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ANNUAL REPORT 2013

Tokyo Medical and Dental University

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2013

2013

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

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Medical Research Institute

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Medical Research Institute

Department of Molecular Epidemiology, Frontier Research Unit Virus Research Unit, Project Research Unit

Medical Research Institute

Highlight

The 11th Surugadai International Symposium/ The MRI Joint Usage/Research Symposium

The Surugadai International Symposium has been held annually since 2002. For 11 years, this symposium has covered current and critically important topics in the biomedical field. This year the symposium was held on July 31, 2012 at Akio Suzuki auditorium by the theme of "New Waves of Omics Research." It was organized by the Division of Medical Genomics.

Human genome data, as well as various omics data, have now rapidly being accumulated. The "human" genome data is rapidly expanding to "personal" genome data, and such omics research will generate useful medical information, which should soon be translated into clinical practice. Cutting edge topics were covered and active discussion was seen throughout the symposium. The speakers participated in the symposium and their presentations were as follows.

Dr. Keiichi Kodama (Stanford Univ. School of Medicine, Dept of Pediatrics, Div. of Systems Medicine)

"Expression-based genome-wide association study links the receptor CD44 in adipose tissue with type 2 diabetes."

Dr. Yusaku Nakabeppu, (Medical Institute of Bioregulation, Kyushu Univ.)

"Altered expression of diabetes-related genes in Alzheimer's disease brains."

Dr. Satoru Miyano, (Institute of Medical Science Univ. of Tokyo, Human Genome Center)

"Whole genome sequencing and supercomputer for personalized genomic medicine: The IMSUT plan"

Dr. Hiroshi Tanaka (MRI, TMDU)

"Systems-pathological approach to cancer – metastasis and drug discovery"

Dr. Rui Chen (Stanford Univ. School of Medicine, Dept. of Genetics)

"Personal omics profiling reveals dynamic molecular and medical phenotypes."

Dr. Fumio Ishino (MRI, TMDU)

"Contribution of LTR retrotransposons to evolution of mammals: a novel view from comparative genomics"

After the Surugadai symposium, The MRI Joint Usage/Research Symposium was held and the following speakers gave a talk.

Dr. Shin Hayashi (MRI, TMDU)
Dr. Masaki Sone (Toho Univ.)
Dr. Takayoshi Suganami (MRI, TMDU)
Dr. Tomoo Ogi (Nagasaki Univ.)
Dr. Nobuyuki Onai (MRI, 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 Pharmacology】

- We found that parathyroid hormone receptor requires β2-Adrenergic receptor for its anabolic action in vivo.
- Identification of Profilin-1 as a migration regulator of skeletal morphology.

【Molecular Cell Biology】

- WNK signaling pathway is involved in neural development via Lhx8 gene expression.
- IQGAP regulates the nuclear localization of Dvl in Wnt signaling.

【Molecular Neuroscience】

- Overstimulation of NMDA receptors impairs early brain development in vivo.
- Hyperactivation of the mouse lateral habenula enhances freezing behavior under acute stress.
- A genetically female brain is required for a regular reproductive cycle in chicken brain chimeras.

【Biodefense Research】

- Discovery of new DC progenitors, which provides significant insight into DC differentiation pathway.
- Establishment of novel IFN-based pre-transplantation conditioning in the treatment of a congenital metabolic disorder.

【Bio-informational Pharmacology】

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

【Stem Cell Regulation】

- We demonstrated that cyclin D1, a major downstream effector of FGF2, is a negative regulator of astroglial differentiation by inhibiting transcriptional activity of the STAT3/p300 complex.
- We showed that $CD45^{low}$/c-Kit^{high} cells in the AGM region have high hematopoietic activity.
- We identified a synthetic polymer that can separate a highly tumorigenic population within C6 glioma cells.

【Bio-informational Pharmacology】

- The interactions between tau protein and peptidyl-prolyl isomerases have been elucidated.
- The crystal structures of vitamin D receptor with some synthetic ligands have been determined.
- We also continued to work with Protein Data Bank Japan (PDBj).

Department of Molecular Pharmacology

Professor	Masaki Noda, M.D., Ph.D.
Associate Professor	Yoichi Ezura, M.D., Ph.D.
Assistant Professor	Tadayoshi Hayata, Ph.D.
GCOE International Coordinator	Tetsuya Nakamoto, M.D., Ph.D.
GCOE Research Instructor	Takuya Notomi, 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 the largest storage site for calcium in a living body and its metabolism is conducted by a complex cell society consisting of bone-forming osteoblasts and bone-resorbing osteoclasts as well as stromal cells and chondrocytes. In our department, we take molecular and cellular biological approaches to study the mechanisms of regulation of the development, differentiation, and function of each group of these cells.

Research Projects

1. Profilin1 regulates sternum development and endochondral bone formation (Miyajima D, Hayata T, Ezura Y, Noda M).

Bone development is a dynamic process that requires cell motility and morphological adaptation under the control of actin cytoskeleton. This actin cytoskeleton system is regulated by critical modulators including actin-binding proteins. Among them, profilin1 (Pfn1) is a key player to control actin fiber structure, and it is involved in a number of cellular activities such as migration. During the early phase of body development, skeletal stem cells and osteoblastic progenitor cells migrate to form initial rudiments for future skeletons. During this migration, these cells extend their process based on actin cytoskeletal rearrangement to locate themselves in an appropriate location within microenvironment. However, the role of Pfn1 in regulation of mesenchymal progenitor cells (MPCs) during skeletal development is incompletely understood. Here we examined the role of Pfn1 in skeletal development using a genetic ablation of Pfn1 in MPCs by using Prx1-Cre recombinase. We found that Pfn1 deficiency in MPCs caused complete cleft sternum. Notably, Pfn1-deficient mice exhibited an absence of trabecular bone in the marrow space of appendicular long bone. This phenotype is location-specific, as Pfn1 deficiency did not largely affect osteoblasts in cortical bone. Pfn1 deficiency also suppressed longitudinal growth of long bone. In vitro, Pfn1 deficiency induced retardation of osteoblastic cell migration. These observations revealed that Pfn1 is a critical molecule for the skeletal development, and this could be at least in part associated with the retardation of cell migration. (J Biol Chem, 2012).

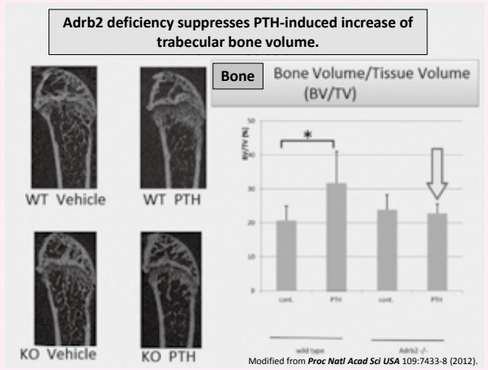
2. Identification of two-pore channel 2 as a novel regulator of osteoclastogenesis. (Notomi T, Ezura Y, Noda M).

Osteoclast differentiation is one of the critical steps that control bone mass levels in osteoporosis, but the molecules involved in osteoclastogenesis are still incompletely understood. Here, we show that two-pore channel 2 (TPC2) is expressed in osteoclast precursor cells, and its knockdown (TPC2-KD) in these cells suppressed RANKL-induced key events including multinucleation, enhancement of tartrate-resistant acid phosphatase (TRAP) activities, and TRAP mRNA expression levels. With respect to intracellular signaling, TPC2-KD reduced the levels of the RANKL-induced dynamic waving of Ca(2+) in RAW cells. The search for the target of TPC2 identified that nuclear localization of NFATc1 is retarded in TPC2-KD cells. Finally, TPC2-KD suppressed osteoclastic pit formation in cultures. We conclude that TPC2 is a novel critical molecule for osteoclastogenesis (J Biol Chem, 2012).

Highlight

Anabolic action of parathyroid hormone regulated by the β_2-adrenergic receptor (Hanyu R, Hayata T, Moriya S, Ezura Y, Noda M).

Parathyroid hormone (PTH), the major calcium-regulating hormone, and norepinephrine (NE), the principal neurotransmitter of sympathetic nerves, regulate bone remodeling by activating distinct cell-surface G protein-coupled receptors in osteoblasts: the parathyroid hormone type 1 receptor (PTHR) and the β_2-adrenergic receptor (β_2AR), respectively. These receptors activate a common cAMP/PKA signal transduction pathway mediated through the stimulatory heterotrimeric G protein. Activation of β_2AR via the sympathetic nervous system decreases bone formation and increases bone resorption. Conversely, daily injection of PTH (1-34), a regimen known as intermittent (i) PTH treatment, increases bone mass through the stimulation of trabecular and cortical bone formation and decreases fracture incidences in severe cases of osteoporosis. Here, we show that iPTH has no osteoanabolic activity in mice lacking the β_2AR. β_2AR deficiency suppressed both iPTH-induced increase in bone formation and resorption. We showed that the lack of β_2AR blocks expression of iPTH-target genes involved in bone formation and resorption that are regulated by the cAMP/PKA pathway. These data implicate an unexpected functional interaction between PTHR and β_2AR, two G protein-coupled receptors from distinct families, which control bone formation and PTH anabolism. (Proc Natl Acad Sci USA, 2012).



Daily injection of parathyroid hormone (PTH) into wild-type (WT) mice increases bone mass. On the other hand, no increase in bone mass is observed in mice (KO) lacking gene encoding Adrenergic receptor β_2 (Adrb2).

PTH is used as a therapeutic agent against severe osteoporosis, but its mechanism of action is not completely understood. In addition, there is a restriction on use. Therefore, development of new drugs has been also expected. In the present study, we revealed that the presence of Adrenergic receptor β_2 is essential for bone anabolic action of PTH. In future, new therapeutic strategy targeting PTH receptor and Adrenergic β_2 receptor will be expected.

Publications

[Original articles]

1. Hanyu R, Wehbi VL, Hayata T, Moriya S, Feinstein TN, Ezura Y, Nagao M, Saita Y, Hemmi H, Notomi T, Nakamoto T, Schipani E, Takeda S, Kaneko K, Kurosawa H, Karsenty G, Kronenberg HM, Vilardaga JP, Noda M. Anabolic action of parathyroid hormone regulated by the β_2-adrenergic receptor. ***Proc Natl Acad Sci U S A*** 109:7433-8, 2012.
2. Hemmi H, Zaidi N, Wang B, Matos I, Fiorese C, Lubkin A, Zbytnuik L, Suda K, Zhang K, Noda M, Kaisho T, Steinman RM, Idoyaga J. Treml4, an Ig superfamily member, mediates presentation of several antigens to T cells in vivo, including protective immunity to HER2 protein. ***J Immunol*** 188:1147-55, 2012.
3. Notomi T, Ezura Y, Noda M. Identification of two-pore channel 2 as a novel regulator of osteoclastogenesis. ***J Biol Chem*** 287:35057-64, 2012.
4. Miyajima D, Hayata T, Suzuki T, Hemmi H, Nakamoto T, Notomi T, Amagasa T, Böttcher RT, Costell M, Fässler R, Ezura Y, Noda M. Profilin1 regulates sternum development and endochondral bone formation. ***J Biol Chem*** 287:33545-53, 2012.
5. Suzuki T, Notomi T, Miyajima D, Mizoguchi F, Hayata T, Nakamoto T, Hanyu R, Kamolratanakul P, Mizuno A, Suzuki M, Ezura Y, Izumi Y, Noda M. Osteoblastic differentiation enhances expression of TRPV4 that is required for calcium oscillation induced by mechanical force. ***Bone*** (in press).
6. Smriti AA, Miyai K, Ezura Y, Hayata T, Notomi T, Nakamoto T, Pawson T, Noda M. Nck1 deficiency accelerates unloading-induced bone loss. ***J Cell Physiol*** (in press).
7. Sakuma T, Nakamoto T, Hemmi H, Kitazawa S, Kitazawa R, Notomi T, Hayata T, Ezura Y, Amagasa T, Noda M. CIZ/NMP4 is expressed in B16 melanoma and forms a positive feedback loop with RANKL to promote migration of the melanoma cells. ***J Cell Physiol*** 227:2807-12, 2012.
8. Izu Y, Ezura Y, Mizoguchi F, Kawamata A, Nakamoto T, Nakashima K, Hayata T, Hemmi H, Bonaldo P, Noda M. Type VI collagen deficiency induces osteopenia with distortion of osteoblastic cell morphology. ***Tissue Cell*** 44:1-6, 2012.
9. Ono N, Nakashima K, Schipani E, Hayata T, Ezura Y, Soma K, Kronenberg HM, Noda M. Constitutively active pth/pthrp receptor specifically expressed in osteoblasts enhances bone formation induced by bone marrow ablation. ***J Cell Physiol*** 227:408-15, 2012.

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.

Wnt signaling plays important roles in multiple developmental events during embryogenesis. Canonical Wnt signaling is initiated by binding of the Wnt ligand to the cell-surface Frizzled and transmembrane LRP complex. This leads to the membrane recruitment and activation of Dishevelled (DVL), which inactivates the APC/Axin/GSK-3 complex in the cytoplasm, responsible for the degradation of β-catenin. As a result, β-catenin accumulates in the cytoplasm, translocates to the nucleus and associates with Tcf transcription factors, which activate the Wnt target genes. In *Xenopus*, Wnt signaling accompanied by β-catenin nuclear localization at the dorsal side is an important for axis formation during early embryogenesis. Ventral over-expression of *Xwnt-8*, *β-catenin* and *DVL2* induces a secondary axis and promotes expression of Wnt target genes, such as *Siamois*, *Xnr3* and *Xtwn*.

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

We investigated roles of IQGAP and DVL in the canonical Wnt signaling pathway, and we have already obtained the following results: [1] xIQGAP1, xDVL2 and β-catenin can form a complex, and each protein contributes to the nuclear localization of each other under the Wnt stimulation. [2] Depletion of xIQGAP1 by antisense morpholino oligonucleotides (*xIQGAP1*-MO) reduced expression of Wnt target genes induced by *Xwnt-8*. [3] Importin-β5 and Ran, which directly bind to IQGAP1, contribute to canonical Wnt signaling pathway, playing a role in nuclear localization of DVL and β-catenin.

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

1. The expression of xIQGAP1 in HEK 293T cells increased GTP-bound activated form of xRan1 in the same way as the effect of xRanGEF.
2. The GTP-bound form of xRan1 was promoted by xRanGEF, but not by xIQGAP1 *in vitro*.
3. The expression of xIQGAP1 inhibited the interaction between active form of xRAN1 and xRanGAP.
4. The hydrolysis of the xRAN1 by xRanGAP was reduced by xIQGAP1.

These results suggest that the direct interaction between xIQGAP1 and xRan1 inhibits xRanGAP function, and is required for nuclear import of DVL, IQGAP1 and β-catenin in Wnt signaling pathway.

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

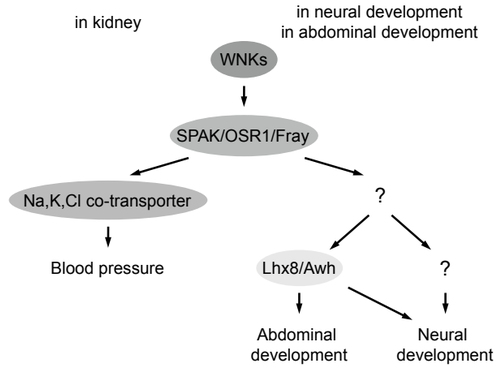
WNK (with no lysine (K)) kinase family that has been recently identified serine/threonin protein kinase family conserved among many species. There are four mammalian WNK family members, and mutations in two of them, WNK1 and WNK4 have been linked to a hereditary form of human hypertension known as Pseudohypoaldosteronism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

1. Evolutional conservation of WNK signaling pathway

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

2. The downstream transcription factor

The mosaic clones of *DWNK* mutant, and the overexpression of the kinase-dead form of DWNK, which might work as a dominant negative, caused the defect of abdominal development. Since these phenotypes are similar to *Arrowhead* (*Awh*) mutant, which encodes the transcription factor, these data suggest that Awh and WNK are genetically involved. When we simultaneously overexpressed the kinase-dead form of DWNK and Awh, the defect of abdominal development, which caused by the



overexpression of the kinase-dead form of DWNK, was rescued. Furthermore, the phenotypes of mosaic clones of *DWNK* mutant were rescued by the overexpression of Awh. In *DWNK* mutant embryos, the abdominal expression of *Awh* was reduced. These results indicate that Awh works at the downstream of WNK signaling pathway.

Awh is conserved in vertebrates as Lhx8. In NIH3T3 cells, Lhx8 expression was induced by the hypertonic condition. And when we knocked down both *WNK1* and *WNK4* by siRNA, the transcription of *Lhx8* was not induced under same hypertonic condition. The overexpression of WNK1, WNK4 or the downstream effecter OSR1 induced *Lhx8* transcription. These results suggest that *Lhx8* is the new downstream target of WNK signaling pathway in mammal. In mouse brain, Lhx8 is known to be involved in the specification of cholinergic neurons. *Lhx8* expression was induced in the differentiated Neuro2A cells. When we knocked down both *WNK1* and *WNK4* in Neuro2A cells, *Lhx8* expression was not induced. These suggest that Lhx8 also works at the downstream of WNK pathway in Neuro2A cells. Furthermore, the knockdown of both *WNK1* and *WNK4* caused the shortening of neurites and the reduction of marker gene expression of cholinergic neurons. These are new findings that WNK pathway is involved in the neural development. Since the pathological conditions of PHAII showed an intellectual impairment, these may suggest that WNK pathway is involved in the pathogenesis of PHAII via Lhx8.

Publications

1. Sato, A. and Shibuya, H. (2013). WNK Signaling Is Involved in Neural Development via Lhx8/Awh Expression. **PLoS One** 8, e55301.

2. Goto, T., Sato, A., Shimizu, M., Adachi, S., Satoh, K., Iemura, S., Natsume, T. and Shibuya, H. (2013). IQGAP1 functions as a modulator of Dishevelled nuclear localization in Wnt signaling. **PLoS One** 8, e60865.

3. Shimizu, M., Goto, T., Sato, A. and Shibuya, H. (2013). WNK4 is an essential effector of anterior formation in FGF signaling. **Genes Cells** in press.

4. Goto T., Michiue T., Ito Y., Asashima M. (2013). Characterization of CXC-type chemokine molecules in early Xenopus laevis development. **Int. J. Dev. Biol.** in press.

Department of Molecular Neuroscience

Professor	Kohichi Tanaka
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Assistant Professor	Tomomi Aida
Assistant Professor	Miho Soma
Assistant Professor	Yukiko Ito
Assistant Professor	Bai Ning

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.

We recently generated glial glutamate transporters GLAST and GLT1 double-knockout (DKO) mice that demonstrate multiple brain defects, suggesting overstimulation of glutamate receptors impairs early brain development. Although all glutamate receptor subunit classes are widely expressed throughout the embryonic brain, by generating GLAST, GLT1 and NR1, an essential subunit of NMDAR, triple knockout mice, we found NMDA receptors (NMDAR) overstimulation is responsible for all of brain defects in DKO mice. These results suggest that offset of excessive NMDAR activity may prevent abnormal brain development due to excess glutamatergic signaling, thereby avoiding later mental disorders.

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

Lateral habenula (LHb) is an epithalamic nucleus regulating the monoaminergic activity. Recent studies reported increased blood flow in the habenula of the patients with depression and facilitation of the excitatory postsynaptic potential in the habenula of rat helpless model for depression. These facts prompted us to hypothesize that the increased activity in the mouse LHb leads to the deterioration of the behaviors associated with depression such as despair and sleep disturbance under the stress.

We previously found that c-Fos was observed in LHb as well as regions sending the axons to LHb such as the entopeduncular nucleus and prefrontal cortex and the regions receiving the inputs from LHb such as rostromedial tegmental nucleus (RMTg). This suggests that multiple brain regions along the LHb pathway are activated in the animals with behavioral despair.

In this fiscal year, to directly address the above hypothesis, we checked whether the hyperactivation of LHb induces the behavioral despair by pharmacological manipulation of neuronal activity. Glutamate mediates the excitatory synaptic transmission in LHb, and glutamate transported play a major role in regulation of glutamate concentration at the synapse. Dihydrokainic acid (DHK), a specific inhibitor for the major glutamate transporter GLT-1, was used to hyperactivate neurons in LHb. For stereotactic application of drug to LHb, we developed head-restrained system which enables us to target the tiny structure like habenula with fine glass capillary. Upon application of DHK to LHb on both sides, a large number of cells expressing c-Fos were observed specifically in LHb as well as the brain region received direct projection from LHb such as RMTg. Thirty minutes after the DHK injection, the mouse despair behavior was examined by the tail suspension test. Results showed that time with immobility during tail suspension test was significantly longer in the DHK-injected mice than PBS-injected mice, suggesting that increase of glutamatergic transmission in LHb exacerbated the behavioral despair induced by acute stress.

