in a similar manner (Fig. 1). Comparison with the structure of the MAO/harmine complex suggested that the difference between INDY and harmine in size and/or polarity might be the source of INDY's higher specificity.

Fig1. Crystal structure of Dyrk1A/INDY complex. INDY (dark color) is bound in the ATP pocket of Dyrk1A (light color).

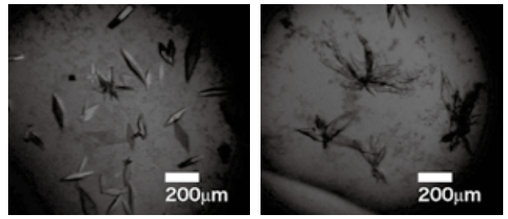
This work was collaboration with Prof. Hagiwara and Prof. Hosoya.

1-2. Structural analysis of a VDR mutant bound to an antagonist ligand

Vitamin D receptor (VDR) utilizes 1α, 25- dihydroxy vitamin 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 VDRand studies of chemical biology dealing with such compounds are expected for application to drug discovery.

Recently, it was reported that vitamin D derivatives with 22-butyl group showed antagonistic activity for VDR. We tried to crystallize the complexes of the ligand binding domain (LBD) of VDR with the derivatives to elucidate their antagonistic mechanism. We, however, could not obtain the crystal with quality enough to determine the structure of the complex.

At lomg last we found that a single mutation of VDR-LBD, K409G, improved the quality of the crystal of the complex (Fig. 2), although the mutation hardly affected the activity of the protein, and we finally succeeded in determining the complex structwre of the VDR-LBD with a 22*S*-butyl antagonist. This complex structure showed that the large structural displacement of the loop 6-7 and the helix 11 of VDR-LBD was caused by the antagonist (Fig. 3), suggesting that the conformation of the helix 11 and the loop 6-7 was very important for the activity of VDR.



(a) (b)

Fig2. One point mutation of the K409G leads to success the crystallization with 22S-butyl type antagonists.Crystals of ternary complex of rat VDR-LBD with the 22S-butyl antagonist and the peptide are shown. Crystals of (A) and (B) were made from rat VDR-LBD with and without the K409G mutation, respectively. Although similar conditions were applied for both crystallizations, large difference was appeared between them. Crystals of (B) were not suitable for diffraction experiment.

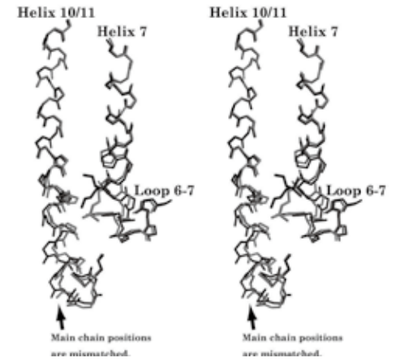


Fig3. 22*S*-butyl antagonist would displace the loop 6-7 and the helix 11 of rat VDR-LBD (Stereo diagram). 22*S*-butyl antagonist-bound K409G (shown by dark grey) and 1,25-D_3-bound K409G (shown by light grey) are superimposed. Mismatched positions on the helices 11 (indicated by an arrow) and the loops 6-7 are shown. Side chains of ligands and the parts of VDR-LBDs are only presented in this figure.

2. Analysis of interactions between tau protein and Pin1

Tau protein is essential to assembly of microtubule, which mainly consists of two types of tubulin. Hyperphosphorylation of tau protein abolishes its ability to bind tubulin and promote microtubule assembly. When it is released from tubulin, the phosphorylated tau protein aggregates into paired helical filaments, which are regarded as the neuropathological hallmarks of Alzheimer's disease. Recently it was revealed that a peptidyl-prolyl isomerase Pin1 restored the ability of the phosphorylated tau protein to bind tubulin 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. We investigated interactions between tau protein and Pin1. As a preliminary result, Pin1 interacted with all the pS/T-P sites of tau protein, but their interactions were too weak and transient to form a stable complex. This suggests that the catalytic function of Pin1 is important to restore the activity of tau protein.

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, contributes to the continuous effort by wwPDB to improve PDB.