Examining the effects of long-lasting LHb activation by genetic manipulation during the chronic defeat stress may clarify the role of LHb in the pathophysiology of depression.

3. Genetically determined sexual differentiation of the brain

Sexual differentiation leads to structural and behavioural differences between males and females. Here we investigate the intrinsic sex identity of the brain by constructing chicken chimeras in which the brain primordium is switched between male and female identities before gonadal development. We find that the female chimeras with male brains display delayed sexual maturation and irregular oviposition cycles, although their behaviour, plasma concentrations of sex steroids and luteinizing hormone levels are normal. The male chimeras with female brains show phenotypes similar to typical cocks. In the perinatal period, oestrogen concentrations in the genetically male brain are higher than those in the genetically female brain. Our study demonstrates that male brain cells retain male sex identity and do not differentiate into female cells to drive the normal oestrous cycle, even when situated in the female hormonal milieu. This is clear evidence for a sex-specific feature that develops independent of gonadal steroids.

Publications

[original papers]

1. Aizawa H, Kobayashi M, Tanaka S, Fukai T, Okamoto H. Molecular characterization of the subnuclei in rat habenula. J Comp Neurol. 2012, 520:4051-4066.
2. Suzuki, K., Maekawa, F., Suzuki, S., Nakamoi, T., Sugiyama, H., Kanamatsu, T., Tanaka, K., Ohki-Hamazaki, H. Elevated expression of brain-derived neurotrophic factor facilitates visual imprinting in chicks. J Neurochem 123. 800-810, 2012.
3. Hayashi, H., Eguchi, Y., Fukuchi-Nakanishi, Y., Takeya, M., Nakagata, N., Tanaka, K., Vance, JE., Tanihara, H. A potential therapeutic function of apolipoprotein E-containing lipoproteins for normal tension glaucoma. J Biol Chem 287. 25395-25406, 2012.
4. Aida, T., Ito, Y., Takahashi, YK., Tanaka, K. Overstimulation of NMDA Receptors Impairs Early Brain Development in vivo. PlosOne 7.eE36853, 2012.
5. Karlsson, R-M., Adwmark, L., Molander, A., Perreau-Lenz, S., Singley, E., Solomon, M., Holmes, A., Tanaka, K., Lovinger, DM., Spanagel, R., Heiling, M. Reduced alcohol intake and reward associated with impaired endocannabinoid signaling in mice with a deletion of the glutamate transporter GLAST. *Neuropsychopharmacology* 63. 181-189, 2012.
6. Tsai, M-C., Tanaka, K., Overstreet-Wadiche, L., Wadiche, JI. Neuronal glutamate transporters regulate glial excitatory transmission. *J Neurosci* 32. 1528-1535, 2012.
7. Maekawa, F., Sakurai, M., Yamashita, Y., Tanaka, K., Haraguchi, S., Yamamoto, K., Tsutsui, K., Yoshioka, H., Mutakami, S., Maeda, T., Tadano, R., Goto, T., Tomonari, K., Oka, T., Ohara, K., Shiraishi, J., Bungo, T., Tsudzuki, M., Ohki-Hamazki, H. A genetically female brain is required for a regular reproductive cycle in chicken brain chimeras. Nature Commun 4. 1372, 2013.

Department of Biodefense Research

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Project Junior Assistant Professor	Jumpei Asano, Ph.D.
Research Technician	Shoko Kuroda
Secretarial Assistant	Hisako Kamioka

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

1. Differentiation and function of dendritic cells

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

Dendritic cells (DCs) have crucial functions in the initiation of innate and adaptive immunity in infection and inflammation, and in the induction of tolerance under steady-state conditions. DCs consist of conventional DCs (cDCs) and plasmacytoid DCs (pDCs). It is currently accepted that DCs are originated from hematopoietic stem cells (HSCs) in the bone marrow (BM) via intermediate progenitors, namely the macrophage and DC precursor (MDP) and common DC precursor (CDP), the latter is a stringently committed to the DC lineage. Notably, both MDPs and CDPs give rise to many fewer pDCs than cDCs, implying the presence of another DC progenitor as a major source of pDCs. Under the background, we have recently succeeded to identify a new DC progenitor with prominent pDC differentiation potential (***Immunity***, in press). Consistent with their pDC differentiation potential, the new DC progenitor expresses elevated levels of E2-2, an essential transcription factor for pDC differentiation and maintenance. Importantly, they appear to be directly derived from either CDPs or lymphoid-primed multi-potent progenitors (LMPPs). On the basis of these findings, we propose a new model for DC development (Fig.1).

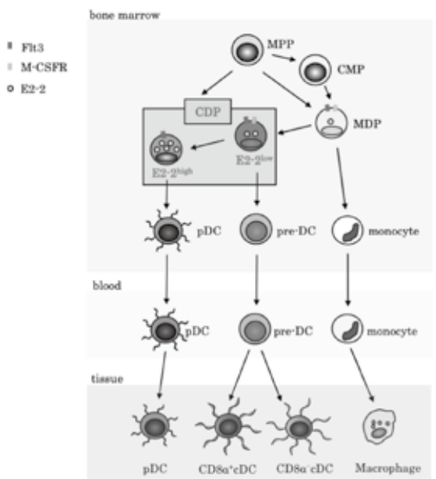

Fig.1 Scheme for pDC and cDC differentiation in the steady-state.

2) Hemophagocytosis by DCs as a new tolerance induction mechanism

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 hemophagocytosis by DCs as a new DC-mediated tolerance mechanism to prevent excessive immune responses under severe infectious and inflammatory conditions, and we are currently analyzing the molecular mechanisms and its physiological relevance.

2. Regulatory mechanism in the gut-associated lymphoid tissues

DCs, composed of pDCs and cDCs, are representative antigen presenting cells (APCs) and play integral roles in balancing tolerance to self-Ags and immunity to pathogens in peripheral lymphoid tissues. In the intestine, DCs are requested to keep the balance even more sharply such that DCs should be tolerogenic in the presence of numerous commensal bacteria while retain the capacity to respond to episodic pathogens. We have been studying the role for DCs in IgA induction in the intestine (***Nature*** 448, 929-933 (2007); ***Immunol Rev*** 234, 247-258 (2010); ***Immunity*** 34, 247-257 (2011)). In addition, using a model for ulcerative colitis (UC), we are currently studying the role of commensal bacteria and autophagy in the development and recovery of the disease.

3. Understanding of disease development on the basis of tissue stem cell disorder

Type I interferons (IFNs), a family of cytokines, are produced by mammalian cells and orchestrate numerous biological and cellular processes. Although it is well known that type I IFNs are essential for establishing the host antiviral state, their role in hematopoietic homeostasis remains unstudied. Importantly, we recently found that type I IFNs induce proliferation and exhaustion in hematopoietic stem cells (HSCs), and that interferon regulatory factor-2 (IRF2), a transcriptional suppressor of type I IFN signaling, preserves the self-renewal and multilineage differentiation capacity of HSCs (***Nat Med*** 15, 696-700 (2009)). Based on our findings, we show that type I IFN preconditioning, without irradiation or DNA alkylating agents, significantly enhanced the HSC engraftment efficiency in wild type (WT) recipient mice. The importance of active type I IFN signaling in HSC recipients was further demonstrated using mice lacking interferon regulatory factor 2 (IRF2), a transcriptional suppressor of type I IFN signaling. In both WT and *Irf2*$^{-/-}$ recipients, active type I IFN signaling greatly enhanced the HSCs' sensitivity to 5-FU or low-dose irradiation. Importantly, IFN-based pre-transplant conditioning was also applicable to the treatment of Sly syndrome, a congenital storage disorder with β-glucuronidase deficiency, in which it restored enzyme expression at the HSC level and reciprocally reduced pathologic glycosaminoglycan storage. Our findings suggest type I IFN-based preconditioning, combined with HSC transplantation, as a novel non-genotoxic treatment for some congenital diseases (**Blood**, in press).

Publications

[Original papers]

1. Liu J, Guo YM, Hirokawa M, Iwamoto K, Ubukawa K, Michishita Y, Fujishima N, Tagawa H, Takahashi N, Xiao W, Yamashita J, Ohteki T, and Sawada K. A synthetic double-stranded RNA, poly I:C, induces a rapid apoptosis of human CD34$^+$ cells. ***Exp Hematol***. Apr 40(4), 330-341,2012.
2. Ichikawa A, Kuba K, Morita M, Chiba S, Tezuka H, Hara H, Sasaki T, Ohteki T, Ranieri V.M , dos Santos C C, Kawaoka Y, Akira S, Luster A D, Lu B, Penninger J M, Uhlig S , Slutsky A S, and Imai Y. CXCL10-CXCR3 enhances the development of neutrophil-mediated fulminant lung injury of viral and non-viral origin. ***Am J Respir Crit Care Med.*** November 9, 2012. (Epub ahead of print)
3. Onai N, Kurabayashi K, Hosoi-Amaike M, Toyama-Sorimachi N, Matsushima K, Inaba, K, and Ohteki T.pA clonogenic progenitor with prominent plasmacytoid dendritic cell developmental potential. ***Immunity***, in press
4. Sato T, Ikeda M, Yotsumoto S, Shimada Y, Higuchi T, Kobayashi H, Fukuda T, Ohashi T, Suda T, and Ohteki T. Novel interferon-based pre-transplantation conditioning in the treatment of a congenital metabolic disorder. ***Blood***, 121(16), 3267-3273

[Presentation at international meetings]

1. Ohteki T. Role for plasmacytoid dendritic cells in gut IgA induction. The 4th Symposium for the Mext Priority Research on Immunological Self. Kyoto 2012.1.28
2. Sato T, Ikeda M, Yotsumoto S and Ohteki T. Combination effects of type-I IFNs and imatinib against Leukemia-initiating cells I mouse CML model. 10th Stem Cell Research Symposium. Awaji 2012.5.31
3. Tezuka H, Abe Y, and Ohteki T. Prominent role for plasmacytoid dendritic cells in mucosal T cell-independent IgA induction. The 20th International Symposium on Molecular Cell Biology of Macrophages 2012 (MMCB2012). Tokyo 2012.6.15
4. Onai N. Monocyate derived dendritic cells perform hemophagocytosis to fine-tune excessive immune responses. The 20th International Symposium on Molecular Cell Biology of Macrophages 2012 (MMCB2012). Tokyo 2012.6.15
5. Ohteki T. Monocyte-derived dendritic cells perform hemophagocytosis to fine-tune excessive immune responses. The 11th Awaji International Forum on Infection and Immunity. Awaji 2012.9.14
6. Ohteki T. Role for monocyte-derived cells in fine-tuning excessive immune responses. The 12th International Symposium on Dendritic Cells. Daegu, Korea 2012.10.9
7. Onai N, Oyagi H. Sato T, Yotsumoto S, Kurabayashi , Hosoi-Amaike M, Sawada K, and Ohteki T. Monocyte-derived dendritic cells perform hemophagocytosis to control ecessive immune response. The 12th International Symposium on Dendritic Cells. Daegu, Korea 2012.10.9
8. Tezuka H, and Ohteki T. Prominent role for plasmacytoid dendritic cells in mucosal T cell-independent IGA Induction. The 12th International Symposium on Dendritic Cells. Daegu, Korea 2012.10.11
9. Sato T, Yotsumoto S, and Ohteki T. Conmibation effects of type-I IFNs and imatinib against Leukemia-initiating cells in mouse CML model. 2012 Annual Meeting of the Japanese Scoiety for Immunology. Kobe 2012.12.6
10. Onai N, Ohyagi H, Sato T, Yotsumoto S, Kurabayashi K, Sawada K, and Ohteki T. Monocyte derived dendritic cells perform hemophagocytosis to fine-tune excessive immune responses during chronic virus infection. 2012 Annual Meeting of the Japanese Scoiety for Immunology. Kobe 2012.12.7

Department of Bio-informational Pharmacology

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

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

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

Susceptibility of several diseases and responsibility to various drugs and therapy exhibit gender-difference. We have previously reported that non-genomic actions of sex hormones play an important role creating gender-difference in cardiac arrhythmia. In this year, we examined the signalosome for the non-genomic effects of sex hormones using molecular imaging technology.

2. Pathogenesis of atrial fibrillation (AF)

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

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

We carry out most extensive GWAS (genome-wide association study) in Japan to determine gene polymorphisms associated with AF. Since 2011, we have participated in the international Meta-analysis called as CHARGE study. We found 10 SNPs associated with AF: among them, 6 SNPs were associated with both European/American and Japanese, and 4 with European/American but not with Japanese (Fig. 1).

		European population				Japanese population			
Locus	Closest gene	SNP	MAF (%)	Relative risk (95% confidence interval)	P-value	SNP	MAF (%)	Relative risk (95% confidence interval)	P-value
1q21	*KCNN3*	rs6666258	29.9	1.18 (1.13-1.23)	2.0×10^{-14}	rs7514452	16.6	0.72(0.62-0.84)	4.9×10^{-5}
1q24	*PRRX1*	rs3903239	44.7	1.14 (1.10-1.18)	9.1×10^{-11}	rs593479	50.3	1.21(1.07-1.37)	2.4×10^{-3}
4q25	*PITX2*	rs6817105	13.1	1.64 (1.55-1.73)	1.8×10^{-74}	rs2634073	31.9	1.84(1.59-2.13)	3.7×10^{-17}
5q31	*WNT8A*	rs2040862	17.8	1.15 (1.09-1.21)	3.2×10^{-8}	rs6878439	12.7	1.20(1.00-1.44)	0.53
7q31	*CAV1*	rs3807989	40.4	0.88 (0.84-0.91)	9.6×10^{-11}	rs3807989	34.5	0.76(0.67-0.87)	7.0×10^{-5}
9q22	*C9orf3*	rs10821415	42.4	1.13 (1.08-1.18)	7.9×10^{-9}	rs6479562	14.7	0.72(0.59-0.87)	4.2×10^{-4}
10q22	*SYNPO2L*	rs10824026	15.8	0.85 (0.81-0.90)	1.7×10^{-8}	rs3180427	7.9	1.16(1.01-1.33)	0.34
14q23	*SYNE2*	rs1152591	47.6	1.13 (1.09-1.18)	6.2×10^{-10}	rs7161192	48.4	0.88(0.78-0.99)	0.041
15q24	*HCN4*	rs7164883	16.0	1.16 (1.10-1.22)	1.3×10^{-8}	rs9920504	2.8	0.68(0.50-0.94)	0.022
16q22	*ZFHX3*	rs2106261	17.6	1.24 (1.17-1.30)	3.2×10^{-16}	rs12932445	37.0	0.80(0.71-0.91)	6.8×10^{-4}

Fig.1 10 SNPs associated with atrial fibrillation

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

b. Inflammatory and immunological mechanisms in atrial fibrillation

AF is a multifactorial disease, and inflammatory response is believed to play a role in linking between these risks and AF. In vitro Boyden chamber experiments and in vivo TAC experiments, we showed that stretch-induced ATP release via a gap-junction channel, pannexin-2, induces recruitment of macrophages, acting as an initial factor to provoke atrial inflammation. This paper was selected as the Best Basic Paper on AF 2012 in Boston AF symposium 2013.

3. Pathogenesis of ventricular tachyarrhythmias and sudden cardiac death

Despite extensive effort by many researchers for years, ventricular tachycardia and fibrillation remain the main cause of sudden death, and the biggest challenge in arrhythmia research. Our laboratory approaches this issue using genetically engineered mice. In this year, we found that the mice with genetic deletion of the His-Purkinje system-specific transcription factor were susceptible to exercise-related atrio-ventricular block and ventricular tachyarrhythmias. In human, genetic mutations in the His-Purkinje system-specific transcription factor are associated with idiopathic ventricular fibrillation related to exercise.

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

4. Use of iPS cells for arrhythmia research

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

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

We established and characterized iPS cell-derived cardiomyocytes from human fibroblasts obtained from familiar sudden death patients (LQT, Brugada syndrome). We have able to establish iPS cells from LQT1, LQT2, LQT3, and Brugada syndrome. Our data showed that hiPS-CM from LQT patients maintain some of electrophysiological phenotype found in LQT patients' hearts.

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

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

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

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

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

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

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

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

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

Publications

[Original articles]

1. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Muller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dorr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW, Wakili R, Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Volker U, Volzke H, Milan DJ, Hofman A, Boerwinjle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE, Witteman JC, Kao WH, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjogren M, Newman AB, Liu Y, Gollob MH, Melander O, Tanaka T, Stricker BH, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kaab S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat. Genet*., 2012;44:670-675.
2. Oishi S, Sasano T, Tateishi Y, Tamura N, Isobe M, Furukawa T. Stretch of atrial myocytes stimulates recruitment of macrophages via ATP released through gap-junction channels. *J. Pharmacol. Sci*. 2012;120:296-304.
3. Egashira T, Yuasa S, Suzuki T, Aizawa Y, Yamakawa H, Matsuhashi T, Ohno Y, Tohyama S, Okata S, Seki T, Kuroda Y, Yae K, Hashimoto H, Tanaka T, Hattori F, Sato T, Miyoshi S, Takatsuki S, Murata M, Kurokawa J, Furukawa T, Makita N, Aiba T, Shimizu W, Horie M, Kamiya K, Kodama I, Ogawa S, Fukuda K. Disease characterization using LQTS-specific induced pluripotent stem cells. *Cardiovasc. Res.* 2012;95:419-429.
4. Takamura C, Ohhigashi H, Ebana Y, Isobe M. New human leukocyte antigen risk allele in Japanese patients with Takayasu arthritis. *Circ. J.* 2012;76:1697-1702.
5. Asayama M, Kurokawa J, Shirakawa K, Okuyama H, Kagawa T, Okada J, Sugiura S, Hisada T, Furukawa T. Effects of an hERG activator, ICA-105574, on electrophysilogical properties of canine hearts. *J. Pharmacol. Sci.* (in press)
6. Furukawa T, Ebana Y. Current overview of genetic background of atrial fibrillation: possible genetically therapeutic targets for the treatment of atrial fibrillation. *J. Arrhyhm*. (in press)
7. Okata S, Yuasa S, Yamane T, Furukawa T, Fukuda K. The generation of induced pluripotent stem cells from a patient with *KCNH2* G603D, without LQT2 disease associated symptom. *J. Med. Dent. Sci*. (in press)

[Review]

1. Kurokawa J, Furukawa T. Non-genomic action of sex steroid hormones and cardiac repolarization. *Biol. Pharm. Bull*. (in press)
2. Kurokawa J, Furukawa T. Region- and condition-dependence of the membrane and Ca^{2+} clocks in the sinus node. *Circ. J*. 2012;76:293-294.
3. Kurokawa J, Kodama M, Furukawa T, Clancy CE. Sex and gender aspects in arrhythmic therapy. *Handb. Exp. Pharmacol*. 2012;214:237-263.
4. Sasano T, Kurokawa J (2012) Remodeling of potassium channels in cardiac hypertrophy In: Molecular ***Mechanisms of Cardiac Remodeling***. Jugdutt BI, Dhalla NS (Eds): Springer, New York, in press.

Department of Stem Cell Regulation

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Research Student	Kazuo TERASHIMA, Wenqian WANG

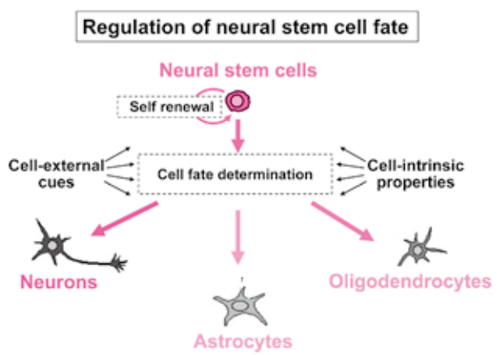
Research Outline

Our research has been conducted to elucidate the mechanisms by which stem cells are regulated. The major focus has been on neural stem cells, hematopoietic stem cells, and cancer stem cells. The study is aimed to understand development, maintenance, and regeneration of the central nervous system and the hematopoietic system, and to obtain a clue to tackle the problem of cancer recurrence. The projects performed in 2012 are categorized into three groups: 1. Understanding of molecular basis for self-renewal and fate determination of neural stem cells, 2. Characterization of fetal hematopoiesis, and 3. Characterization of cancer stem cells and their niche.

Research Projects

1. Understanding of molecular basis for self-renewal and fate determination of neural stem cells

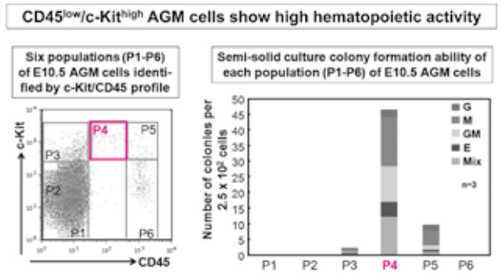
Neurons, astrocytes, and oligodendrocytes, the three major cell types in the nervous system, are generated from common neural stem cells (NSCs) during development. As illustrated below, self-renewal and fate determination of neural stem cells are governed by cell-external cues (such as cytokines) and cell-intrinsic programs (including chromatin modification). Self-renewing proliferation of neural stem cells (NSCs) is intimately linked to inhibition of neuronal and glial differentiation, however, their molecular linkage has been poorly understood. We have previously proposed a model explaining partly this linkage, in which fibroblast growth factor 2 (FGF2) and Wnt signals cooperate to promote NSC self-renewal via β-catenin accumulation, which leads to the promotion of proliferation by LEF/TCF-mediated cyclin D1 expression and at the same time to the inhibition of neuronal differentiation by β-catenin-mediated potentiation of Notch signaling.



To fully understand the mechanisms underlying NSC self-renewal, it needs to be clarified how these growth factor signals inhibit glial differentiation as well. We recently demonstrated that a NSC growth promoting signaling component and also a common component of FGF2 and Wnt, i.e. cyclin D1, inhibits astroglial differentiation of NSCs. Interestingly, this effect of cyclin D1 is mediated even though its cell cycle progression activity is blocked. Forced down-regulation of cyclin D1 enhanced astrogliogenesis of NSCs in culture and in vivo. We further demonstrated that cyclin D1 binds to STAT3, a transcription factor downstream of astrogliogenic cytokines, and suppresses its transcriptional activity on the glial fibrillary acidic protein gene (*Gfap*). Taken together with our previous finding, we provide a novel molecular mechanism for NSC self-renewal in which growth promoting signaling components activated by FGF2 and Wnts inhibit neuronal and glial differentiation.

2. Characterization of fetal hematopoiesis

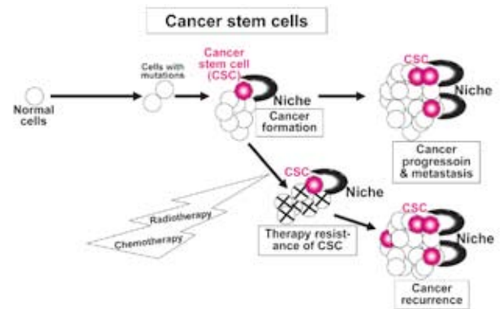
During mouse development, hematopoiesis starts in the yolk sac (YS) and the site of hematopoiesis then changes during ontogeny, e.g. from the YS to the aorta-gonad-mesonephros (AGM) region, placenta, fetal liver, spleen, thymus, and bone marrow. Hematopoiesis during development is divided into two phases, comprising early primitive hematopoiesis and later-occurring definitive hematopoiesis. The definitive hematopoiesis is characterized by the potential for enucleated erythropoiesis and lymphopoiesis and is known to firstly arise from the AGM region at midgestation.



Based on the expression profiles of CD45 and c-Kit in freshly dissociated AGM cells from embryonic day 9.5 (E9.5) to E12.5 and aorta cells in the AGM from E13.5 to E15.5, we recently defined six cell populations. As illustrated above (left part), the populations 1 through 6 identified in E10.5 AGM cells (P1-P6: CD45－/c-Kit－; CD45－/c-Kit low; CD45－/c-Kit high; CD45 low/c-Kit high; CD45 high/c-Kit high; and CD45 high/c-Kit low and －). Among these six populations, CD45 low/c-Kit high cells (P4) were most able to form hematopoietic cell colonies. The same figure (right part) shows the prominent semi-solid colony forming activity of CD45 low/c-Kit high cells (P4) from E10.5 AGM cells. The hematopoietic ability of CD45 low/c-Kit high cells decreased after E11.5 and was undetectable at E13.5 and later. The CD45 low/c-Kit high cells showed multipotency in vitro. We demonstrated further enrichment of hematopoietic activity in the Hoechst dye-effluxing side population among the CD45 low/c-Kit high cells. We determined that CD45 low/c-Kit high cells arise from the lateral plate mesoderm using embryonic stem cell-derived differentiation system. In conclusion, CD45 low/c-Kit high cells are the major hematopoietic cells in the mouse AGM. CD45 low/c-Kit high cells with hematopoietic activity were also found in the yolk sac and the placenta. We are currently working on the transcription factor Sox17 that we found involved in AGM hematopoiesis. This line of study casts new insights into the development of definitive hematopoiesis.

3. Characterization of cancer stem cells and their niche

"Cancer stem cells" (CSCs), a functional subset of tumour cells, are characterized by radio- and chemo-resistance and have been postulated as key drivers of tumour relapse and progression as shown below. CSCs reside in a specialized microenvironment known as the niche composed of, for instance, various stromal cells. However, to date, very little is known about the identity of niche components. As we have previously reported, C6 glioma cell line contains a sub-population of CSCs, which is enriched in the "side population (SP)" by Hoechst 33342 staining and FACS analysis. As we published in 2004, SP cells in C6 are tumorigenic, but cells in the major population (main population, MP) are not. In the recent couple of years, we searched for CSC niche mimicries from hundreds of synthetic polymers in collaboration with Professor Mark Bradley (University of Edinburgh). Out of nearly 400 polymers arrayed on slides, six polymers were identified to preferentially support the proliferation of SP over MP cells, suggesting these polymers mimic in vivo niche. C6 SP cells (C6 CSCs) were able to be separated on Polymer #10 (Pol10) into two populations; Pol10 adherent (Pol10 Ad) and Pol10 non-adherent (Pol10 NAd) cell populations. The Pol10 Ad cells showed dramatically higher tumorigenic activity when transplanted into the NOD/SCID mouse brain. This approach will provide clues to understand the molecular basis for the CSC niche and to develop effective therapeutic strategies.



Publications

[Original Article]

1. Nobuhisa I, Yamasaki S, Ramadan A and Taga T: CD45low c-Kit high cells have hematopoietic properties in the mouse aorta-gonad-mesonephros region. Exp. Cell Res., 318:705-715, 2012.
2. Uemura M, Ozawa A, Nagata T, Kurasawa K, Tsunekawa N, Nobuhisa I, Taga T, Hara K, Kudo A, Kawakami H, Saijoh Y, Kurohmaru M, Kanai-Azuma M and Kanai Y. Sox17 haploinsufficiency results in perinatal biliary atresia and hepatitis in C57BL6 background mice. Development, 140:639-648, in press.

[Review and Book]

1. Tabu K, Taga T, and Tanaka S. Tumor stem cells: CD133 gene regulation and tumor stemness. In Stem Cells and Cancer Stem Cells, Volume 2, Part 2, (Springer.) 145-153, 2012.
2. Tabu K, Bizen N, Taga T, and Tanaka S. Gene regulation of Prominin-1 (CD133) in normal and cancerous tissues. In Prominin-1 (CD133): New Insights on Stem & Cancer Stem Cell Biology. D. Corbeil Ed. (Springer) Adv. Exp. Med. Biol., in press.

Department of Structural Biology

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Adjunct Assistant Professor	**Minako Abe**
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Postgraduate Student	**Kenrou Shinagawa, Michika Miyashita**

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 Bone Morphogenetic Protein 10

Bone morphogenetic protein 10 (BMP-10) is a member of the TGF-β superfamily and plays a critical role in heart development. A substitution variant of BMP-10 gene has been found to be associated with hypertensive dilated cardiomyopathy. To elucidate the detailed molecular mechanisms of maturation, activation, and pathogenesis of BMP-10, we have initiated crystallographic studies for human BMP-10. BMP-10 is translated as a precursor protein with an N-terminal propeptide and a C-terminal region (active BMP-10) that is cleaved by proteases such as furin. The mature protein consists of both the propeptide and BMP-10 as a complex, and interactions of this mature protein with the target receptors would promote releasing the activated BMP-10.

We have shown that two molecules of propeptide and two molecules of BMP-10 form a stable mature protein in vitro, and the mature protein complex can interact with the extracellular matrix proteins, forming supra-macromolecules. We have also obtained crystals of the propeptide and BMP-10 complex. Self-rotation function from the X-ray diffraction data at the synchrotron radiation facilities suggests that the propeptide and BMP-10 complexes form a 24-mer assembly with non-crystallographic 432 symmetry in the crystal. To determine the structure at higher resolution, we have attempted to enhance the crystal quality using the crystallization in high magnetic force fields. In gradient magnetic fields, diamagnetic waters and protein molecules receive a magnetic force and the upward magnetic force can suppress the natural convection due to gravity. Therefore, crystallization under the high magnetic force fields would provide favorable effects for protein crystallization because the relatively slow supply rate of protein molecules due to reduced convection could decrease the undesired impurity uptake during crystal growth. Now the optimization of crystallization conditions under the high magnetic force fields is in progress.

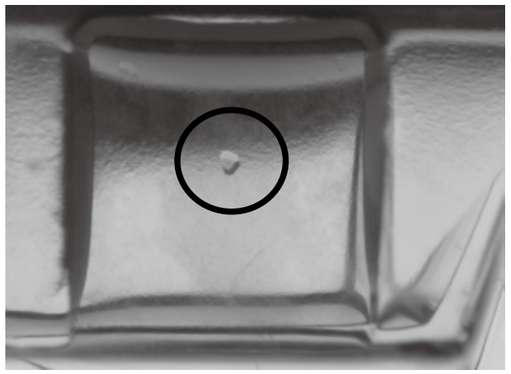


Figure 1: Crystallization of BMP-10 complex in magnetic field (a crystal is shown in circle).

2. Analysis of interactions between tau protein and peptidyl-prolyl isomerases

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, phosphorylated tau protein aggregates into paired helical filaments (PHF), which are characterized as the neuropathological hallmarks of Alzheimer's disease. Peptidyl-prolyl isomerases (PPIases), FKBP51 and FKBP52, interact with tau protein, and then regulate length of microtubule, while other PPIases, Pin1 and FKBP12, interact with PHF. Recently, it was revealed that Pin1 restored the ability of phosphorylated tau protein to bind tublin and promote microtubule assembly. We do not well know molecular mechanism of the interaction between tau protein and PPIases because these researches were performed only in vivo.

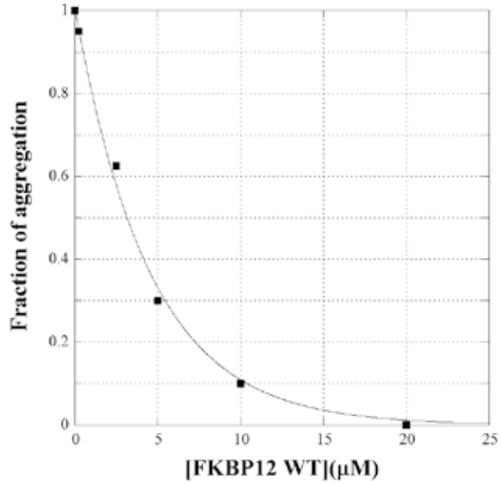


Figure 2: Inhibition of aggregation of tau protein by FKBP12

Thus we investigated the interaction between them in vitro. We focused on the microtubule-binding region of tau protein because it had a high propensity for aggregation and it was suspected of causing PHF. Then we analyzed systematically how PPIases affect aggregation of tau protein. As results, FKBP12 as well as Pin1 inhibited tau protein from aggregating by PPIase-activity. FKBP12 also recover tau protein from aggregation.

3. Improvement in Protein Data Bank

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

Publications

[Research Papers]

1. Tamashiro T, Tanabe Y, Ikura T, Ito N, Oda M: Critical roles of Asp270 and Trp273 in the α-repeat of the carbohydrate-binding module of endo-1,3-β-glucanase for laminarin-binding avidity. Glycoconj J 29: 77-85, 2012.
2. Iwaya N, Akiyama K, Goda N, Tenno T, Fujiwara Y, Hamada D, Ikura T, Shirakawa M, Hiroaki H: Effect of Ca(2+) on the microtubule-severing enzyme p60-katanin: Insight into the substrate-dependent activation mechanism. FEBS J 279: 1339-1352, 2012.
3. Nomura W, Masuda, A, Ohba, K, Urabe A, Ito N, Ryo A, Yamamoto N, Tamamura H: Effects of DNA Binding of Zinc Finger and Linkers for Domain Fusion on Catalytic Activity of Sequence-Specific Chimeric Recombinases Determined by a Facile Fluorescent System. Biochemistry 51: 1510–1517, 2012.
4. Yoshimoto N, Sakamaki Y, Haeta M, Kato A, Inaba Y, Itoh T, Nakabayashi M, Ito N, Yamamoto K: Butyl Pocket Formation in the Vitamin D Receptor Strongly Affects the Agonistic or Antagonistic Behavior of Ligands. J. Med. Chem. 55: 4373–4381, 2012.

[Domestic conferences]

1. Ito N; Protein Data Bank & Structure Deposition at PDBj, Fukuoka, 2012.

Frontier Research Unit
Laboratory of Oxygen Biology

Associate Professor　Koh Nakayama, Ph.D.

Aim of the Research

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

Research Projects

1. Signal transduction of hypoxia response

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

2. Tumor invasion under chronic hypoxic conditions

HIF has been studied broadly as a central regulator of hypoxic responses. However, we recently demonstrated that expression and activity of HIF decreases under chronic hypoxic conditions. Therefore, we initiated a new study to clarify the molecular mechanism regulating the chronic hypoxic response. Matrix Metalloproteinase *MMP1* was identified in the DNA microarray analyses as a gene up-regulated during chronic hypoxia. Induction of *MMP1* was found in 24 – 48 hrs of hypoxic treatment, and it was found to be regulated by two transcription factors; CREB and NF-κB. These transcription factors are highly activated during chronic phase of hypoxia. Depletion of CREB/NF-κB by siRNA decreased the expression of MMP1, which led to significant reduction of cell migration and invasion on the collagen gel. Thus, we concluded that CREB- and NF-κB-mediated induction of MMP1 would be one of mechanisms to induce malignant transformation of cancer cell under chronic hypoxic conditions (Figure). It is possible that co-inhibition of HIF and CREB/NF-κB serves as a more effective way to inhibit the tumor progression during hypoxia.

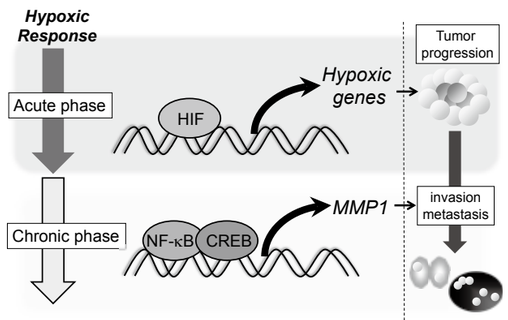


Figure Regulation of *MMP1* expression during chronic hypoxia

Presentation at the meetings

Koh Nakayama
Regulation of matrix metalloproteinase *MMP1* expression by NF-κB pathway during prolonged hypoxic conditions.
Keystone Symposia: Advances in Hypoxic Signaling
Feb 14th, Banff, Canada.

Koh Nakayama
Activation of NF-κB/CREB transcription factors induces *Matrix Metalloproteinase (MMP) 1* expression and promotes the invasive ability of cancer cells during chronic hypoxia.
The 35th Annual Meeting of the Molecular Biology Society of Japan, Dec 12th, Fukuoka.

Koh Nakayama
Acute and prolonged phase of hypoxic responses mediated by transcription factors.
The 85th Annual Meeting of the Japanese Biochemical Society, Dec 14th, Fukuoka.

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】

- Establishment of technical basis to identify pathological phosphorylation signaling.
- Discovery of the relationship between PQBP1's expression level and lifespan.

【Pathological Cell Biology】

- Elucidation of novel p53 function that regulate neovascularization through regulation of VEGF.
- Elucidation of genetic evidence demonstrating that epithelial cell death is crucial for the development of chronic colitis.

【Developmental and Regenerative Biology】

- Discovery of a novel function of the stress kinase MKK7 in the regulation of the mammalian circadian clock.
- Discovery of a novel acetylation cycle of the transcription co-activator Yes-associated protein that is downstream of the Hippo pathway.

【Stem Cell Bilogy】

- Identification of melanocyte stem cells in sweat glands of volar skins.
- Mechanisms of hair loss with aging in mice.

【Immunology】

- Elucidation of a novel B lymphocyte tolerance mechanism that regulates pathogenic autoantibody production in lupus.
- Identification of a modifier gene that regulates development of autoimmune disease in a mouse model for autoimmune lymphoproliferative syndrome (ALPS).

【Molecular Pathogenesis】

- Myopalladin mutations caused hypertrophic cardiomyopathy, dilated cardiomyopathy and restrictive cardiomyopathy via abnormal assembly of sarcomere.
- SLMAP mutations cause Brugada syndrome via defective cytoplasmic trafficking of cardiac sodium channel Nav1.5.

【Virus Research Unit】

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

Department of Neuropathology

Professor	Hitoshi Okazawa
Associate Professor	Kazuhiko Tagawa
Adjunct Lecturer	Nobuyuki Nukina, Masaki Sone, Toshiki Uchihara
Assistant Professor	Takuya Tamura
Project Assistant Professor	Hikaru Ito, Toshikazu Sasabe, Chisato Yoshida, Kyota Fujita, Kazumi Motoki, Xigui Chen
Technicians	Tayoko Tajima, Chiharu Mizoi, Yuko Uyama, Kimiko Ibagawa
Secretary	Mari Kishimoto
Graduate Students	Ying Mao, Min Xu, Chan Li, Hong Zhang,
Research Trainees	Asuka Katsuta

Research contents

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

1) Intellectual disability gene PQBP1 affects lifespan

PQBP1 (polyglutamine binding protein-1) was originally identified by our group as a new factor binding to polyQ tract sequence by yeast two-hybrid screening. PQBP1 is now shown to be a nuclear protein involved in transcription and splicing. It is a component of spliceosome and shuttles to cytosol and forms stress granule when cells are under stress. PQBP1 mutations cause one of the most frequent intellectual disability/mental retardation diseases (ID/MR). This year, we investigated the relationship between phenotypes and PQBP1 gene expression levels in order to prepare for the future therapeutics.

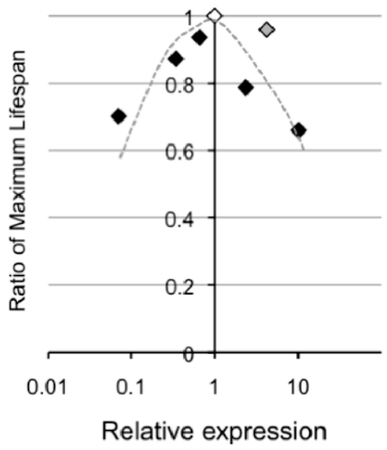


Figure 1. Relationship between lifespan and PQBP1 expression level

Previously we made PQBP1 mutant fly that possesses transposon insertion within Drosophila homologue PQBP1(dPQBP1) gene. Expression was extremely decreased and the fly showed learning disturbance. We newly found this year that lifespan of the mutant fly was shortened. We asked relationship of dPQBP1 expression level and lifespan or learning ability with various rescue flies using neuron-specific or general drivers, and finally found that optimum level of PQBP1 expression exists near the normal level. Below the normal expression level, lifespan and learning ability are both decreased. Above the normal level, lifespan is shortened but learning ability remains normal (Figure 1, 2). The knockdown of PQBP1 in neurons does not remarkably affect lifespan but general knock down using tubulin-driver induces remarkable shortening of lifespan (Figure 3).

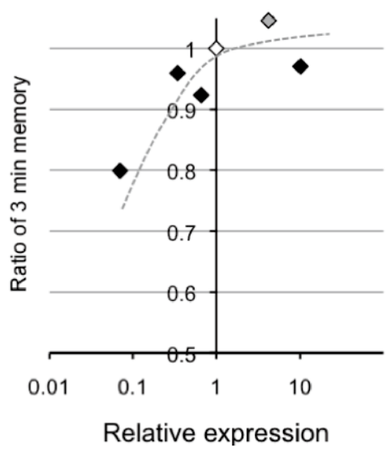


Figure 2. Relationship between learning ability and PQBP1 expression level

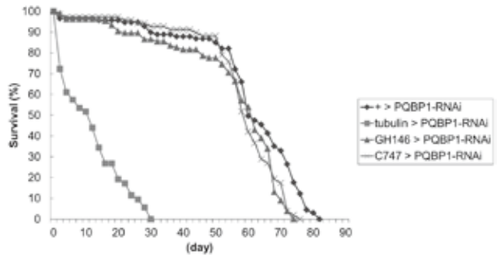


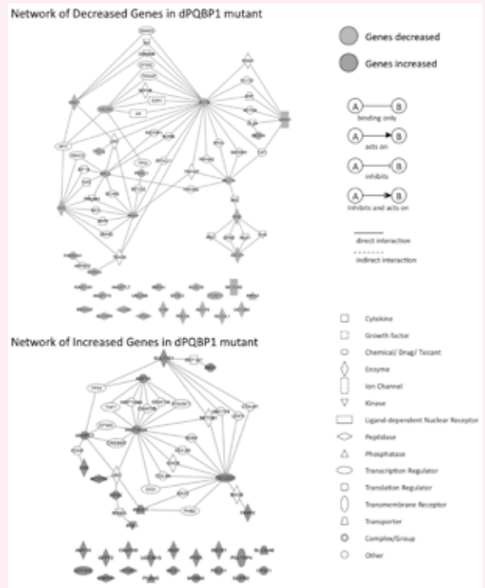
Figure 3. Effects of tissue specific knock down of PQBP1 on lifespan

These results indicate that normal or excessive expression of PQBP1 in neurons is not harmful, while the excessive expression in non-neural tissues might be harmful. The knowledge will be useful for future development of therapeutics against the PQBP1 spectrum of ID.