Research Papers

1. Inaba Y, Nakabayashi M, Itoh T, Yoshimoto N, Ikura T, Ito N, Shimizu M, Yamamoto K; 22S-Butyl-1alpha,24R-dihydroxyvitamin D(3): Recovery of vitamin D receptor agonistic activity. J. Steroid Biochem. Mol. Biol., 121, 146-150 (2010).
2. Ogawa Y, Nonaka Y, Goto T, Ohnishi E, Hiramatsu T, Kii I, Yoshida M, Ikura T, Onogi H, Shibuya H, Hosoya T, Ito N, Hagiwara M; Development of a novel selective inhibitor of the Down syndrome-related kinase Dyrk1A. Nat. Commun., 1, 1-9 (2010).

International conferences

1. Ito N: PDBj. CCP4 Seminar and Workshop, Osaka, November 2010.

Domestic conferences

1. Nonaka Y, Ogawa Y, Ikura T, Yoshida M, Hiramatsu T, Onogi H, Hosoya T, Ito N, Hagiwara M: A structural analysis of a potential Down syndrome drug discovery lead compound. The 5th Annual Meeting of Japanese Society for Chemical Biology, Yokohama, 2010.
2. Nakabayashi M, Inaba Y, Sakamaki Y, Yoshimoto N, Ito M, Shimazaki M, Masuno H, Shimizu M, Yamamoto K, Ikura T, Ito N: What 22S-butyl-vitamin D3 tells us about its functional mechanism? The 62nd Annual Meeting of the Vitamin Society of Japan, Morioka, 2010
3. Masuno H, Kazui Y, Ikura T, Nakabayashi M, Ito N, Fujii S, Kawachi E, Tanatani A, Kagechika H: Synthesis of LCA derivatives with VDR activities and their complex structure with VDR. The 62nd Annual Meeting of the Vitamin Society of Japan, Morioka, 2010

Laboratory of Chemical Bioscience

Professor **Takamitsu Hosoya**
Assistant Professor **Suguru Yoshida**
Assistant Professor **Yuto Sumida**

Strain-promoted double-click reaction: a novel method for chemical modification of biomolecules

"Click reaction," copper(I)-catalyzed azide–alkyne [3+2] cycloaddition, is an emerging method for conjugating molecules in the fields of chemistry and biology. However, cytotoxicity by the copper catalyst and the slow rate of the reaction have restricted its application. Recently, to overcome these limitations, the strain-promoted azide–alkyne [3+2] cycloaddition, a copper-free variant of click reaction, has been introduced exploiting the spontaneous reactivity of cyclooctynes (Fig. 1, **1**, **2a–c**) toward an azide owing to the ring strain.

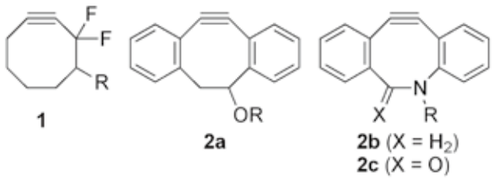


Fig. 1 Strained-alkynes.

In order to make this technique more practical, we developed a novel method for chemical modification of azido-biomolecules by introducing strain-promoted "double-click" reaction, a dual click reaction mediated by *sym*-dibenzo-1,5-cyclooctadiene-3,7-diyne (**3**, Sondheimer diyne), possessing two highly-strained alkyne bonds (Fig. 2). We found that both triple bonds of diyne **3** undergo spontaneous cycloaddition with various azides, giving a unique saddle-shaped bis-cycloadduct in high yield. The mono-cycloadduct, the presumed monoyne intermediate, was neither isolated nor detected even when an equimolar amount of azide was used, indicating that the monoyne intermediate is more reactive toward azides than the starting diyne. Treatment of an azido-incorporated protein with diyne **3** followed by a small azido-compound provided the desired hetero-cycloadduct in high efficiency. Under these conditions, the signal for the homo-dimer of the azido-incorporated protein was substantially undetected.

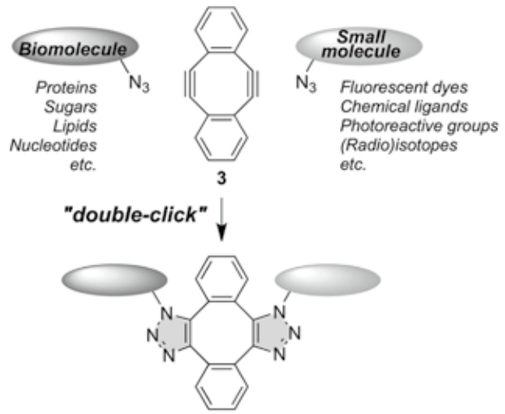


Fig. 2 Strain-promoted double-click reaction mediated by Sondheimer diyne (**3**).