We used systems biology to analyze the pathological network of abnormal gene expression profiles in dPQBP1 mutant flies (Highlight).

Highlight

We used systems biology analyses to analyze expression profiles of dPQBP1 mutant flies. The decreased genes are linked to some key genes like actin.



Publications

1. Nakamura, Y., Tagawa, K., Oka, T., Sasabe, T., Ito, H., Shiwaku, H., La Spada, A.R. and Okazawa, H. (2012). Ataxin-7 associates with microtubules and stabilizes the cytoskeletal network. ***Hum Mol Genet***. 21 (5): 1099-1110. doi: 10.1093/hmg/ddr539

2. Ress, M., Gorba, C., Gorba, C., de Chiara, C., Bui, T.T.T., Garcia-Maya, M., Drake, A.F., Okazawa, H., Pastre, A., Svergun, D. and Chen, Y.W. (2012). The solution model of the intrinsically disordered polyglutamine tract binding protein-1 (PQBP-1). ***Biophys J***. 102:1608–1616. doi: 10.1016/j.bpj.2012.02.047

3. Tamura T., Sone M., Nakamura Y., Shimamura T., Imoto S., Miyano S. and Okazawa H. (2012). A restricted level of PQBP1 is needed for the best longevity of Drosophila. ***Neurobiology of Aging***. 2013 Jan;34(1):356.e11-20. doi: 10.1016/j.neurobiolaging.2012.07.015

Department of Pathological Cell Biology

Professor	Shigeomi SHIMIZU
Junior Associate Professor	Akimitsu KONISHI
Tokunin Junior Associate Professor	Tatsushi YOSHIDA
Assistant professor	Satoko ARAKAWA
Tokunin Assistant Professor	Michiko MUROHASHI, Shinya HONDA, Hirofumi YAMAGUCHI

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

〈**Research Projects**〉

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

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

2, 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.

We recently identified the role of cell death on inflammatory bowel diseases. Interleukin-10-deficient (IL-10 KO) mice spontaneously develop intestinal inflammation, which has many similarities to Crohn's disease. Several reports suggest that epithelial cell death may increase the severity of colitis, however decisive evidence is lacking. Therefore, we addressed whether and how epithelial cell death plays a role in the development of chronic colitis. We first examined the morphology of intestines of IL-10 KO mice and found the two forms of epithelial cell death: typical apoptosis and necrosis-like cell death, in colitis (Fig. 1A). To elucidate the pathological roles of epithelial cell death, we crossbred IL-10 KO mice with Bcl-2 transgenic mice, in which the anti-apoptosis protein Bcl-2 was overexpressed in intestinal epithelial cells, but

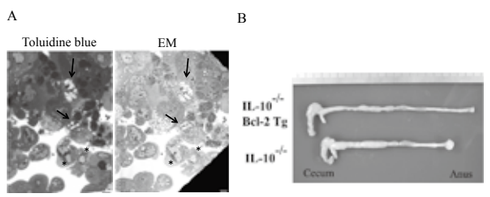


Fig.1 Morphological analysis of the colons of IL-10 KO mice
A. Colonic section occurring cell death in IL-10 KO mice at 16 weeks of age. Colonic sections were fixed and stained with toluidine blue. The serial section was also examined by EM. Both apoptotic cells (asterisks) and necrotic cells (arrows) were observed.
B. Inhibition of IL-10 deficiency-induced colitis by expression of Bcl-2 in intestinal epithelial cells. Representative image of colon tissue from IL-10 KO mice and IL-10 KO/Bcl-2 Tg mice at 16 weeks of age. The colons of IL-10 KO mice were short and thick, whereas those of IL-10 KO/Bcl-2 Tg mice looked nearly normal.

not in immune cells. Epithelial cell-specific Bcl-2 protected IL-10 deficiency-induced colitis and markedly reduced their symptoms (Fig. 1B). Interestingly, morphological analysis revealed that Bcl-2 suppressed not only apoptosis but also necrosis-like cell death, and better-maintained mucosal barrier in IL-10 KO mice. These results provide genetic evidence demonstrating that epithelial cell death is crucial for the development of chronic colitis.

We also identified the novel function of p53 tumor suppressor gene. The p53 is one of the most frequently mutated genes in cancers, and its mutations affect to various biological actions, including tumor growth, apoptosis, and so on. Recently, we showed that expression of wild type p53, but not null or mutated p53, significantly suppressed HIF-1 activity as well as production of VEGF, which is mostly depended on the HIF-1b protein level. Consistently, in tumor xenograft model, we found that loss of p53 promotes VEGF production, neovascularization, and tumor progression (Fig. 2), via accumulation of HIF-1b protein. Furthermore, in clinical pancreas cancer, tumors with mutated p53 have significantly higher levels of vascularity than that of wild type p53. These results indicate that loss of p53 contributes to the neovascularization through regulation of HIF-1.

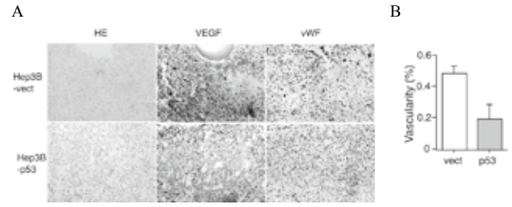


Fig.2 Effect of p53 on angiogenesis and tumor growth in solid tumors.
A. Hep3B-v and Hep3B-p53 cells were injected subcutaneously into the BALB/c (nu/nu) mice. Expression of VEGF and vWF in Hep3B-v and Hep3B-143 tumors (day 11) was analyzed by immunohistochemistry.
B. Blood vessel density in the tumors was quantitated.

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. We are trying to prolong the life of these mice.

List of Publications

[Original paper]

1. Yoshioka Y, **Shimizu S**, Ito T, Taniguchi M, Nomura M, Nishida T, Sawa Y. p53 Inhibits Vascular Endothelial Growth Factor Expression in Solid Tumor. ***J Surg Res***. 174: 291-297, 2012
2. Konishi A, Arakawa S, Yue Z, **Shimizu S**. Involvement of Beclin 1 in the engulfment of apoptotic cells. ***J. Biol. Chem***. 287: 13919-29, 2012
3. Fukamatsu M, Ogawa M, Arakawa S, Ashida H, Suzuki M, Furuse M, Nakayama K, **Shimizu S**, Kin M, Mimuro H, Sasakawa C. Shigella targets epithelial tricellular junctions to spread between cells via a noncanonical clathrin-dependent endocytic pathway. ***Cell Host Microbe***. 11: 325-336, 2012
4. Miyaoka Y, Ebato K, Kato H, Arakawa S, **Shimizu S**, Miyajima A. Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. ***Curr. Biol***. 22: 1166-75, 2012
5. Nakase I, Okumura S, Katayama S, Hirose H, Pujals S, Yamaguchi H, Arakawa S, **Shimizu S**, Futaki S. Transformation of an antimicrobial peptide into a plasma membrane-permeable, mitochondria-targeted peptide via the substitution of lysine with arginine ***Chemical Commun***. 48: 11097-99, 2012

[Review paper]

1. **Shimizu S**, Arakawa S, Nishida Y, Yamaguchi H, Yoshida T.: Mammalian autophagy can occur through an Atg5/Atg7-independent pathway. ***AUTOPHAGY: Cancer, Other Pathologies, Inflammation, Immunity, and Infection.*** *in press*

Department of Developmental and Regenerative Biology

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

Our goal is to define the molecular basis for the mechanism of organ formation, regeneration, and maintenance using knockout mice and mutant fishes. To accomplish this goal, we have focused on defining signaling molecules and pathways that regulate liver and brain 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 the development of new rational therapy for the diseases.

1. SAPK/JNK Signaling Pathway

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. Hippo Signaling Pathway

The Yes-associated protein (YAP) is a transcriptional co-activator that contributes to the regulation of multiple cellular processes by activating several transcription factors. Recently, YAP was shown to play an important role in organ size control and to be inhibited by the Hippo signaling pathway. Either transgenic overexpression of YAP or knockout of Hippo pathway genes in mouse liver results in enlargement of this organ and the eventual development of hepatic tumors. *In vitro*, YAP overexpression promotes cell proliferation and induces oncogenic transformation by activating TEAD family transcription factors. In certain tumor-derived cell lines exposed to various genotoxic stimuli, YAP promotes apoptosis via the induction of pro-apoptotic genes controlled by the transcription factor p73.

The functions of YAP are regulated by the Hippo pathway. The Hippo pathway component Lats kinase phosphorylates serine 127 (S127) of human YAP (hYAP), promoting its recognition and cytoplasmic retention by 14-3-3 protein. In addition, phosphorylation of hYAP S381 by Lats primes subsequent phosphorylation of hYAP S384 by casein kinase 1, leading to hYAP ubiquitination and degradation. We are studying on the regulation and function of YAP.

3. Circadian Clock

Circadian clocks are intrinsic, time-tracking systems that endow organisms with a survival advantage. The molecular mechanism underlying the circadian clock is based on interconnected transcriptional–translational feedback loops. In vertebrate CLOCK : BMAL1 complex transactivates Per and Cry genes, whereas PER and CRY inhibit CLOCK : BMAL1-mediated transcription. It is important to note that the CLOCK–BMAL1 heterodimer also stimulates the transcription of many other clock-controlled genes, which, in turn, influence functions external to the oscillatory mechanism itself. This accounts for the circadian rhythmicity of many physiological processes. The molecular pathways that light uses to influence the clock mechanism are of central importance. We have been studying the light signaling pathway for clock entrainment using zebrafish as the model animal.

Highlight

1) The stress kinase MKK7 is a specific activator of c-Jun N-terminal kinase (JNK), which controls various physiological processes. Here we show that genetic inactivation of MKK7 resulted in an extended period of oscillation in circadian gene expression in mouse embryonic fibroblasts. MKK7-JNK pathway induced phosphorylation of PER2, an essential circadian component. Furthermore, MKK7-mediated JNK activation increased the half-life of PER2 protein by inhibiting its ubiquitination. Notably, the PER2 protein stabilization induced by MKK7-JNK fusion protein reduced the degradation of PER2 induced by casein kinase 1e (CK1 ε). Taken together, our results support a novel function for the stress kinase MKK7 as a regulator of the circadian clock in mammalian cells (Fig. 1).

2) Yes-associated protein (YAP) is a transcriptional co-activator that acts downstream of the Hippo signaling pathway and regulates multiple cellular processes. Here we report the discovery of a novel cycle of acetylation/deacetylation of nuclear YAP induced in response to S_N2 alkylating agents. We show that, following treatment of cells with the S_N2 alkylating agent methyl methanesulfonate, YAP phosphorylation mediated by the Hippo pathway is markedly reduced, leading to nuclear translocation of YAP and its acetylation. This YAP acetylation occurs on specific and highly conserved C-terminal lysine residues and is mediated by the nuclear acetyltransferases CBP and p300. Conversely, the nuclear deacetylase SIRT1 is responsible for YAP deacetylation. Intriguingly, we found that YAP acetylation is induced specifically by S_N2 alkylating agents and not by other DNA-damaging stimuli. These results identify a novel YAP acetylation cycle that occurs in the nucleus downstream of the Hippo pathway. Intriguingly, our findings also indicate that YAP acetylation is involved in responses to a specific type of DNA damage (Fig. 2).

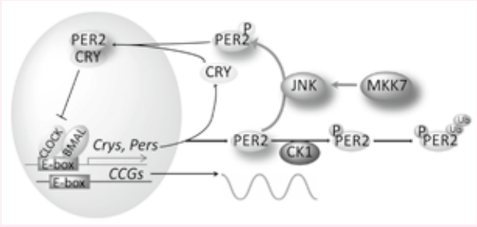


Fig.1. A proposed model for mammalian circadian clock regulation by MKK7.

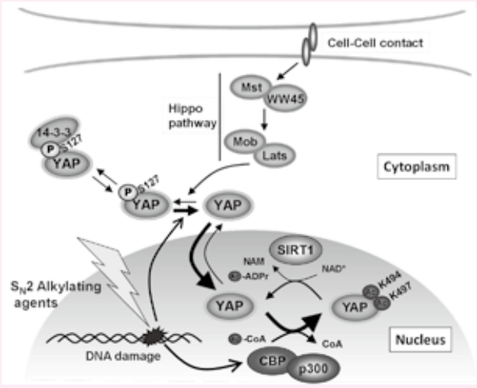


Fig.2. A proposed model for hYAP regulation in response to S_N2 alkylating agents.

Publications

1. Shoji Hata, Jun Hirayama, Hiroaki Kajiho, Kentaro Nakagawa, Yutaka Hata, Toshiaki Katada, Makoto Furutani-Seiki and Hiroshi Nishina (2012) A novel acetylation cycle of the transcription co-activator Yes-associated protein that is downstream of the Hippo pathway is triggered in response to SN2 alkylating agents. ***J. Biol. Chem***. 287, 22089-22098.
2. Yoshimi Uchida, Tomomi Osaki, Tokiwa Yamasaki, Tadanori Shimomura, Shoji Hata, Kazumasa Horikawa, Shigenobu Shibata, Takeshi Todo, Jun Hirayama and Hiroshi Nishina (2012) Involvement of the Stress Kinase Mitogen-activated Protein Kinase Kinase 7 in the Regulation of the Mammalian Circadian Clock. ***J. Biol. Chem***. 287, 8318-8326.
3. Yoshimi Uchida, Tadanori Shimomura, Jun Hirayama and Hiroshi Nishina (2012) Light, reactive oxygen species, and magnetic fields activate ERK/MAPK signaling pathway in cultured zebrafish cells. ***Appl. Magn. Reson***. 42, 69-77.
4. Miki Nishio, Koichi Hamada, Kohichi Kawahara, Masato Sasaki, Fumihito Noguchi, Shuhei Chiba, Kensaku Mizuno, Satoshi O. Suzuki, Youyi Dong, Masaaki Tokuda, Takumi Morikawa, Hiroki Hikasa, Jonathan Eggenschwiler, Norikazu Yabuta, Hiroshi Nojima, Kentaro Nakagawa, Yutaka Hata, Hiroshi Nishina, Koshi Mimori, Masaki Mori, Takehiko Sasaki, Tak W. Mak, Toru Nakano, Satoshi Itami, and Akira Suzuki (2012) Cancer Susceptibility and embryonic lethality in Mob1A/1B double mutant mice. ***J. Clin. Invest***. 122(12):4505-4518.
5. Tadashi Yokoi, Yuko Seko, Tae Yokoi, Hatsune Makino, Shin Hatou, Masakazu Yamada, Tohru Kiyono, Akihiro Umezawa, Hiroshi Nishina, Noriyuki Azuma (2012) Establishment of Functioning Human Corneal Endothelial Cell Line with High Growth Potential. ***PLoS ONE*** 7(1):e29677.
6. Ken Okada, Akihide Kamiya, Keiichi Ito, Ayaka Yanagida, Hidenori Ito, Hiroki Kondou, Hiroshi Nishina and Hiromitsu Nakauchi (2012) Prospective isolation and characterization of bipotent progenitor cells in early mouse liver development. ***Stem Cells and Development*** 21, 1124-1133.
7. Takuya Iwamoto, Shuji Terai, Yuko Mizunaga, Naoki Yamamoto, Kaoru Omori, Koichi Uchida, Takahiro Yamasaki, Yasuhiko Fujii, Hiroshi Nishina, and Isao Sakaida (2012) Splenectomy enhances the anti-fibrotic effect of bone marrow cell infusion and improves liver function in cirrhotic mice and patients ***J. Gastroenterol***. 47, 300-312.
8. Toshiyuki Oishi, Shuji Terai, Shinya Kuwashiro, Koichi Fujisawa, Toshihiko Matsumoto , Hiroshi Nishina and Isao Sakaida (2012) Ezetimibe reduces fatty acid quantity in liver and decreased inflammatory cell infiltration and improved NASH in medaka model. ***Biochem. Biophys. Res. Commun***. 422, 22-27.
9. Shoji Hata and Hiroshi Nishina (2012) [Letters to the Editor] Reply to Sun et al.: Targeting YAP acetylation in cancer. ***J. Biol. Chem***. 287, 35443.
10. Tokiwa Yamasaki, Hiroshi Kawasaki and Hiroshi Nishina (2012) [review] Diverse roles of JNK and MKK pathways in the brain. ***J. Signal Trans***. 2012: 459265.
11. Hiroshi Nishina (2012) [commentary] hDlk-1: A cell surface marker common to normal hepatic stem/progenitor cells and carcinomas. ***J. Biochem***. 152, 121-123.

Department of Stem Cell Biology

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Assistant Professor	Hiroyuki Matsumura, Ph. D.
JSPS Research Fellow	Yasuaki Mohri, Ph. D.

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

1) Identification of stem cells in the skin: follicular melanocyte stem cells vs. volar melanocyte stem cells.

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" (McSCs/MelSCs), which are located in the hair follicle bulge and supply mature melanocytes required for hair pigmentation (Nishimura EK et al. Nature 2002) (Figure 1). We are currently trying to identify melanocyte stem cells in hairless areas of skin by generating Dct-H2B・GFP transgenic mice in which melanocyte stem cells can be stably visualized. This year we discovered an unprecedented melanocytic population in the mouse footpad skin using the transgenic mice. We are currently trying to identify and characterize the population and test whether those cells satisfy the criteria for somatic stem cells and whether the population can be an origin of melanoma in the acral volar skin which contain abundant eccrine sweat glands instead of hair follicles.

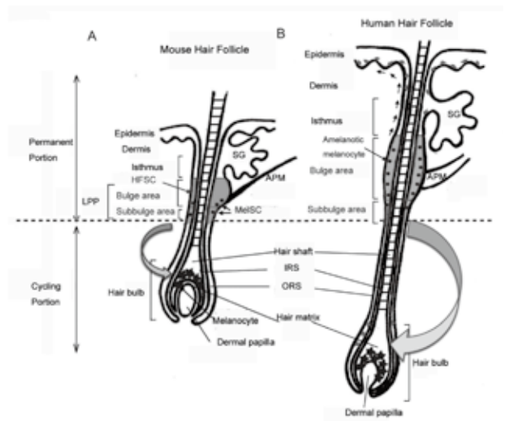

Figure 1

2) Mechanisms of stem cell maintenance

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

While we previously found that the niche microenvironment plays a dominant role in McSC fate determination (Nishimura EK et al. Nature 2002), the identity of the niche cells and the underlying molecular mechanisms have been largely unknown. McSCs and hair follicle stem cells (HFSCs), which are originally derived from a completely different developmental origin, are located in the bulge area of mammalian hair follicles. Our recent study revealed that HFSCs provide a functional niche for McSCs through transforming growth factor β (TGF-β) signaling to prevent premature hair graying (Tanimura S. et al. Cell Stem Cell 2011). 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 McSC maintenance, we analyzed deficient mice of *Col17a1* gene and *Tgfbr2* gene. *Tgfbr2* null mice show progressive hair graying but not hair loss[3], while *Col17a1* deficient mice show premature hair loss as well as premature hair graying. Analysis of HFSCs and McSCs of the *Col17a1* null mice showed that *Col17a1* is critical for maintenance not only of HFSCs but also of McSCs, which do not express *Col17a1* but directly adhere to HFSCs through maintaining their quiescence and immaturity (Figure 2). This potentially explains the mechanism underlying hair loss in human *COL17A1* deficiency. Interestingly, *Col17a1*-deficient mice show defective TGF-β production by HFSCs. TGF-β signaling is activated in McSCs when they reenter the quiescent non-cycling state during hair cycles. Therefore, we analyzed McSCs in conditional *Tgfbr2* deficient mice in which *Tgfbr2* gene that encodes TGF-β type II receptor is knocked out specifically in the melanocyte lineage and found that *Tgfbr2* is essential for the maintenance of McSC immaturity and quiescence to prevent hair graying[3]. These data indicate that HFSC-derived TGF-β is a critical niche factor that regulates McSC immaturity and quiescence. Finally, forced expression of *COL17A1* in basal keratinocytes, including HFSCs, in *Col17a1* null mice rescues McSCs from premature differentiation and restores TGF-β signaling, demonstrating that HFSCs function as a critical regulatory component of the McSC niche through TGF-β signaling (Figure 2). The interactions between different lineages of stem cells thus turned out to be crucial for cyclic regenerative growth of pigmented hair and point to a complex but efficient crosstalk in stem cell niches. The maintenance of somatic stem cell populations by another type of somatic stem cells in a coherent cell mass with might be a recurring strategy for somatic stem cell maintenance.

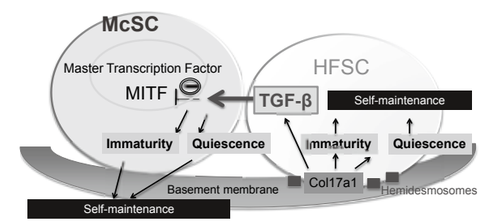

Figure 2

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

Physiological hair graying is the most obvious outward sign of aging even in normal mammals. We previously demonstrated that physiological hair graying is caused by incomplete self-renewal/maintenance of McSCs (Nishimura EK et al. Science 2005) (Figure 1). However, it was still not known what causes the self-renewal of McSCs to become defective during the course of aging. We recently found that genotoxic stress abrogates renewal of McSC by triggering their differentiation. Stem cell differentiation but not stem cell apoptosis nor senescence turned out to be the major fate of McSCs under irreparable/excessive genotoxic stress or with ageing. Our findings indicated that a "stem cell renewal checkpoint" exists to maintain the quality of the McSC pool (Inomata K., Aoto T. et al. Cell 2009) (Figure 3).

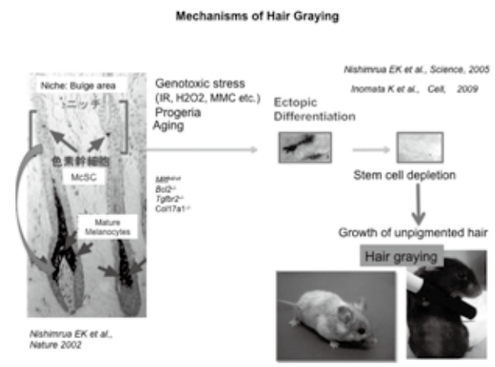

Figure 3

Publications

1. Mohammad S, Matsumura H, Okubo-Suzuki R, Ohkawa N, Inokuchi K. Neuronal stimulation induces autophagy in hippocampal neurons that is involved in AMPA Receptor degradation after chemical long-term depression. J Neurosci. 32(30): 10413-10422. 2012
2. Mohri Y, Oyama K, Sone M, Akamatsu A, Nishimori K. LGR4 is required for the cell survival of the peripheral mesenchyme at the embryonic stages of nephrogenesis. Biosci Biotechnol Biochem. 76(5): 888-91. 2012

Invited lecture/presentation at international meetings

Nishimura EK : Stem cell regulation by Stem cells in hair follicles : SID Annual Meeting: May 9–12, 2012, North Carolina, USA.

Poster Presentation at international meetings

Aoto T, Okamoto N, Uhara H, Akutsu H, Umezawa A, Miyachi Y, Saida T, Nishimura EK. : Identification of eccrine gland melanocyte stem cells in mouse acral skin as a potential source of acral melanoma : ISSCR 10th Annual Meeting : July 14, 2012, YOKOHAMA.

Department of Immunology

Professor	Takeshi Tsubata, M.D., Ph.D.
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	Yusuke Kishi, Ph.D.
	Xu Miduo, Ph.D.

The nature of immune responses depends on whether they respond to protein or non-protein antigens because T lymphocytes recognize only protein antigens. Normal immune system removes pathogens and cancer cells but does not respond to non-microbial foreign substances or self-antigens. Immune responses to non-microbial foreign substances and self-antigens cause allergy and autoimmune diseases, respectively. How immune system distinguishes pathogens from non-microbial antigens and self-antigens is already clarified for protein antigens. However, little is know about such distinction for non-protein antigens. Immune responses to non-protein antigens play crucial roles in host defense against pathogens such as tuberculosis bacilli and meningococci, and autoimmune diseases such as lupus and immuno-neurological disorders. Thus, immune responses to non-protein antigens constitute a remaining frontier in immunology research. Followings are our research subjects.

1) Elucidation of the mechanisms for humoral immune responses to glycans, glyco-lipids and nucleic acids-related antigens.
2) Elucidation of the role of glycan signals in the regulation of humoral immune responses, and development of modified glycan signals for therapy.
3) Elucidation of the mechanisms for autoantibody production in lupus and immuno-neurological disorders.

1. Elucidation of regulatory mechanisms for pathogenic autoantibody production in lupus.

Systemic lupus erythematosus is a prototype of systemic autoimmune diseases, and characterized by production of autoantibodies to various nuclear components. Among these autoantibodies, those reactive to RNA-related antigens such as the Sm antigen play a pathogenic role. Self-reactive B lymphocytes (B cells) that produce autoantibodies are regulated by various self-tolerance mechanisms including deletion and inactivation so that autoantibodies are not produced in healthy individuals. In collaboration with Professor Weigert at Chicago University, we recently found that anti-Sm B cells are regulated by a distinct mechanism (Kishi et al. PNAS, 2012).

Self-reactive B cells that produce anti-DNA antibodies are deleted or inactivated, or undergo alteration of antigen specificity at the immature B cell stage in bone marrow where B cells develop from their precursors. Anti-Sm B cells escape from these self-tolerance mechanisms in the bone marrow by a yet unknown mechanism, and emerge in the peripheral lymphoid tissue such as spleen. Anti-Sm B cells then undergo apoptosis in the peripheral lymphoid tissue, leading to maintenance of self-tolerance. However, the peripheral apoptosis of anti-Sm B cells is perturbed by excess CD40L, which is often found in patients with SLE, leading to autoantibody production. Thus, anti-Sm B cells are regulated by a distinct mechanism that is abrogated by excess CD40L.

2. Genetic factors that regulate autoantibody production in SLE and immuno-neurological disorders.

Guillain-Barre syndrom (GBS), an immuno-neurological disease, is often triggered by Campylobactor infection, but may involve genetic factors. Patients with GBS often produce autoantibodies to gangliosides, sialic acid-containing glycolipids. We are analyzing the Siglec family genes encoding negative regulators of cell activation expressed in various immune cells in patients with GBS in collaboration with Professor Kusunoki at Kinki University. Also we are analyzing, genetic factors in mouse lupus, and demonstrated that *CD72*^{c} is a modifier gene for mouse lupus (see Highlights).

3. Development of sialic acid derivatives for immune regulation.

Although various immuno-modulating compounds have been developed, no such compound that targets B cells is available. We are developing the compounds that specifically regulate B cells by synthesizing sialic acid derivatives.

CD22, a member of the siglec family that predominantly express in B cells, negatively regulates signaling through B cell antigen receptor. Although CD22 specifically binds to a2,6 sialic acid, CD22 is strongly phosphorylated when B cells interact with antigen regardless of whether the antigen contains sialic acids. Thus, CD22 is a predominant negative regulator of B cell activation. In collaboration with Professors Kiso and Ishida at Gifu University, we developed a sialic acid derivate that binds to CD22 with affinity 10000 fold higher than the natural ligand. We are currently analyzing its biological activities.

Highlight

Cd72^c is a modifier gene for SLE.

Modifier genes constitute genetic factors other than disease-causing genes, and regulate penetration, severity and disease manifestation of the disease. Modifier genes are extensively studied in the diseases such as cancer and arrythmia, but not in immunological diseases.

Fas (CD95) is a member of the TNF receptor family that transmits apoptotic signaling in variety of cell types including immune cells. Loss of function mutation of Fas causes autoimmune lymphoproliferative syndrome (ALPS) characterized by autoimmune diseases and systemic swelling of lymphoid tissues. MRL/lpr mice carrying a loss-of-function mutation of Fas (lpr mutation) is a mouse model of ALPS. Development of the autoimmune disease by the lpr mutation depends on the mouse strains, suggesting an involvement of modifier genes.

CD72 is an inhibitory membrane molecule mainly expressed in B cells. CD72 contains a C-type lectin-like domain in the extracellular part and an immunoreceptor tyrosine-based inhibition motif (ITIM) in the cytoplamic region. By recruiting SH2-containing protein tyrosine phosphatase (SHP) 1 at the ITIM, CD72 negatively regulates B cell activation. Previously, CD72 was shown to be associated with development of autoimmune disease in MRL/lpr mice. There are three allelic forms of CD72, i.e., CD72^a, CD72^a and CD72^c. MRL mice carry CD72^c, which contain numbers of amino acid substitutions and seven amino acid deletion compared to CD72^a or CD72^a.

By using B cell lines expressing CD72^a or CD72^c, we demonstrated that CD72^c is hypofunctional in B cell signal inhibition. We then generated congenic B6.CD72^c mice by introducing the chromosomal interval containing *Cd72*^{c} derived from MRL mice into C57BL/6 mice through repeated back crossing. Whereas B6.CD72^c mice are healthy, B6.CD72^c /lpr mice carrying the lpr mutation develop moderate lupus-like disease, suggesting that *Cd72*^{c} is a modifier gene that regulates lpr-induced autoimmune disease.

We further established CD72-deficient mice, and demonstrated that these mice develop moderate lupus-like disease whereas CD72-deficient lpr mice develop severe disease. Thus, *Cd72* is crucial for preventing development of lupus-like disease, and *Cd72*^{c} does not cause autoimmune disease by it self probably because it partially retains signal inhibition capacity.

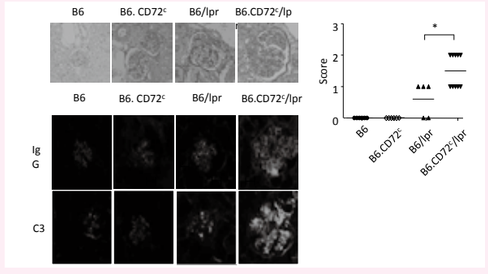

Fig. CD72c induces lupus nephritis in the presence of the lpr mutation. Indicated mice were analyzed at 12 mo of age. Kidney sections were analyzed by PASH staining and immunohistology for IgG and C3. Significant deposition of PASH positive substance and immunecomplex containg IgG and C3 is shown in B6.CD72c/lpr mice. Severity of glomerular damage was scored (right panel).

Publications

[Original papers]

1. Klionsky, D.J., Abdalla, F. C. Tsubata, T. et al. (2012): Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 8: 1-100.
2. Kishi, Y., Higuchi, T., Phoon, S., Kamiya, K., Riemekasten, G., Akiyoshi, K., Weigert, M. and Tsubata, T. (2012): Apoptotic marginal zone deletion of anti-Sm/ribonucleoprotein B cells. *Proc. Natl. Acad. Sci. USA* 109: 7811-7816.
3. Tsubata, T. (2012): Role of inhibitory BCR coreceptors in immunity. *Infect Disord Drug Targets* 12:181-190.
4. Maeno, E., Tsubata, T. and Okada, Y (2012): Apoptotic volume decrease (AVD) is independent of mitochondrial dysfunction and initiator caspase activation. *Cells* 1: 1156-1167.
5. Hitomi, Y., Adachi, T., Tsuchiya, N., Honda, Z.-I., Tokunaga, K and Tsubata, T. (2012): Human CD72 splicing isoform responsible for resistance to systemic lupus erythematosus regulates serum immunoglobulin level and is localized in endoplasmic reticulum. *BMC Immunol.* 13: 72.
6. Shimoda, M., Bolduc, A., Takezaki, M., Amtani, Y., Huang, L., Nutt S. L., Kamanaka, M., Flavell, R. A., Mellor A. L, Tsubata, T., Koni, P. (2013): Constitutively CD40-activated B cells regulate CD8 T cell inflammatory response by IL-10 induction. *J. Immunol.* (in press).
7. Adachi T, Harumiya S, Takematsu H, Kozutsumi Y, Wabl M, Fujimoto M, Tedder TF. (2012): CD22 serves as a receptor for soluble IgM. *Eur J Immunol.* 2012 42:241-7.

Department of Molecular Pathogenesis

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Assistant Professor	Daisuke Sakurai, Ph.D.
Research Associate	Taeko Naruse, Ph.D.

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

1. Molecular mechanisms for hereditary cardiomyopathy

We have searched for mutations of myopalladin gene (MYPN) in patients with hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) or restrictive cardiomyopathy (RCM), who have no mutations in the known disease genes, as an international collaboration study. Several disease-associated mutations were identified in the patient population, including Y20C and Q529X; the former was found in different HCM patient and DCM patient, while the latter was detected in RCM patients showing HCM-like phenotype or DCM-like phenotype. Functional studies revealed different functional alterations for the mutations, including that Q529X impaired sarcomere assembly in rat cardiomyocytes (see Highlight).

2. Molecular mechanisms for atherosclerosis

A genome-wide screening of loci for myocardial infarction (MI) identified a SNP of MKL1, which induced higher expression, as the disease gene for coronary artery disease (CAD). We generated transgenic mouse lines expressing human MKL1 under the CD68 promoter and found that these mice exhibited abnormality in differentiation of macrophages. In addition, an international collaboration study revealed the impact of 9p21 locus on the coronary atherosclerosis and not on myocardial infarction.

3. Molecular mechanisms for arrhythmia

We have searched for mutations of SLMAP gene in patients with Brugada syndrome, who had no mutations in the known disease genes, as an international collaboration study. We identified two mutations in Japanese patients. Functional studies revealed that the SLMAP mutations impaired intracellular trafficking of Nav1.5 channel and decreased inward currents.

4. Analysis of MHC in human and old world monkeys

We revealed the diversity of MHC class I genes in Rhesus macaque and Crab-eating macaque, consist of specific haplotypes with multiple alleles on the same chromosomes. In addition, NFKBIL1 gene was found to regulate alternative splicing of human and viral genes, which may be involved in the immunological host-pathogen interaction.

5. Genome diversity in association with HIV/AIDS

We have investigated natural selection on immune-related genes in the primate evolution. The gene showing under the most strong selection pressure was TIM1, which showed extensive polymorphisms in the Old World monkey lineage which were associated with production level of neutralizing antibodies after SIV vaccination in Rhesus macaques. In addition, specific TIM1 haplotype was found to be associated with good prognosis of HIV infection in Indian population. Moreover, TIM1 was found to be had become pseudogene in the New World monkey lineage by multiple independent events.

Highlight

By a candidate gene approach, we identified MYPN mutations in patients with different phenotypes of cardiomyopathy; hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and restrictive cardiomyopathy (RCM). Quite interestingly Q529X mutation was found in sibling cases of RCM; one showed HCM-like phenotype and the other suffered from DCM-like phenotype, whereas Y20C mutation was detected in HCM patient and DCM patient. Introduction of MYPN of wild-type (WT), Q529X, or Y20C mutation revealed that Q529X impaired sarcomere organization and induced apoptosis of rat cardiomyocytes, while Y20C did not induced such abnormalities (Figure 1). On the other hand, transgenic mice expressing Y20C-MYPN showed sarcomere disruption and HCM phenotype. Furthermore, Y20C mutation increased binding of MYPN to CARP, which was not found for Q529X, indicating different mutations caused different functional alterations.

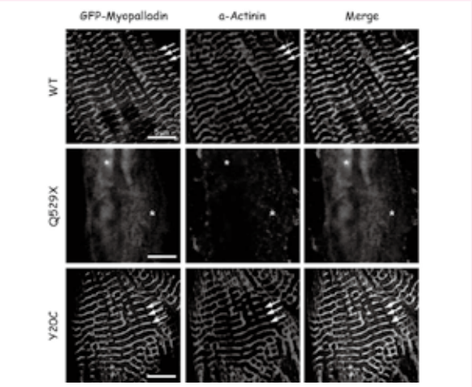


Fig.1. Q529X mutation of MYPN impaired the sarcomerogenesis
GFP-tagged MYPN gene of wild-type or mutant was introduced into primary rat cardiomyocytes. GFP signals indicate the localization of MYPN in cardiomyocytes which were stained for Z-discs (alpha-actinin). Note that MYPN with Q529X mutation diminished sarcomere assemblies.

Publications

(#; equal contribution, *; corresponding author)

1. Ishii H, Kawada M, Tsukamoto T, Yamamoto H, Matsuoka S, Shiino T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Naruse TK, Kimura A, Takiguchi M, *Matano T. Impact of vaccination on cytotoxic T lymphocyte immunodominance and cooperation against simian immunodeficiency virus replication in rhesus macaques. J Virol. 2012; 86(2): 738-745.
2. Takaki A, Yamazaki A, Maekawa T, Shibata H, Hirayama K, Kimura A, Hirai H, *Yasunami M. Positive selection of Toll-like receptor 2 polymorphisms in two closely related old world monkey species, rhesus and Japanese macaques. Immunogenetics. 2012; 64(1): 15-29.
3. #Saito Y, #Naruse TK, Akari H, Matano T, *Kimura A. Diversity of MHC class I haplotypes in cynomolgus macaques. Immunogentics. 2012; 64(2): 131-141.
4. #Otsuka H, #Arimura T, Abe T, Kawai H, Aizawa Y, Kubo T, Kitaoka H, Nakamura H, Nakamura K, Okamoto H, Ichida F, Ayusawa M, Nunoda S, Isobe M, Matsuzaki M, Doi YL, Fukuda K, Sasaoka T, Izumi T, Ashizawa N, *Kimura A. Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. Circ J. 2012; 76(2): 453-461.
5. #Purevjav E, #Arimura T, Augustin S, Huby A-C, Takagi K, Nunoda S, Kearney DL, Taylor MD, Terasaki F, Bos JM, Ommen SR, Shibata H, Takahashi M, Itoh-Satoh M, McKenna W, Murphy RT, Labeit S, Yamanaka Y, Machida N, Park JE, Alexander PMA, Weintraub RG, Kitaura Y, Ackerman MJ, *Kimura A, *Towbin JA. Molecular basis for clinical heterogeneity in inherited cardiomyopathies due to myopalladin mutations. Hum Mol Genet. 2012; 21(9): 2039-2053.
6. *Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, Nagao S, Yagihara N, Takehara N, Kawamura Y, Sato A, Okamura K, Sato M, Hosaka Y, Fukae S, Chinushi M, Oda H, Okabe H, Kimura A, Maemura K, Watanabe I, Kamakura S, Aizawa Y, Shimizu W, Makita N. Similarities and differences in genetic and clinical characteristics between early repolarization syndrome and Brugada syndrome. Circ Arrhythm Electrophysiol. 2012; 5(2): e60-e61.
7. Nomura T, Terahara K, Yamamoto H, Shiino T, Takahashi N, Nakane T, Iwamoto N, Ishii H, Tsukamoto T, Kawada M, Matsuoka S, Takeda A, Terahara K, Tsunetsugu-Yokota Y, Iwata-Yoshikawa N, Hasegawa H, Sata T, Naruse TK, Kimura A, *Matano T. Association of major histocompatibility complex class I haplotypes with disease progression after simian immunodeficiency virus challenge in Burmese rhesus macaques. J Virol. 2012; 86(12): 6481-6490.
8. Ohtani H, Naruse TK, Iwasaki Y, Ishida T, Akari H, Matano T, *Kimura A. Lineage-specific evolution of T-cell immunoglobulin and mucin domain 1 gene in the primates. Immunogenetics. 2012; 64(9): 669-678.
9. *Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, Makiyama T, Nagao S, Yagihara N, Takehara N, Kawamura Y, Sato A, Okamura K, Hosaka Y, Sato M, Fukae S, Chinushi M, Oda H, Okabe M, Kimura A, Maemura K, Watanabe I, Kamakura S, Horie M, Aizawa Y, Shimizu W, Makita N. Clinical characteristics and risk of arrhythmia recurrences in patients with idiopathic ventricular fibrillation associated with early repolarization. Int J Cardiol. 2012; 159(3): 238-240.
10. Xi Y, *Ai T, De Lange E, Li Z, Wu G, Brunelli L, Kyle WB, Cheng J, Ackerman MJ, Kimura A, Weiss JN, Qu Z, Kim JJ, Faulkner G, *Vatta M. Loss-of-function of hNav1.5 by ZASP1-D117N associated with intraventricular conduction disturbances in left ventricular noncompaction. Circ Arrhythm Electrophysiol. 2012; 5(5): 1017-1026.
11. Sato A, *Sakamoto N, Ando K, Kaneshiro T, Uekita H, Sugimoto K, Yamaki T, Kunii H, Nakazato K, Suzuki H, Saitoh S, Sato M, Tamagawa K, Arimura T, Kimura A, Takeishi Y. Dilated phase of hypertrophic cardiomyopathy caused by two different sarcomere mutations, treated with surgical left ventricular reconstruction and cardiac resynchronization therapy with a defibrillator. Intern Med. 2012; 51(18): 2559-2564.
12. #Ishikawa T, #Sato A, Marcou CA, Tester DJ, Ackerman MJ, Crotti L, Schwartz PJ, On YK, Park JE, Nakamura K, Hiraoka M, Nakazawa K, Sakurada H, Arimura T, *Makita N, *Kimura A. A novel disease gene for Brugada syndrome: sarcolemmal membrane-associated protein gene mutations impair intracellular trafficking of hNav1.5. Circ Arrhythm Electrophysiol.2012; 5(6): 1098-1107.
13. Minami T, *Kuwahara K, Nakagawa Y, Takaoka M, Kinoshita H, Nakao K, Kuwabara Y, Yamada Y, Yamada C, Shibata J, Usami S, Yasuno S, Nishikimi T, Ueshima K, Sata M, Nakano H, Seno T, Kawahito Y, Sobue K, Kimura A, Nagai R, Nakao K. Reciprocal expression of MRTF-A and myocardin is crucial for pathological vascular remodeling in mice. EMBO J. 2012; 31(23):4428-4440.
14. Sharma G, Ohtani H, *Kaur G, Naruse TK, Sharma SK, Vajpayee M, *Kimura A, Mehra NK. Status of TIM-1 exon 4 haplotypes and CD4+T cell counts in HIV-1 seroprevalent North Indians. Hum Immunol. 2013, In Press (doi: 10.1016/j.humimm.2012.11.013)
15. Ishikawa T, Takahashi N, Ohno S, Sakurada H, Nakamura K, On YK, Park JE, Makiyama T, Horie M, Arimura T, Makita N, *Kimura A. Novel SCN3B mutation associated with Brugada syndrome affects intracellular trafficking and function of Nav1.5.Circ J. 2013, In Press (doi:10.1253/circj.CJ-12-0995)
16. Takahashi N, Nomura T, Takahara Y, Yamamoto H, Shiino T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Sakawaki H, Miura T, Igarashi T, Koyanagi Y, Naruse TK, Kimura A, *Matano T. A Novel Protective MHC-I Haplotype Not Associated with Dominant Gag-Specific CD8+ T-Cell Responses in SIVmac239 Infection of Burmese Rhesus Macaques. PLoS ONE 2013, In Press (doi: 10.1371/journal.pone.0054300)
17. #Chan K, #Patel RS, Newcombe P, Nelsone CP, Qasim A, Epstein SE, Burnett S, Vaccarino V, Zafari AM, Shah SH, Anderson JL, Carlquist JF, Hartiala J, Allayee H, Hinohara K, Lee BS, Erl A, Ellis KL, Goel A, Schaefer AS, Eddine N, Mokhtari NEE, Goldstein BA, Hlatky MA, Go AS, Shen GQ, Gong Y, Pepine C, Laxton RC, Wittaker JC, Tang WHW, Johnson JA, Wang QK, Assimes TL, Nöthlings U, Farrall M, Watkins H, Richards MA, Cameron VA, Muendlein A, Drexel H, Koch W, Park JE, Kimura A, Shen WF, Simpson IA, Hazen SL, Horne BD, Hauser ER, Quyyumi AA, Reilly MP, Samani NJ, *Ye S. Association between the chromosome 9p21 locus and angiographic coronary artery disease burden - a collaborative meta-analysis. J Am Col Cardiol. 2013, In Press (doi:10.1016/j.jacc.2012.10.051)
18. #Nakayama EE, #Nakajima T, Kaur G, Miyama J, Terunuma H, Mehra NK, Kimura A, *Shioda T. A naturally occurring single amino acid substitution in human TRIM5a linker region affects its anti-HIV-1 activity and susceptibility to HIV-1 infection. AIDS Res Hum Retroviruses. 2013, In Press, (doi:10.1089/AID.2012.0369)