Furthermore, this reaction also worked efficiently for fluorescence visualization of azido-incorporated glycoconjugates in cultured cells. Considering the availability of the diyne as well as various small functional azido-compounds, the strain-promoted double-click modification of azido-biomolecules would be a valuable method to accelerate various researches in the field of chemical biology.

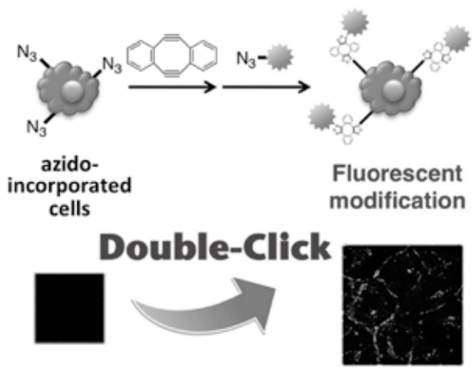


Fig. 3 Fluorescence visualization of azido-incorporated glycoconjugates in cultured cells by means of strain-promoted double-click reaction.

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. Kii I, Shiraishi A, Hiramatsu T, Matsushita T, Uekusa H, Yoshida S, Yamamoto M, Kudo A, Hagiwara M, Hosoya T. Strain-promoted double-click reaction for chemical modification of azido-biomolecules. Org Biomol Chem 8(18): 4051-4055, 2010.
2. Inouye S, Iimori R, Sahara Y, Hisada S, Hosoya T. Application of New Semi-Synthetic Aequorins with Long Half-Decay Time of Luminescence to G-Protein-Coupled Receptor Assay. Anal Biochem 407(2): 247-252, 2010.
3. Ogawa Y, Nonaka Y, Goto T, Ohnishi E, Hiramatsu T, Kii I, Yoshida M, Ikura T, Onogi H, Shibuya H, Hosoya T, Ito N, Hagiwara M. Development of a novel selective inhibitor of the Down syndrome-related kinase Dyrk1A. Nat Commun 1:86 doi: 10.1038/ncomms1090, 2010.
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5. Abe Y, Okumura E, Hosoya T, Hirota T, Kishimoto T. A single starfish Aurora kinase performs the combined functions of Aurora-A and Aurora-B in human cells. J Cell Sci 123(22): 3978-3988, 2010.

School of Biomedical Science, Laboratory of Organic and Medicinal Chemistry

Professor Hiroyuki Kagechika
Assistant Professor Shinya Fujii
Assistant Professor Shuichi Mori

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).

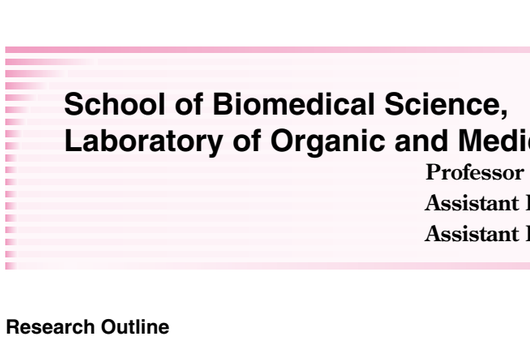
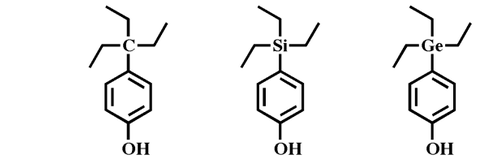


Fig. 1. Action mechanism of retinoic acid and synthetic retinoid, Am80.

We also developed novel hydrophobic pharmacophore. In this year, we examined the function of various silyl- or germyl-containing substituents as hydrophobic part of nuclear receptor ligand structures. For example, triethylsilyl and triethylgermyl groups are more hydrophobic than 2,2-diethylpropyl group, and *p*-(triethylgermyl)phenol are more potent estrogen than *p*-(2,2-diethylpropy)phenol (Fig. 2).



log *P*	5.0	5.7	5.7
EC_{50} (nM)*	23	3.4	2.1

*EC_{50} value was deteremined by the cell proliferation assay of MCF7 cells.