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

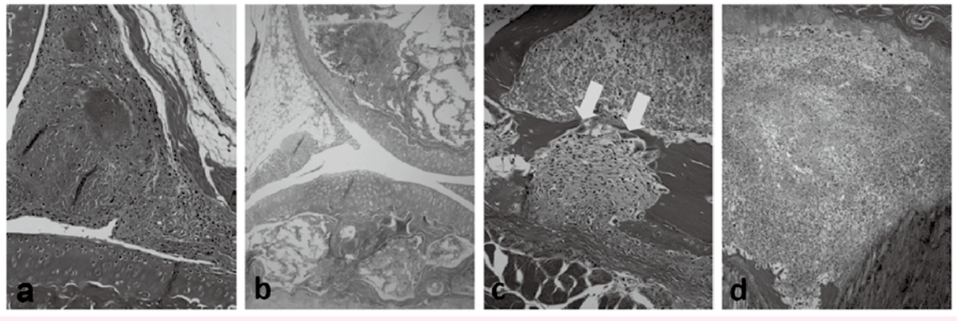
The functional human immune system is reconstituted in NOD/Shi-*scid*/IL-2R γ null(NOG) mice that receive hematopoietic stem cell transplants (hNOG mice). Histologic analysis of major joints of hNOG mice revealed evidence of synovial proliferation and inflammatory cell infiltration in 65% of EBV-infected mice but none of the control mice showed these features. These results strongly implicate EBV as a causative agent in rheumatoid arteritis (RA), and demonstrate the potential of EBV-infected hNOG mice in the future study of RA pathogenesis.

2. Development of an exhaustive pathogenic microbe screening system

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

Highlight

Experimental arthritis: EBV induced erosive arthritis in hNOG mice



Histopathology of joint tissues in hNOG mice infected with EBV. a: EBV(+) synovial proliferation, b: EBV(-) , c: EBV(+) a pannus-like lesion, d:EBV(+) bone marrow near the knee joint showing edema

Publications

[Original papers]

1. Ramakrishman R, et al. Epstein-Barr virus BART9 miRNA modulates LMP1 levels and affects growth rate of nasal NK T cell lymphomas. ***PLoS One***. 2011:e27271.
2. Ogawa M, et al. Broad-range real-time PCR assay for detection of bacterial DNA in ocular samples from infectious endophthalmitis. ***Jpn J Ophthalmol***. 56(6):529-535, 2012.
3. Sugita S, et al. Virological analysis in patients with human herpes virus 6-associated ocular inflammatory disorders. ***Invest Ophthalmol Vis Sci***. 12;53(8):4692-8. 2012.
4. Ogawa M, et al. Novel diagnosis of fungal endophthalmitis by broad-range real-time PCR detection of fungal 28S ribosomal DNA. ***Graefes Arch Clin Exp Ophthalmol***. 250(12):1877-1883, 2012.
5. Sugita S, et al. Detection of Candida & Aspergillus species DNA using broad-range real-time PCR for fungal endophthalmitis. ***Graefes Arch Clin Exp Ophthalmol***. 250:391-398, 2012.

Division of Medical Genomics

【Aim and Scope】

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 2012 we contributed as follows;

1. Identification of novel genes including microRNAs responsible for cancer and unknown genetic diseases.
2. Understanding the pathogenesis of intractable cancers and genetic disorders based on Integrative Omics approach including Systems Biology.
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. Role of stress response gene ATF3, a target of p53, in TRAIL-based pro-apoptotic cancer therapy. Further, the stress code of p53-ATF3 axis was investigated by genome-wide system biology.
2. Transcriptional properties of elongation factor Elongin A was elucidated in stress response and cranial nerve development.
3. Biochemical and biological role of FCP1, a causative gene for CCFDN, was studied and shown to be essential for transcription cycle.

【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】

1. Among recently identified coronary artery disease (CAD) susceptible genes, we replicated the effect of variations in LTA and LGALS2 and showed that those were associated with pathological coronary stenosis.
2. We showed that a functional SNP in the catechol-O-methyltransferase (COMT) gene associated with the severity of systemic atherosclerosis in Japanese elderly population, whereby the influence of the genotype appears to be stronger in females than in males.

【Genome Pathology】

1. We are analyzing the global profiling of cancer-stromal interactions by massively-parallel sequencing of cancer xenograft transcriptome.
2. We started to develop analysis method for immune-genomics to characterize immune-mediated disease and immunotherapy.
3. We started to develop technology to do genomic analysis of clinical samples.
4. We made an effort to create laboratory environment since Jan 2013, department start-up.

【Epigenetics】

1. We reported the existence of many LTR retrotranposon-derived genes in eutherian mammals, such as sushi-ichi-related retrotransposon homologue family of genes (*SIRH* family genes). Among them, we demonstrated that *Peg10*, *Peg11/Rtl1* and *Sirh7*, play essential eutherian-specific functions, namely, multiple aspects of placental function.
2. We have recently reported that distribution of *SIRH* genes and another LTR retrotranposon-derived genes, *PNMA* family genes, are much abundant in the eutherian mammals but only few in marsupial mammals, another group in mammals, suggesting that these LTR retrotransposon-derived genes deeply contributed to diversification and establishment of these two viviparous mammalian groups.
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 are tend to be drug targets, while almost no drug targets were found among hub proteins.
2. 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 prognosis prediction in hepatocellular carcinoma patients, 2) development of predictive marker for metastatic relapse in colorectal cancer.
3. We have constructed a publicly available pathway map called "AlzPathway" that comprehensively catalogs signaling pathways in the field of AD. AlzPathway is composed of 1347 molecules and 1070 reactions in neuron, brain blood barrier, presynaptic, postsynaptic, astrocyte, and microglial cells and their cellular localizations.
4. Comparative genomic analysis of olfactory receptors (OR) genes from the draft genome sequences of 38 diverse mammals demonstrated that the estimated numbers of functional OR genes are extremely variable, ranging from only ~10 in dolphins to ~2,000 in elephants. Identification of orthologous gene sets among 13 eutherian mammals with the genome of deep coverage (>6x) revealed that hundreds of gene gains and losses have occurred during eutherian evolution.

Department of Molecular Cytogenetics

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

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

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

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

2. Identification of cancer-related micro RNAs

MicroRNAs (miRNAs) have started a revolution in molecular biology and emerged as key players in the cancer process. To date, we have successfully identified novel tumor-suppressive miRNAs (TS-miRNAs), *miR-137*, *miR-193a*, *miR-124*, *miR-203*, *miR-218* and *miR-152*, which are frequently silenced by tumor-specific DNA hypermethylation in oral squamous cell carcinoma (OSCC), hepatocellular carcinoma (HCC) or endometrial cancer (EC), using expression-, methylation- or function-based screenings, resulting in the identification of direct targets of these TS-miRNAs and the elucidation of these mechanisms. Based on these studies, we have considered that the tumor-specific DNA hypermethylation of CpG islands located immediately 5'-upstream of miRNA genes is a useful landmark to explore novel TS-miRNAs silenced epigenetically in cancer cells, similar to classical tumor suppressor genes. Recently, by using methylation-based screening of TS-miRNAs in OSCC, we identified *miR-596* and *LGALS3BP* as a novel TS-miRNAs and its direct target. On the other hand, we performed integrative function- and expression-based screenings of TS-miRNAs in HCC, resulting in the identification of *miR-195* and *miR-497* showing significant growth-suppressive activity with induction of G1 arrest. Moreover, comprehensive exploration of their targets using Argonute2-immunoprecipitation-deep-sequencing (Ago2-IP-seq) and genome-wide expression profiling after their overexpression, successfully identified a set of cell-cycle regulators, including *CCNE1*, *CDC25A*, *CCND3*, *CDK4*, and *BTRC*.

3. Cancer pathogenesis relevant to impaired autophagy

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

4. Molecular cytogenetic investigation of MCA/MR

Array-CGH (aCGH) is one of the most powerful tools to detect cryptic pathogenic copy number variants (pCNVs) in genomic disorders including multiple congenital anomalies and mental retardation (MCA/MR). We have explored pCNVs in 646 patients with clinically uncharacterized MCA/MR, whose karyotypes showed normal, for two-stage screening using two types of bacterial artificial chromosome-based microarray. We detected pCNV in 69 (10.7%) cases in the 1st screening using a targeted array and in 61 cases (9.4%) in the 2nd screening using a genome-wide array [Hayashi et al. J Hum Genet 2010]. Also, we are currently analyzing the remaining negative cases using a SNP array as the 3rd screening. It is notable that we indicated the *CASK* gene as a causative gene of microcephaly and pontine and cerebellar hypoplasia (MICPCH), and we continue to recruit new patients of MICPCH and analyze a *CASK* mutation [Hayashi et al. Hum Genet 2012].

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

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

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

Articles

1. Furuta M, Kozaki K, Tanimoto K, Tanaka S, Arii S, Shimamura T, Niida A, Miyano S, Inazawa J: The tumor-suppressive miR-497-195 cluster targets multiple cell-cycle regulators in hepatocellular carcinoma. PLOS ONE, in press.
2. Endo H, Muramatsu T, Furuta M, Uzawa N, Pimkhaokham A, Amagasa T, Inazawa J, Kozaki K: Potential of tumor-suppressive miR-596 targeting LGALS3BP as a therapeutic agent in oral cancer. Carcinogenesis, in press.
3. Miyawaki Y, Imoto I, Tokairin Y, Kawada K, Nakajima Y, Nishikage T, Nagai K, Kajiwara M, Inazawa J, Kawano T: Esophageal Squamous Cell Carcinoma Developed 11 Years After Allogeneic Bone Marrow Transplantation for Acute Lymphatic Leukemia. Jpn J Clin Oncol 43: 69-73, 2013.
4. Takanashi J, Okamoto N, Yamamoto Y, Hayashi S, Arai H, Takahashi Y, Maruyama K, Mizuno S, Shimakawa S, Ono H, Oyanagi R, Kubo S, Barkovich AJ, Inazawa J: Clinical and radiological features of Japanese patients with a severe phenotype due to CASK mutations. Am J Med Genet A 158A: 3112-3118, 2012.
5. Miyawaki Y, Kawachi H, Ooi A, Eishi Y, Kawano T, Inazawa J, Imoto I: Genomic copy-number alterations of MYC and FHIT genes are associated with survival in esophageal squamous-cell carcinoma. Cancer Sci 103: 1558-1566, 2012.
6. Matsumura S, Imoto I, Kozaki K, Matsui T, Muramatsu T, Furuta M, Tanaka S, Sakamoto M, Arii S, Inazawa J: Integrative array-based approach identifies MZB1 as a frequently methylated putative tumor-suppressor in hepatocellular carcinoma. Clin Cancer Res 18: 3541-3551, 2012.
7. Honda S, Hayashi S, Nakane T, Imoto I, Kurosawa K, Mizuno S, Okamoto N, Kato M, Yoshihashi H, Kubota T, Nakagawa E, Goto Y, Inazawa J: The incidence of hypoplasia corpus callosum in patients with dup (X) (q28) involving MECP2 is associated with the location of distal breakpoints. Am J Med Genet A 158A: 1292-1303, 2012.
8. Akamatsu S, Takata R, Haiman CA, Takahashi A, Inoue T, Kubo M, Furihata M, Kamatani N, Inazawa J, Chen GK, Le Marchand L, Kolonel LN, Katoh T, Yamano Y, Yamakado M, Takahashi H, Yamada H, Egawa S, Fujioka T, Henderson BE, Habuchi T, Ogawa O, Nakamura Y, Nakagawa H: Common variants at 11q12, 10q26 and 3p11.2 are associated with prostate cancer susceptibility in Japanese. Nat Genet 44: 426-429, 2012.
9. Ono H, Imoto I, Kozaki K, Tsuda H, Matsui T, Kurasawa Y, Muramatsu T, Sugihara K, Inazawa J: SIX1 promotes epithelial-mesenchymal transition in colorectal cancer through ZEB1 activation. Oncogene 31: 4923-4934, 2012.
10. Okamoto N, Hayashi S, Masui A, Kosaki R, Oguri I, Hasegawa T, Imoto I, Makita Y, Hata A, Moriyama K, Inazawa J: Deletion at chromosome 10p11.23-p12.1 defines characteristic phenotypes with marked midface retrusion. J Hum Genet 57: 191-196, 2012.
11. Bai H, Inoue J, Kawano T, Inazawa J: A transcriptional variant of the LC3A gene is involved in autophagy and frequently inactivated in human cancers. Oncogene 31: 4397-4408, 2012.
12. Honda S, Satomura S, Hayashi S, Imoto I, Nakagawa E, Goto Y, Inazawa J: Concomitant microduplications of MECP2 and ATRX in male patients with severe mental retardation. J Hum Genet 57: 73-77, 2012.
13. Kurasawa Y, Kozaki K, Pimkhaokham A, Muramatsu T, Ono H, Ishihara T, Uzawa N, Imoto I, Amagasa T, Inazawa J: Stabilization of phenotypic plasticity through mesenchymal-specific DNA hypermethylation in cancer cells. Oncogene 31: 1963-1974, 2012.
14. Hayashi S, Okamoto N, Chinen Y, Takanashi J, Makita Y, Hata A, Imoto I, Inazawa J: Novel intragenic duplications and mutations of CASK in patients with mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH). Hum Genet 131: 99-110, 2012.

[Review articles]
Kozaki K, Inazawa J: Tumor-suppressive microRNAs silenced by tumor-specific DNA hypermethylation in cancer cells. Cancer Sci 103: 837-845, 2012.

Department of Molecular Genetics

Professor	Yoshio Miki, MD. Ph.D.
Associate Professor	Akira Nakanishi, Ph.D.
Assistant Professor	Katsuya Takenaka, Ph.D.
Assistant Professor	Ken Miyaguchi, 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. Synthetic lethality effect for chemotherapy using BRCA1/2-deficient breast cancers

In this study, we aimed to establish novel synthetic lethal interactions between BRCA1/2 and four well-known anti-cancer agents (adriamycin, cisplatin, paclitaxel and 5-fluorouracil) in the hope that the use of chemotherapy in BRCA1/2-deficient breast cancer patients may be optimized on the basis of the tumor cell genotype. In order to identify potential synthetic lethality relationships between anti-cancer agents and BRCA1/2, WST-1-based cytotoxicity assays were performed on the background of BRCA1/2-targeted RNA interference and treatment with anti-cancer agents (5-fluorouracil, adriamycin, cisplatin and paclitaxel). Carriage of loss-of-function mutation of BRCA1/2 was successfully mimicked by transfection with siRNA targeted against BRCA1 and two different components of BRCA. Varying concentrations of 5-fluorouracil, adriamycin, cisplatin and paclitaxel were simultaneously screened for on 96-well plates containing BRCA1/2-knocked down MCF7 breast cancer cells to determine the minimum dose for significant cytotoxic activity.

Notably, application of paclitaxel at 5 μM and 10 μM reduced the proportion of living cells where BRCA2 (both BRCA2-1 and BRCA2-2) was knocked down to a greater extent compared to the controls (transfected with and without non-targeting siRNA) in MCF7 cells (*p<0.01*; Figure 1). This indicates a synergistic effect on cell viability between paclitaxel and BRCA2-knock down. Such sensitivity to paclitaxel was not conferred to BRCA2-knocked down MCF7 cells at 20 μM, suggesting that at a higher concentration, cell death is induced by the action of paclitaxel alone. At concentrations of 12.5 μg/ml and above, adriamycin and cisplatin displayed cytotoxic effects to similar extents in MCF7 cells where BRCA2 was intact or knocked down (Figure 1). This suggests that cell death may have been induced by the actions of adriamycin and cisplatin alone, potentially masking any possible synthetic lethality. Therefore, WST-1 assays were performed with treatment of cells at lower concentrations (3.13, 6.25 and 12.5 μg/ml) of adriamycin and cisplatin (data not shown). Although cytotoxicity was observed across the concentration range with both agents, reduction in cell viability again occurred to similar degrees. On the other hand, 5-fluorouracil conferred little cytotoxicity and cell viability was compromised only at 100 μM. Furthermore, BRCA1-knock down did not sensitize MCF7 cells to any of the four drugs. We speculate that the established paclitaxel/BRCA2 synthetic lethal pair operates against centrosome regulation and this provides rationale for the use of paclitaxel in BRCA2-deficient breast cancer patients.

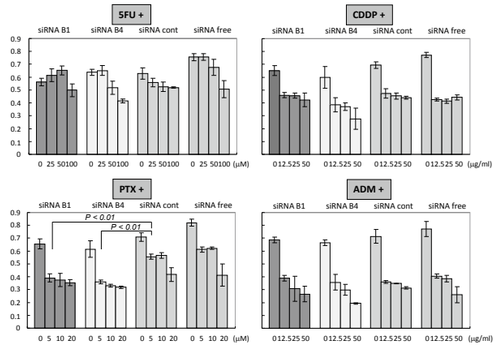


Fig.1. Treatment of BRCA2 knocked-down MCF7 cells with 5FU, CDDP, PTX or ADM

2. Identification of novel BRCA2-associated proteins functioning in DNA damage repair.

The product of a major hereditary breast cancer susceptibility gene BRCA2 is involved in DNA double strand break repair by homologous recombination. We recently identified novel candidates of gene products that associate BRCA2 by mass-spectrometric analysis of BRCA2 co-precipitates. These candidates might contain genes functioning in homologous recombination. Some genes were knocked-down by siRNA and homologous recombination efficiency was measured by the DR-GFP reporter gene assay. The efficiency diminished when the expression of valosin-containing protein (VCP) was decreased. Since VCP later became known to be involved in double strand bread repair, it may be possible that it acts in homologous recombination repair in association with BRCA2. The same strategy will be applied for further identification of novel functional BRCA2-associated proteins.