Fig. 2. Hydrophobicity and estrogenic activity of silyl- or germyl-containing phenols

3. 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 fluorescent sensors bearing a bodipy group by control of intramolecular complex formation between a bodipy moiety and another molecular species (Fig. 3). In this strategy, almost complete quenching of fluorescence was observed in aqueous media, due to the intramolecular heterodimer formation, and the fluorescence is restored by disruption of the heterodimerization in response to change of solvent polarity.

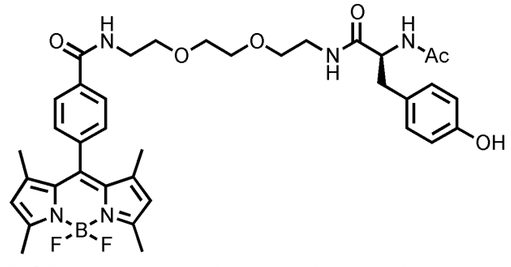


Fig. 3. Example of a bodipy derivative in response to environmental change

4. 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 *trans* structures of most secondary amides, such as acetanilide and benzanilide, 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 the benzhydroxamic acid derivative changed its amide conformation depending on the solvent property, which could be detected by the fluorescence spectra (Fig. 4). These properties could be applied to the molecular switch or fluorescence sensor.

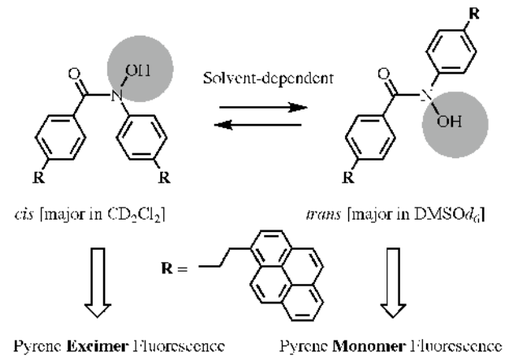


Fig. 4. Solvent-dependent conformational change of benzhydroxamic acids

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Invited Lectures at International Symposium

1. Kagechika, H. Development of Novel Synthetic Ligands for Nuclear Receptors. The 14th International Congress of Endocrinology (ICE2010), Kyoto, Mar. 2010.
2. Kagechika, H. Development of Novel Non-seco-Stroid-type Vitamin D Derivatives. ICE2010 Official Satellite Symposium - Nuclear Receptor and its Frontier-, Kyoto, Mar. 2010.

Presentation at International Symposium

1. Fujii, S., Ohta, K., Endo, Y., Kagechika, H. Development of Novel Nonsteroidal Androgen Receptor Ligands Based on Hydrophobic Boron Clusters. ICE2010 Official Satellite Symposium - Nuclear Receptor and its Frontier-, Kyoto, Mar. 2010.
2. Tanatani, A., Sakai, H., Mori, S., Fujii, S., Hirano, T., Kagechika, H. Development of Novel Nonsteroidal Androgen Receptor Ligands Based on Hydrophobic Boron Clusters. ICE2010 Official Satellite Symposium - Nuclear Receptor and its Frontier-, Kyoto, Mar. 2010.
3. Matsumura, M., Muranaka, A., Uchiyama, M., Masu, H., Azumaya, I., Kagechika, H. Tanatani, A. Development of Novel Porphyrin Derivatives Based on Steric Properties of Amide Bond. ISCD-22, Nara, June, 2010.
4. Kudo, M., Hanashima, T., Muranaka, A., Sato, H., Uchiyama, M., Kagechika, H., Tanatani, A. Synthesis and Conformational Analysis of Helical Aromatic Multilayered Ureas. ISCD-22, Aug. 2010.
5. Fujii, S., Yamada, A., Ohta, K., Endo, Y., Kagechika, H. Development of Novel Androgen Antagonists Based on Hydrophobic Boron Clusters. EFMC-ISMC2010 21st International Symposium on Medicinal Chemistry, Brussels, Bergium, Sep. 2010.

Laboratory of Biosystem Modeling, School of Biomedical Science

Associate Professor Yutaka Fukuoka

1. Bioinformatics

1) Local 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) Adaptive threshold for detecting differentially expressed genes in microarray data

To detect significant changes in gene expression, a fixed threshold is used in various studies. However, it is not always guaranteed that a threshold which is appropriate for highly expressed genes is suitable for genes with low expression. In this study, aiming at detecting truly differentially expressed genes from a wide range of expression levels, we proposed an adaptive threshold method. The proposed method employs two independent measurements in the same condition to model the total error and divides the data sets into some bins according to the expression level in one data set. Then based on local variance of the data, upper and lower thresholds are calculated in each bin. The minimum requirement of the proposed method is two independent measurements in the same condition. The method is designed to detect truly differentially expressed genes from a small size data set. However, in such a data set, there is a trade-off between suppressing the number of false positives and achieving the perfect detection of all meaningful changes. We focus on suppressing the number of false positives because it would make interpretation of the result easier.

To investigate the performance of the proposed method, we have simulated some data sets in which we know the subsets of genes that are up-regulated, down-regulated and unregulated, respectively. The data sets were constructed as follows. The mean values of 13 experiments from human hippocampus were used as the true values of the control data. Then zero-mean additive and multiplicative noises were added to the control data. The variance of the additive noise was varied in a range between 0.01 and 20 and that of the multiplicative noise was between 0 and 0.1. Figure 1 illustrates a typical distribution of the ratio between two control measurements and the adaptive thresholds. As shown in the figure, ratios of most genes from the two control measurements are between the upper and lower thresholds, indicating that the adaptive thresholds are appropriate to detect truly differentially expressed genes in comparison with measurements between different conditions. Then, using the upper and lower thresholds, we compared another data set, in which 1000 genes were randomly selected and up- or down-regulated. The specificity in the investigated conditions was always greater than 99%, indicating that the adaptive threshold method can suppress the number of false positives and thus make interpretation of the result easier.

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 accurately 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 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.

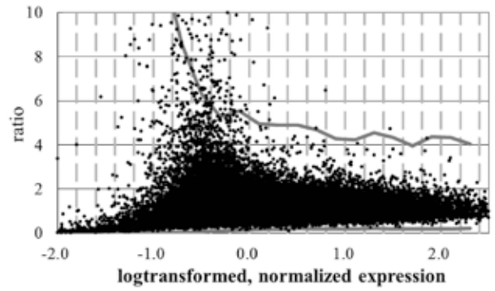

Fig.1. A example of distribution of the ratio between two control measurements and the adaptive thresholds. A dot represents a ratio of the two expression values of a gene. The bins used to calculate the adaptive thresholds are illustrated by the dashed lines. The grey solid lines represent the upper and lower thresholds. Only few genes were greater/lower than the upper/lower threshold.

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1. Tsutsumi T, Murakami M, Kawaishi J, Chida W, Fukuoka Y, Watanabe K. Postural stability during visual stimulation and the contribution from the vestibular apparatus. Acta Oto-Laryngologica, 130:464-471, 2010

Presentation at International Meetings

1. Suzuki S, Takai-Igarashi T, Fukuoka Y, Tanaka H, Fusaro V, Pivovarov R, Tonellato PJ. Clinical gene network analysis on inflammatory bowel disease. International Omics Symposium, Yokoyama, Feb. 2010
2. Fukuoka Y, Inaoka H, Noshiro M. Adaptive Threshold for detecting differentially expressed genes in microarray data-a simulation study to investigate its performance. 32nd International IEEE EMBS Conference, Buenos Aires, Sep. 2010
3. Kobayashi K, Tanaka M, Inaoka H, Kokubo K, Nebuya S, Fukuoka Y, Kobayashi H, Noshiro M. Temporal changes in gene expressions and cytokine productions caused by stretching of normal human pulmonary artery endothelial cells. Europeean Respiratory Society Annual Congress, 281, Sep. 2010
4. Inaoka H, Fukuoka Y, Noshiro M. Genome-wide DNA methylation analysis in tumor and normal tissues from lung squamous cell carcinoma and serous cystadenocarcinoma. IEEE International Conference on Bioinfomatics & Biomedicine, Hong Kong, Dec 2010
5. Kobayashi K, Tanaka M, Inaoka H, Kokubo K, Nebuya S, Fukuoka Y, Kobayashi H, Noshiro M. Effects of cyclic stretch on early response gene expressions in normal human pulmonary artery endothelial cells in vitro. IEEE International Conference on Bioinfomatics & Biomedicine, Hong Kong, Dec 2010

Department of Medical Omics Informatics

Professor	Hiroshi MIZUSHIMA
Lecturer	Eiko UCHIYAMA
Assistant Professor	Mahmut YASEN, Afraz Ul QRAISH
Staff	Ken MIYAGUCHI, Shota NEMOTO, Mizuho ISHII, Miho MAKIMURA

Department of Medical Omics Informatics was established as an endowed department in Graduate School of Biomedical Science of Tokyo Medical and Dental University in Oct. 2009. The main theme of this department is to apply omics information to medicine and to enhance the research and the development of medical omics informatics.