3. Identification of UVSSA gene as the responsible gene for UV-sensitive syndrome.

Cells from individuals with UV-sensitive syndrome are very UV sensitive and are deficient in transcription-coupled nucleotide-excision repair (TC-NER). Some cases carry mutations in the Cockayne syndrome genes but remaining individuals formed a separate complementation group. By exome sequencing we identified the functionally unknown gene KIAA1530 is the responsible gene for this syndrome and was renamed UV-stimulated scaffold protein A (UVSSA). This finding would explain the different clinical features across TC-NER-deficient disorders. This project was carried out by collaboration with Nagasaki University.

Publications

1. Kawazu, M., Ueno, T., Kontani, K., Ogita, Y., Ando, M., Fukumura, K., Yamato, A., Soda, M., Takeuchi, K., Miki, Y., Yamaguchi, H., Yasuda, T., Naoe, T., Yamashita, Y., Katada, T., Choi, Y.L. and Mano, H. (2013) Transforming mutations of RAC guanosine triphosphatases in human cancers. *Proc Natl Acad Sci U S A*.
2. Wang, L., Tsutsumi, S., Kawaguchi, T., Nagasaki, K., Tatsuno, K., Yamamoto, S., Sang, F., Sonoda, K., Sugawara, M., Saiura, A., Hirono, S., Yamaue, H., Miki, Y., Isomura, M., Totoki, Y., Nagae, G., Isagawa, T., Ueda, H., Murayama-Hosokawa, S., Shibata, T., Sakamoto, H., Kanai, Y., Kaneda, A., Noda, T. and Aburatani, H. (2012) Whole-exome sequencing of human pancreatic cancers and characterization of genomic instability caused by MLH1 haploinsufficiency and complete deficiency. *Genome Res*, **22**, 208-19.
3. Taira, N., Mimoto, R., Kurata, M., Yamaguchi, T., Kitagawa, M., Miki, Y. and Yoshida, K. (2012) DYRK2 priming phosphorylation of c-Jun and c-Myc modulates cell cycle progression in human cancer cells. *J Clin Invest*, **122**, 859-72.
4. Suzuki, K., Dashzeveg, N., Lu, Z.G., Taira, N., Miki, Y. and Yoshida, K. (2012) Programmed cell death 6, a novel p53-responsive gene, targets to the nucleus in the apoptotic response to DNA damage. *Cancer Sci*, **103**, 1788-94.
5. Satoh, Y., Sugai, S., Uehara, H., Mun, M., Sakao, Y., Okumura, S., Nakagawa, K., Ishikawa, Y., Miki, Y. and Miyata, S. (2012) Clinical impact of intraoperative detection of carcinoembryonic antigen mRNA in pleural lavage specimens from nonsmall cell lung cancer patients. *Thorac Cardiovasc Surg*, **60**, 533-40.
6. Sakamoto, K., Fujii, T., Kawachi, H., Miki, Y., Omura, K., Morita, K., Kayamori, K., Katsube, K. and Yamaguchi, A. (2012) Reduction of NOTCH1 expression pertains to maturation abnormalities of keratinocytes in squamous neoplasms. *Lab Invest*, **92**, 688-702.
7. Khanom, R., Sakamoto, K., Pal, S.K., Shimada, Y., Morita, K., Omura, K., Miki, Y. and Yamaguchi, A. (2012) Expression of basal cell keratin 15 and keratin 19 in oral squamous neoplasms represents diverse pathophysiologies. *Histol Histopathol*, **27**, 949-59.
8. Iyevleva, A.G., Kuligina, E., Mitiushkina, N.V., Togo, A.V., Miki, Y. and Imyanitov, E.N. (2012) High level of miR-21, miR-10b, and miR-31 expression in bilateral vs. unilateral breast carcinomas. *Breast Cancer Res Treat*, **131**, 1049-59.
9. Elgazzar, S., Zembutsu, H., Takahashi, A., Kubo, M., Aki, F., Hirata, K., Takatsuka, Y., Okazaki, M., Ohsumi, S., Yamakawa, T., Sasa, M., Katagiri, T., Miki, Y. and Nakamura, Y. (2012) A genome-wide association study identifies a genetic variant in the SIAH2 locus associated with hormonal receptor-positive breast cancer in Japanese. *J Hum Genet*, **57**, 766-71.
10. Nakazawa Y, Sasaki K, Mitsutake N, Matsuse M, Shimada M, Nardo T, Takahashi Y, Ohyama K, Ito K, Mishima H, Nomura M, Kinoshita A, Ono S, Takenaka K, Masuyama R, Kudo T, Slor H, Utani A, Tateishi S, Yamashita S, Stefanini M, Lehmann AR, Yoshiura K, Ogi T. (2012) Mutations in UVSSA cause UV-sensitive syndrome and impair RNA polymerase IIo processing in transcription-coupled nucleotide-excision repair. *Nature Genetics* **44**, 586-592.

Department of Molecular Epidemiology

Professor Masaaki Muramatsu, M.D. & Ph.D.
Associate Professor Noriko Sato M.D.& Ph.D.
Assistant Professor Shinobu Ikeda, DMD. Ph.D.

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

1. Polymorphisms of LTA, LGALS2, and PSMA6 genes and coronary atherosclerosis: a pathological study of 1503 consecutive autopsy cases.

Recent genome-wide association studies have identified polymorphisms of lymphotoxin-α (LTA), galectin-2 (LGALS2), and proteasome subunit a type 6 (PSMA6) genes as genetic risk factors for myocardial infarction (MI). However, their effects on coronary atherosclerosis, an intermediate phenotype of MI, remain largely unknown.

METHODS: We investigated the correlation between polymorphisms of the LTA, LGALS2, and PSMA6 genes and the severity of pathological coronary stenosis index (CSI) and MI in 1503 consecutive autopsy cases of Japanese elderly patients.

RESULTS: The polymorphisms LTA rs1041981 and LGALS2 rs7291467 were associated with CSI with odds ratios of 1.54 (95% CI, 1.17-2.01; AA+CA over CC) and 1.62 (95% CI, 1.11-2.37; TT over CC+CT), respectively. PSMA6 rs1048990 was not associated with CSI. None of the SNPs was associated with MI in our sample.

CONCLUSION: Our findings indicate that the LTA and LGALS2 polymorphisms affect the subclinical phenotype of the coronary artery, which predisposes to the incidence of MI.

2. Association of COMT gene polymorphisms with systemic atherosclerosis in elderly Japanese.

AIM: Atherosclerotic disease is a major health problem among the elderly, which arises from a complex interaction between genetic and environmental factors. The catechol-O-methyltransferase (COMT) gene encodes an enzyme that degrades catecholamines and estrogens to less active metabolites. The objective of this study was to examine whether polymorphisms of the COMT gene affected the severity of atherosclerotic disease in a Japanese elderly population.

METHOD: A total of 1536 autopsy cases of hospital deaths were assessed for the degree of pathological atherosclerotic index (PAI), coronary stenotic index (CSI) and intracranial stenotic index (ICAI), which were obtained by macroscopic examination of the luminal surface of formalin-fixed arteries. Two single nucleotide polymorphisms (SNPs) in the COMT gene, rs4633 (C/T) and rs4680 (G/A) were genotyped. The rs4680 (G/A) corresponds to a functional SNP with the substitution of valine to methionine.

RESULT: The CC genotype of rs4633 (C/T) and the GG genotype of rs4680 (G/A) showed a significantly higher degree of PAI and the association remained positive after adjustment for age, hypertension, diabetes, smoking and drinking (p=0.035 and p=0.031, respectively). There were no significant associations between COMT genotypes and CSI or ICAI. When male and female subjects were analyzed separately, the association was observed only in female subjects (p=0.012 and p=0.027) after adjustment for age, hypertension, diabetes, smoking and drinking.

CONCLUSION: The functional SNP in the COMT gene associated with the severity of atherosclerosis in a Japanese elderly population, whereby the influence of the genotype appears to be stronger in females than in males.

3. Association of GLUT4 gene variants with HbA1c level in Japanese men.

GLUT4 is a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis. Recent studies in south Indian populations revealed that haplotypes of the GLUT4 gene associated with type 2 diabetes. A total of 734 middle aged apparently healthy Japanese men were recruited from two separate occupational cohorts from Kanagawa and Kyoto. Participants were genotyped for GLUT4 variants, rs5418 (A/G) and rs2654185 (C/A), and association with HbA1c level was analyzed. The HbA1c value was determined by JDS method which is 0.4% lower than NGSP value. The G allele carrier of rs5418 and A allele carrier of rs2654185 associated with significantly higher HbA1c level (AG + GG vs. AA carriers; 5.2 ± 0.8 vs. 4.9 ± 0.4, $P < 0.002$, and AA + AC vs. CC; 5.2 ± 0.9, vs. 4.9 ± 0.4, $P < 0.002$, respectively). G allele, AG + GG genotype of rs5418 and A allele, AA + AC genotype of rs2654185 showed a significant association with higher HbA1c (β=0.215, P=0.026; β=0.215, P=0.026; β=0.190, P=0.042; β = 0.190, P=0.042, respectively). These two SNPs are in high linkage disequilibrium (LD) of r(2) = 0.67. In haplotype analysis, four haplotypes were estimated. HbA1c is significantly higher in the most frequent GA haplotype compared with the second frequent AC haplotype (5.2% vs. 5.1%, P = 0.004). Genetic variations, rs5418 and rs2654185 in GLUT4 gene are associated with HbA1c level in Japanese men.

4. Effects of COMT and MTHFR on normal variation of mental health in a Japanese population

Maintenance of mental health requires adequate prefrontal cortex (PFC) function. Optimal PFC dopamine level is important to attain its function while high or low levels have adverse effects. The "inverted U-shaped" PFC dopamine function is influenced by modulators of dopamine, such as catechol-O-methyltransferase (COMT) and methylenetetrahydrofolate reductase (MTHFR). We have investigated how functional COMT and MTHFR polymorphisms affect inter-individual mental health difference, and tested whether COMT haplotype analysis increases detection of the difference. The mental health status of general Japanese men was measured by Mental Health Inventory (MHI)-5 score. Either COMT Val158Met genotype or rs4633-rs4818-rs4680 haplotypes were used to rank the COMT activity, and association to their mental health status was analyzed together with the interaction of MTHFR C677T. We identified a "curvilinear" correlation between COMT activity–ranked diplotypes and MHI-5 scores within MTHFR-CC genotype ($P < 0.001$), but not in T-allele carriers. Within MTHFR-CC, the intermediate COMT diplotype group showed significantly higher score in mean MHI-5 than the extreme diplotype groups ($P < 0.001$). No association was observed for single Val158Met analysis. Our pilot study implies the distinctive influence of COMT and MTHFR genotypic combination on the normal variation of mental health status. Because there are diverse COMT haplotypic lineages among different ethnic populations, our data strengthen the advantage of haplotype-based analysis of COMT activity in the (specific) East Asian population. The use of the COMT haplotypes produces a better fit to a PFC dopamine function curve than that of the single Val158Met polymorphism.

Publications

1. Ikeda S, Tanaka N, Arai T, Chida K, Muramatsu M, Sawabe M. Polymorphisms of LTA, LGALS2, and PSMA6 genes and coronary atherosclerosis: a pathological study of 1503 consecutive autopsy cases. Atherosclerosis. 221:458-60 (2012)
2. Ko MK, Ikeda S, Mieno-Naka M, Arai T, Zaidi SA, Sato N, Muramatsu M, Sawabe M. J Atheroscler Thromb. Association of COMT gene polymorphisms with systemic atherosclerosis in elderly Japanese.19:552-8 (2012)
3. Xi C, Miyaki K, Ikeda S, Song Y, Sinbo T, Muramatsu M. Association of GLUT4 gene variants with HbA1c level in Japanese men. Endocr J.59:677-84 (2012)
4. Ishii T, Hagiwara K, Kamio K, Ikeda S, Arai T, Mieno MN, Kumasaka T, Muramatsu M, Sawabe M, Gemma A, Kida K. Involvement of surfactant protein D in emphysema revealed by genetic association study. Eur J Hum Genet.20:230-5 (2012)
5. Ishii T, Hagiwara K, Ikeda S, Arai T, Mieno MN, Kumasaka T, Muramatsu M, Sawabe M, Gemma A, Kida K. Association between genetic variations in surfactant protein d and emphysema, interstitial pneumonia, and lung cancer in a Japanese population. COPD 9:409-16 (2012)
6. Honma N, Yamamoto K, Ohnaka K, Morita M, Toyomura K, Kono S, Muramatsu M, Arai T, Ueki T, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Maekawa T, Yasunami Y, Takenaka K, Ichimiya H, Terasaka R. Estrogen receptor-β gene polymorphism and colorectal cancer risk: effect modified by body mass index and isoflavone intake. Int. J, Cancer Epub 2012 Jul 3.
7. Honma N, Mori S, Zhou H, Ikeda S, Mieno MN, Tanaka N, Takubo K, Arai T, Sawabe M, Muramatsu M, Ito H. Association between estrogen receptor-β dinucleotide repeat polymorphism and incidence of femoral fracture. J Bone Miner Metab.Epub 2012 Sep 5.

Department of Biochemical Genetics

Professor	Shigetaka Kitajima MD, PhD.
Associate Professor	Yujiro Tanaka MD, PhD.
Assistant Professor	Junya Kawauchi MD, PhD.

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.

Key words

・To provide novel paradigm of transcriptional regulation

・To understand the role of transcription factor in cell fate determination

Research 1 : Transcription

Transcription proceeds from initiation via elongation to termination, and eventually Pol II is recycled for the next rounds of transcription when gene expression is activated. Among many protein factors that regulate transcription cycle, TFIIF and Elongin A function during elongation phase and the dysregulation of Elongin A may cause to develop 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) has a dual function, one is activation of transcriptional elongation by RNA polymerase II (Pol II) and the other is the degradation of Rpb1, the largest subunit of Pol II. ChIP analysis revealed that Elongin A is recruited to the HSP70 and ATF3 gene over their promoter through 3'-downstream region, implicating that it travels with the transcribing Pol II. By contrast, Elongin A forms E3-ligase complex that targets Pol II causing the ubiquitin-mediated degradation upon DNA damage. These two properties seem to be opposite functions as a point of view to regulate the transcript amount. Elongin A may function as one of safety net mechanism in mammalian cells. In collaboration with Kochi University, we also showed Elongin A plays a crucial role in neuronal development.

1-2 A novel function of FCP1

FCP1 is the firstly identified Pol II CTD phosphatase, which is believed as a transcriptional recycling factor. We found that FCP1 physically associates with tumor suppressor p53 and is required for the p53 target gene expression in response to DNA damage. In order to uncover the biological function of FCP1, we are now investigating the functional interaction of FCP1 and p53. Eventually, we aim to find the strategy for the treatment of CCFDN.

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 the 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

Screening of ATF3 target gene(s) upon DNA damage as in 2-2 revealed death receptor gene DR5 as a candidate for the clinical application related to ATF3. 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 remarkably impaired apoptosis upon TRAIL/CPT. By demonstrating that ATF3 co-operates with p53 to induce the DR5 transcription on the chromatin, we provided novel paradigm of both hierarchical and horizontal role of p53-ATF3 axis in pro-apoptotic process by DNA damage or combination therapy of TRAIL/DNA damage. Furthermore, we are now revealing that ATF3 is also implicated in p53-independent cell death by other natural products such as zerumbone, celecoxib, and baicalein. The latter agents are expected as less toxic anti-cancer therapeutics.

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

ATF3 functions as both oncogene and tumor suppressor. For example, in prostate cancer and Hodgkin disease, ATF3 expression is positively correlated with cell proliferation and also enhanced metastasis. Conversely, ATF3 inhibits p53 degradation and stabilizes its expression level. As a first step, we performed genome-wide ChIP-chip and expression profile analysis of 1) cells after DNA-damage of human colorectal HCT116 cells, 2) human prostate cancer cells LNCap, 3) Hodgkin Reed-Sternberg cells. In HCT116 cells, ATF3 binds over 6,000 gene promoters upon DNA damage (MMS), while it binds to ~1,300 gene promoters in ATF3-expressing LNCap cells. We also performed the genome-wide expression study 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. Further, we generated genetically engineered mouse model of p53 and ATF3 gene knockout to unravel genetic codes of p53-ATF3 axis regulation. The genome-wide analysis of these mice is now revealing intriguing regulatory networks between these two transcription factors in cancer and stress response.

2-3 ATF3 complex; transcriptional repressor or activator

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

2-4 ATF3 transcriptionally regulates microRNA.

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

Research 3: H3K36-sepcific histone methyltransferase ASH1.

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

Publications

1. Yasukawa T, Bhatt S, Takeuchi T, Kawauchi J, Takahashi H, Tsutsui A, Muraoka T, Inoue M, Tsuda M, Kitajima S, Conaway RC, Conaway JW, Trainor PA, Aso T. Transcriptional Elongation Factor Elongin A Regulates Retinoic Acid-Induced Gene Expression during Neuronal Differentiation. *Cell Reports* 10.1016/j.celrep.2012.09.031

2. Kawauchi J, Kitajima S. "Mechanism of Transcriptional Termination" in Encyclopedia of Systems Biology chapter 1408 (W. Dubitzky, O. Wolkenhauer, K. Cho & H. Yokota (eds.), DOI 10.1007/978-1-4419-9863-7, *Springer Science+Business Media LLC*, 2012

3. Cabianca DS, Casa V, Bodega B, Carvalho C, Ginelli E, Tanaka Y, Carmo-Fonceca M, Gabellini D. A ncRNA regulating a Polycomb/Trithorax epigenetic switch in muscular dystrophy. *Cell* in press, 2012

4. Taketani K, Kawauchi J, Tanaka-Okamoto M, Ishizaki H, Tanaka Y, Sakai T, Miyoshi J, Maehara Y, Kitajima S. Key role of ATF3 in p53-dependent DR5 induction upon DNA damage of human colon cancer cells. Oncogene. 2012 Apr 26;31(17):2210-21

Department of Genomic Pathology

Professor	**Shumpei Ishikawa**
Assistant Professor	**Takayuki Isagawa**
Technical Assistant	**Reiko Sato**
Secretary	**Miharu Tamukai**

Research Field:

Department of Genomic Pathology started when Shumpei Ishikawa was appointed as professor on January 1, 2013. Reiko Sato, Miharu Tamukai has been adopted as a technical assistant and a secretary on January 16. Also by making a public offering, Takayuki Isagawa was chosen as assistant professor and, was adopted on February 16. We are planning to adopt one more assistant professor in April.

Location of the laboratory is the north side of the 24th floor of the M&D tower, and in fiscal 2012 laboratory start-up work was done, including the establishment and carrying research equipment and general office supplies, and the development of information infrastructure.

Genomic Pathology is intended to conduct a genomics-based approach, and research to help clarify the pathology of refractory disease and its diagnosis and treatment. A large amount of data measurement is performed comprehensively to reveal the dynamics & mechanism of a complex system that consists of a variety of cells, like inflammatory and neoplastic disease. Also through the analysis of the actual human disease specimens, we are willing to do infrastructure and technology development for the application of genomics in clinical practice.

We also participate in a meeting of Bio-Resource Center, TMDU project, and involved in development of infrastructure and making related documents.

Belongs to the Advanced Therapeutic Sciences of Graduate School of Medical and Dental Sciences from March 2013, we are responsible for graduate education, as “Disease Genomics” section. Goal of our program is to learn about the adaptation, techniques and interpretation of the disease genomics, to know about technology development, infrastructure and guidelines for human genome analysis, and, through the analysis of actual human disease specimens, to learn the applied aspect of genomic analysis,

Department of Epigenetics

Professor	**Fumitoshi ISHINO**
Associate Professor	**Takashi KOHDA**
GCOE lecturer	**Jiyoung LEE**
Adjunct lecturer	**Shin KOBAYASHI**
Assistant Professor	**Ryuichi ONO, Mie NARUSE**
Technical assistant	**Masayuki ISHII**

Introduction of Department of Epigenetics

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

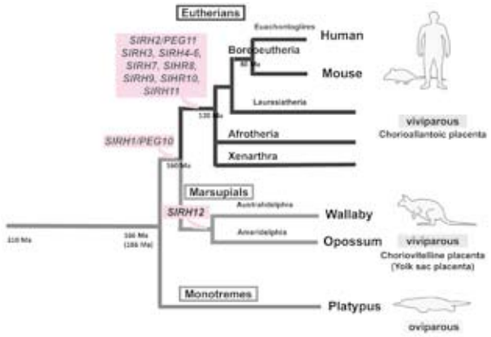
Latest researches

1. Analyses of Mammalian-Specific Genomic Functions

We have been focusing mammalian-specific genes and epigenetic mechanisms to elucidate mammalian-specific functions, such as viviparity and maternal nursing behavior. We previously demonstrated that mammalian-specific retrotransposon- derived genes, *Peg10* and *Peg11/Rtl1*, play essential roles in mammalian development via formation and maintenance of placentas that are unique to mammals. By collaboration with Prof. Kaneko-Ishino at Tokai University, analyses of other *SIRH* (sushi-ichi retrotransposon homologue) genes, such as *Sirh3* to *Sirh11*, are under investigation. Last year, we reported that another *SIRH12* gene specific to marsupial mammals. It is present in an Australian marsupial species, tammar wallaby. However, its orthologue in a South American marsupial species, grey short-tailed opossum, have degenerative protein coding frames, suggesting it is only functional in the tammar wallaby (Ono *et al*. DNA Res 2011). This year, we have expanded our research to another LTR retrotransposon-derived genes, *PNMA* (paraneoplastic Ma antigen) and confirmed that most eutherian species possess 19 *PNMA* genes including novel genes. Although no their homologues are present in marsupial species, we identified a marsupial-specific *PNMA-MS1* gene and an opossum-specific *PNMA-MS2* gene (Iwasaki *et al*. DNA Res 2013). Thus, it is clear that the eutherians and the marsupials have completely different sets of *SIRH* and *PNMA* genes during evolution, suggesting that these newly acquired genes contributed to diversification of these two viviparous mammalian groups.