Research

Integrated Database

We have been collecting clinical and Omics (Genomics, Transcriptomics, Proteomics, Epigenomics, Glycomics, etc.) data as the Integrated Database Project. This includes both comprehensive molecular Omics information and comprehensive clinical information from almost 1,000 patients at TMD-Hospital, who has either colon cancer, liver cancer or oral cancer. This project has been performed by the collaboration with Department of Systems Biology (Prof. Tanaka), Department of Surgical Oncology (Prof. Sugihara), Department of Hepato-Biliary-Pancreatic surgery (Prof. Arii) and Department of Maxillofacial Surgery (Prof. Amagasa). Data cleaning and integrated analysis, along with molecular biological analysis has been performed. As the outcome of this project, we established "integrated Clinical Omics Database: iCOD (http://omics.tmd.ac.jp/)" in 2008.

Database Analysis

Updating and analyzing this database, we have found several genes related to oral, colon and liver cancer. This was done not only by analyzing genomics or transcriptomics data, but by integrating multiple omics data along with the detailed clinical data with them. Especially, in case of colon cancer, it is very difficult to find the related gene for subgroups of colon cancer, but could find possible relation using copy combination of number aberration and expression analysis.

Developing easy detection device for personalized medicine

We are also developing an instrument which can analyze the molecular information in fast, easy and low-cost way, with the collaboration with Micro-Blood-Science Inc. Developing this kind of device is very important, as medical tests in most clinics is outsourcing the analysis and requires several days to get the result. Aim of our instrument is to get the result while patient is in the clinic. We are also adopting this device for food safety.

Information systems for Medicine

Our research also includes developing an easy system using mobile tablet computers, which can be used by the doctors or co-medicals easily and efficiently everywhere. This activity includes organizing a Symposium " Mobile Health 2010" on Oct.23th 2010 at our campus.

Publications

Tsukamoto S, Ishikawa T, Iida S, Ishiguro M, Mogushi K, Mizushima H, Uetake H, Tanaka H, Sugihara K. Clinical Significance of Osteoprotegerin Expression in Human Colorectal Cancer. Clin Cancer Res. 2011 Jan 26. PMID: 21270110.

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Presentation at International Conferences.

Taika Muto, Hiroshi Mizushima, Setsuo Hirohashi, Shin Fujita and Tadashi Kondo (National Cancer Center Research Institute) 4th Euro Proteomics Association Meeting 2010.10.23 COLORECTAL CANCER PROTEOMICS IDENTIFIED PROTEINS ASSOCIATED WITH CARCINOGENSIS AND CANCER PROGRESSION

Satoshi Nagaie, Kazuo Terashima, Kaoru Mogushi, Yasen Mahmut, Ken Miyaguchi Noriaki Nakamura, Shinji Tanaka, Masanobu Kitagawa, Shigeki Arii, Hiroshi Mizushima, Hiroshi Tanaka. Comprehensive analysis of gene expression in hepatocellular carcinoma with special reference to hepatic stellate cells, 2011, 9th Asian Pacific Bioinformatics Conference (Inchon, Korea)

Shireli muhammad, Mahmut Yasen, Kaoru Mogushi, Maynur Abdurahman, Gulanbar Obulhasim, Shinji Tanaka, Shigeki Arii. Gene expression analysis of high serum a-fetoprotein associated gene in human hepatocellular carcinoma. 33th (2010) Annual Meeting of the Molecular Biology Society of Japan. Kobe Port Island. Kobe December 7-10. 4P-0838.

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Advisory Committee Members of Medical Research Institute, School of Biomedical Science, and Biomedical Science Ph D Program

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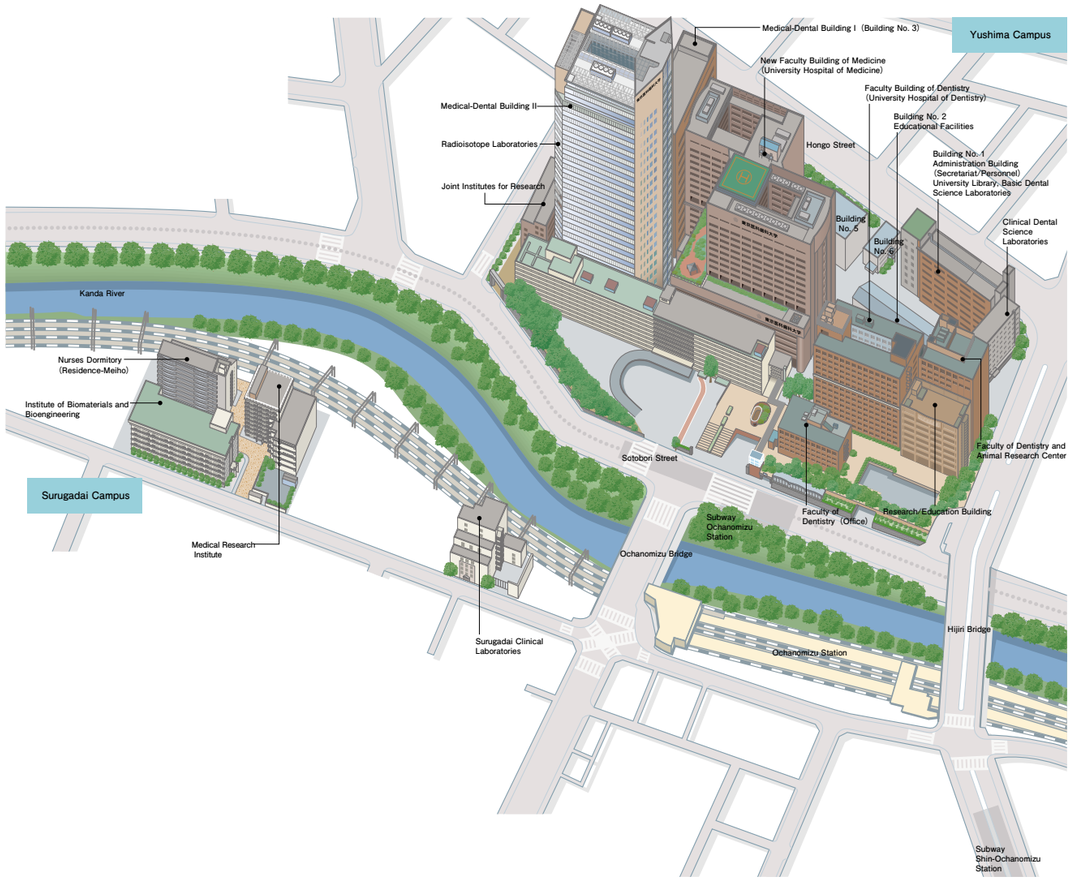
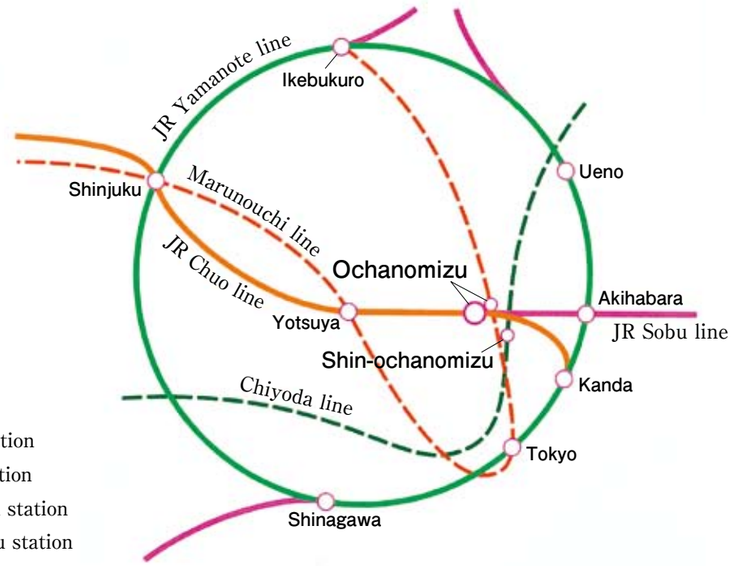
Access Map

Tokyo Medical and Dental University





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