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 reported that *Xist*, a noncoding RNA that inactivates one of the two X chromosomes in females, was ectopically expressed from the active X (Xa) chromosome in cloned mouse embryos of both sexes. Therefore, We showed that knockout or knockdown of *Xist* on Xa were very good method to improve global gene expression profiles resulting in about an eight- to ninefold increase in cloning efficiency. This year, we have analyze effect of an epigenetic agent, Trichostatin A, that also improve the efficiency of clone production. We demonstrated that the mouse pups treated with Trichostatin A for short time at early zygotic period display almost normal gene expression profiles. Thus, we believe that these improvements are consistent steps toward the practical use of the SCNT method in many domesticated animals.



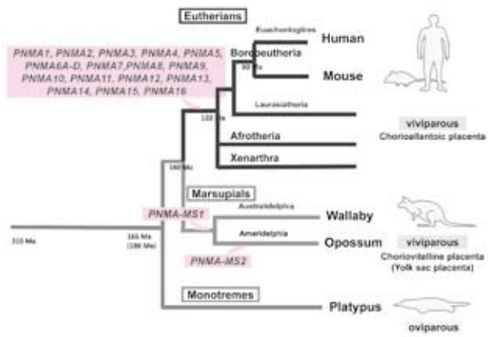


Fig.1. **LTR retrotransposon-derived genes in mammals**
A: *SIRH* family genes: Twelve *SIHR* family genes were domesticated in mammals. *PEG10/SIRH1* is conserved in both eutherians and marsupials while *PEG11/SIRH2* and *SIRH3-SIRH11* are present only in the eutherians. *SIRH12* is a domesticated gene derived from a marsupial-specific insertion event.
B: *PNMA* family genes: Nineteen *PNMA* family genes are present in most of the eutherian species but *PNMA6A-D* on X chromosome is lost in a rodent lineage. Interestingly, marsupials have different *PNMA* genes, *PNMA-MS1* and *PNMA-MS2*. Ma: million years ago.

Publications (Original papers)

1. Kohda, T., Kishigami, S., Kaneko-Ishino, T., Wakayama T. and Ishino F. Gene expression profile normalization in cloned mice by trichostatin A treatment. Cellular Reprogramming **14**(1), 45-55 (2012).
2. Kaneko-Ishino, T. and Ishino, F. The role of genes domesticated from LTR retrotransposons and retroviruses in mammals. Frontiers Microbiol **3**, Article 262 (1-10) (2012).
3. Kaneko-Ishino, T. and Ishino, F. Evolution of viviparity and genomic imprinting in mammals by retrotransposons. *In* Evolutionary Biology: Mechanisms and Trends (ed. Pontarotti, P.), Springer-Verlag Berlin Heidelberg, pp.265-282 (2012).
4. Ishino, F., Shinkai, Y. and Whitelaw, E. Mammalian epigenetics in biology and medicine. *In* Mammalian Epigenetics in Biology and Medicine (eds. Ishino, F., Shinkai, Y. and Whitelaw, E.), Royal Society Publishing, Philos Trans R Soc Lond B Biol Sci, 368(1609):20120386 (2012).
5. Kohda, T. and Ishino, F. Embryo manipulation via assisted reproductive technology and epigenetic asymmetry in mammalian early development. *In* Mammalian Epigenetics in Biology and Medicine (eds. Ishino, F., Shinkai, Y. and Whitelaw, E.), Royal Society Publishing, Philos Trans R Soc Lond B Biol Sci, 368(1609):20120353 (2012).
6. Oikawa, M., Matoba, S, Inoue, K., Kamimura, S., Hirose, M., Ogonuki, N., Shiura, H., Sugimoto, M., Abe, K., Ishino, F. and Ogura, A. Incomplete Rescue of the Impaired Development of Female Cloned Mouse Embryos by RNAi-mediated Knockdown of *Xist*. J Reprod Dev (in press).
7. Wakayama, S., Kohda, T., Obokata, H., Tokoro, M., Li, C., Terashita, Y., Mizutani, E., Nguyen, V. T., Kishigami, S., Ishino, F. and Wakayama, T. Successful serial recloning in the mouse over multiple generations. *Cell Stem Cell* (in press).

Presentation at International meetings

1. Fumitoshi Ishino, Ryuichi Ono, Shunsuke Suzuki, Yoichi Sekita, Mie Naruse, Masahito Irie, Masayuki Ishii, Sawa Iwasaki, Moe Kitazawa, Takashi Kohda and Tomoko Kaneko-Ishino. Role of mammalian-specific retrotransposon-derived genes in mammalian reproductive system, The 2nd SKLRB Symposia in Reproductive Biology, May 6-10, 2012, Beijing Friendship Hotel (Beijing, China).
2. Fumitoshi Ishino and Tomoko Kaneko-Ishino: Contribution of LTR retrotransposons to evolution of mammals: a novel view from comparative genomics, 11th Surugadai Symposium, July 31, 2012 (TMDU, Tokyo, Japan).
3. Fumitoshi Ishino: Functions of mammalian-specific *SIRH* family genes- LTR retrotransposon-derived genes in mammalian reproducion system-, Fujihara Seminar 2012, A New Horizon of Retroposon Research, July 31-August 3, 2012 (Shiran-Kaikan, Kyoto, Japan).
4. Fumitoshi Ishino: Evolution of Genomic Imprinting, Viviparity and Placentation in Mammals Suggest Critical Contribution of LTR-Retrotransposons and/or Exogenous DNAs, Quantitative Evolutionary and Comparative Genomics 2012, August 6-10, 2012 (Okinawa Inst Sci Tech, Okinawa, Japan).

Department of Bioinformatics

Professor Hiroshi Tanaka
Associate Professor Yoshihito Niimura
Assistant Professor Kaoru Mogushi

Research Subjects

Our mission is "system-level understanding of biological systems" in molecular biology and evolution (systems evolution) and medicine (omics-based medicine, systems pathology). Recently, the whole genome sequences of diverse organisms have become available. Moreover, various "omics" 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 omics information. Genomic and omics 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 omics-based medicine and systems pathology.

1. Analysis of disease mechanism using omics-based approaches

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 prognosis prediction in hepatocellular carcinoma patients, 2) development of predictive marker for metastatic relapse in colorectal cancer, and 3) analysis of spinocerebellar ataxia and hepatocellular carcinoma using next generation sequencing technologies.

2. Systems pathology analyses on disease progression of cancer, metastasis, and Alzheimer's disease

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

3. i2b2: A novel technology of clinical databases as an infrastructure of translational informatics

Translational informatics facilitates the computational technology for translation of genome information into the clinical application. It targets collection and computation of clinical and genomic information on the basis of mathematical models for diseases. Among the ongoing projects, the i2b2 provides an ontology-based object-oriented database system for integration of clinical information dispersed in different laboratories and different hospitals. We constructed i2b2 database with clinical and biomedical data of patients in the university hospital of TMDU (iCOD). We transferred 8,580 English and 54,579 Japanese descriptions into i2b2 by our NLP pipeline for extraction of clinical terms.

4. Analyses of the human protein-protein interaction networks and their applications to drug discovery

Since proteins exert their functions though interaction to other proteins, protein-protein interaction networks (PINs) are inevitable to discover novel drug-target genes. To discover novel targets, it is of use to understand topological characteristics of PINs, and how the targets are distributed over the PINs. To uncover the topological features of PINs, we used a novel method to decompose the genome-wide human PIN into simple sub-networks. A sub-network contains almost 60% of targets of small molecule drugs for cancerous diseases. Further, genes in the sub-network are involved in cancer-related signaling pathways. These results indicate that the listing of genes in the sub-network may help drug companies to search more efficiently for novel targets for cancerous diseases.

5. Diversity of olfactory receptor gene repertoires among mammals

Odor molecules in the environment are detected by olfactory receptors (ORs) encoded by a large multigene family. To investigate the diversity of OR gene repertoires among mammals, I extensively identified the OR genes from the draft genome sequences of 38 diverse mammals. The results demonstrated that the estimated numbers of functional OR genes are extremely variable, ranging from only ~10 in dolphins to ~2,000 in elephants. Identification of orthologous gene sets among 13 eutherian mammals with the genome of deep coverage (>6x) revealed that hundreds of gene gains and losses have occurred during eutherian evolution, suggesting dynamic changes of OR gene repertoires depending on each species' living environment.

Publications

[Original Articles]

1. Boeck M, Ogishima S, Tanaka H, Kramer S, Kaderali L: Hub-centered gene network reconstruction using automatic relevance determination. PLoS ONE, 7(5):e35077, 2012
2. Katayama Y, Maeda M, Miyaguchi K, Nemoto S, Yasen M, Tanaka S, Mizushima H, Fukuoka Y, Arii S, Tanaka H: Identification of pathogenesis-related microRNAs in hepatocellular carcinoma by expression profiling. ONCOLOGY LETTERS, 4:817-23, 2012
3. Khamas A, Ishikawa T, Mogushi K, Iida S, Ishiguro M, Tanaka H, Uetake H, Sugihara K: Genome-wide screening for methylation-silenced genes in colorectal cance. International Journal of Oncology, 41:490-6, 2012
4. Khamas A, Ishikawa T, Shimokawa K, Mogushi K, Iida S, Ishiguro M, Mizushima H, Tanaka H, Uetake H, Sugihara K: Screening for epigenetically masked genes in colorectal cancer using 5-Aza-2'-deoxycytidine, microarray and gene expression profile. Cancer Genomics & Proteomics, 9:67-75, 2012
5. Kikuchi A, Ishikawa T, Mogushi K, Ishiguro M, Iida S, Mizushima H, Uetake H, Tanaka H, Sugihara K: Identification of NUCKS1 as a colorectal cancer prognostic marker through integrated expression and copy number analysis. International Journal of Cancer, Doi:10.1002/ijc.27911, 2012
6. Mayinuer A, Yasen M, Mogushi K, Obulhasim G, Xieraili M, Aihara A, Tanaka S, Mizushima H, Tanaka H, Arii S: Upregulation of Protein Tyrosine Phosphatase type IVA member 3 (PTP4A3/PRL-3) associated with tumor differentiation and a poor prognosis in human hepatocellular carcinoma. Annals of Surgical Oncology, DOI 10.1245/s10434-012-2395-2, 2012
7. Miyaguchi K, Uzawa N, Mogushi K, Takahashi K.I, Michikawa C, Nakata Y, Sumino J, Okada N, Mizushima H, Fukuoka Y, Tanaka H: Loss of NKX3-1 as potential marker for an increased risk of occult lymph node metastasis and poor prognosis in oral squamous cell carcinoma. International Journal of Oncology, 40:1907-14, 2012
8. Mizuno S, Iijima R, Ogishima S, Kikuchi M, Matsuoka Y, Ghosh S, Miyamoto T, Miyashita A, Kuwano R, Tanaka H: AlzPathway: a comprehensive map of signaling pathways of Alzheimer's disease. BMC Syst Biol, 6:52, 2012
9. Nomura F, Sogawa K, Noda K, Seimiya M, Matsushita K, Miura T, Tomonaga T, Yoshitomi H, Imazeki F, Takizawa H, Mogushi K, Miyazaki M, Yokosuka O: Serum anti-Ku86 is a potential biomarker for early detection of hepatitis C virus-related hepatocellular carcinoma. Biochem Biophys Res Commun, 421:837-43, 2012
10. Nukaya S, Shino T, Kurihara Y, Watanabe K, Tanaka H: Noninvasive Bed Sensing of Human Biosignals via Piezoceramic Devices Sandwiched Between the Floor and Bed. IEEE SENSORS JOURNAL , 12:431-438, 2012
11. Obulhasim G., Yasen M, Kajino K, Mogushi K, Tanaka S, Mizushima H, Tanaka H, Arii S, Hino O: Up-regulation of dbpA mRNA in hepatocellular carcinoma associated with metabolic syndrome. Hepatology International, Doi: 10.1007/s12072-012-9357-4, 2012
12. Okazaki S, Ishikawa T, Iida S, Ishiguro M, Kobayashi H, Higuchi T, Enomoto M, Mogushi K, Mizushima H, Tanaka H, Uetake H, Sugihara K: Clinical significance of UNC5B expression in colorectal cancer. International Journal of Oncology, 40:209-16, 2012
13. Shimada S, Mimata A, Sekine M, Mogushi K, Akiyama Y, Fukamachi H, Jonkers J, Tanaka H, Eishi Y, Yuasa Y: Synergistic tumour suppressor activity of E-cadherin and p53 in a conditional mouse model for metastatic diffuse-type gastric cancer, Gut, 61:344-53, 2012
14. Sekiya I, Ojima M, Sizuki S, Yamaga M, Horie M, Koga H, Tsuji K, Miyaguchi K, Ogishima S, Tanaka H, Muneta T: Human mesenchymal stem cells in synovial fluid increase in the knee with degenerated cartilage and osteoarthritis, Journal of Orthopaedic Research, 30:943-9, 2012
15. Suzuki S, Takai-Igarashi T, Fukuoka Y, Wall D.P, Tanaka H, Tonellato P.J: Systems analysis of inflammatory bowel disease based on comprehensive gene information. BMC Medical Genetics, Apr 5; 13:25, 2012
16. Xieraili M, Yasen M, Mogushi K, Obulhasim G, Mayinuer A, Aihara A, Tanaka S, Mizushima H, Tanaka H, Arii S: Villin 1 is a predictive factor for the recurrence of high serum alpha-fetoprotein-associated hepatocellular carcinoma after hepatectomy, Cancer Science, 103:1493-501, 2012
17. Yae T, Tsuchihashi K, Ishimoto T, Motohara T, Yoshikawa M, Yoshida GJ, Wada T, Masuko T, Mogushi K, Tanaka H, Osawa T, Kanki Y, Minami T, Aburatani H, Ohmura M, Kubo A, Suematsu M, Takahashi K, Saya H, Nagano O: Alternative splicing of CD44 mRNA by ESRP1 enhances lung colonization of metastatic cancer cell, Nature Communications, 6:883, 2012
18. Yasen M, Obulhasim G., Kajino K, Mogushi K, Mizushima H, Tanaka S, Tanaka H, Hino O, Arii S: DNA binding protein A expression and methylation status in hepatocellular carcinoma and the adjacent tissue, International Journal of Oncology, 40:789-97, 2012

[Reviews]

1. Niimura Y: Olfactory receptor multigene family in vertebrates: from the viewpoint of evolutionary genomics. Current Genomics 13, 103-111, 2012

[Book chapters]

1. Niimura Y: Evolution of chemosensory receptor genes in primates and other mammals. Post-Genome Biology of Primates, Primatology Monographs (eds. Hirai H, Imai H, Go Y), Springer, 2012
2. Hase T, Niimura Y: Protein-protein interaction networks: Structures, evolution, and application to drug design. Protein Interaction / Book 2 (ed. Weibo Cai), InTech, 2012

Frontier Research Unit
Redox Response Cell Biology

Associate Professor **Shun-Ichi Kurata**

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

1. p63 enhances Wnt target gene expression by nuclear complex formation with beta-catenin and TCF.

P63 is expressed in keratinocyte stem cells and squamous cell carcinomas (SCC) to control cell growth and differentiation. Multiple cellular regulatory pathways may be influenced by p63. Despite an earlier report that p63 activates Wnt signaling through protein phosphatase 2A (PP2A) and GSK-3b (Patturajan, A Cancer Cell 1:369, 2002), a recent study indicated a direct interaction of DNp63a with TCF/LEF/b-catenin for suppression (Drewelus I, Cell Cycle 9:580, 2010). Our experiments of gene expression profiling in p63-sknockdown SCC indicated that p63 activated Wnt target genes, SNAI2, CCND2, and DKK3, with strong affinity Wnt response elements (WRE, WTYYYCTTTGATSTT) (Atcha FA, Mol Cell Biol 27:8352, 2007), but not MMP7 with weak WREs. Only the DNp63a isoform significantly enhanced reporter gene expression with WREs. Although DNp63a was co-precipitated with PP2A, neither the phosphatase activity nor the nuclear localization of GSK-3b and b-catenin was altered. TCF-4 was associated with DNp63a and b-catenin. Taken together, DNp63a enhances Wnt target gene expression by nuclear complex formation with b-catenin and TCF to control cancer progression.

2. Endotoxin-induced metabolic acidosis and lung inflammation

Acute respiratory distress syndrome (ARDS) is a severe form of lung injury and inflammation that frequently occurs during pneumonia and sepsis. In septic lung injury, acute lung inflammation may have deleterious effects on remote organs such as the kidney. The nuclear enzyme poly (adenosine diphosphate-ribose) polymerase (PARP) enhances the nuclear factor (NF)-κB-dependent transcription of inflammatory cytokines. The objective of this study was to elucidate whether a PARP inhibitor, 3-aminobenzamide (3-AB), could attenuate lipopolysaccharide (LPS)-induced inflammation of the lung and kidney in rats. *Methods*: Male Sprague-Dawley rats were anesthetized and ventilated. The rats were divided into three groups; 1) a control group (n=8), 2) an LPS group (n=12) intratracheally instilled with LPS (16mg/kg), and 3) an LPS+3-AB group (n=12) given the same dose of LPS by the same method followed by an injection of 3-AB (10 mg/kg). Hemodynamics, arterial blood gas, and plasma lactate levels were measured at 0,1,2,3, and 4h. The mRNA expression of TNF-α, interleukin (IL)-1β and IL-6 in the lung and kidney were measured. NF-κB in the lung and kidney was histologically examined. *Results*: LPS induced metabolic acidosis, hypotension, and increases in the mRNA expression of IL-1β and IL-6 in the lung and IL-1β in the kidney. These actions were associated with a high NF-κB expression score in the lung. Treatment with 3-AB prevented LPS-induced metabolic acidosis and mRNA expression of IL-1β and IL-6 in the lung. *Conclusion*: The PARP inhibitor prevented LPS-induced metabolic acidosis and lung inflammation, possibly via an inhibition of NF-κB dependent proinflammatory cytokines.

Project Research Unit
Affiliated Institutes

Project Research Unit

Project Research Unit (Advanced Molecular Medicine)

Associate Professor　Takayoshi Suganami M.D., Ph.D.

Research project: role of chronic inflammation in the metabolic syndrome

Obesity may be viewed as a state of chronic, low-grade inflammation, which plays an important role in the development of the metabolic syndrome. Indeed, unbalanced production of pro- and anti-inflammatory adipocytokines observed in visceral fat obesity plays a critical role in the pathophysiology of the metabolic syndrome. Recent evidence has revealed that adipose tissue macrophages may participate in obesity-induced chronic inflammation, thereby contributing to the pathogenesis of metabolic derangements of multiple organs. We have provided evidence that a paracrine loop involving saturated fatty acids (FAs) and tumor necrosis factor-alpha derived from adipocytes and macrophages, respectively, aggravates obesity-induced adipose tissue inflammation. Saturated FAs, which are released from hypertrophied adipocytes via the macrophage-induced lipolysis, activate macrophages through Toll-like receptor 4 (TLR4). We also identified novel regulators of adipose tissue inflammation such as activating transcription factor 3 (ATF3) and macrophage-inducible C type lectin (Mincle). Understanding the molecular mechanism of chronic inflammation in obese adipose tissue would lead to the identification of novel therapeutic strategies to prevent or treat the metabolic syndrome.

Publications

1. Watanabe Y, Nakamura T, Ishikawa S, Fujisaka S, Usui I, Tsuneyama K, Ichihara Y, Wada T, Hirata Y, Suganami T, Izaki H, Akira S, Miyake K, Kanayama HO, Shimabukuro M, Sata M, Sasaoka T, Ogawa Y, Tobe K, Takatsu K, Nagai Y. The Radioprotective 105/MD-1 complex contributes to diet-induced obesity and adipose tissue inflammation. **Diabetes** 61: 1199-1209, 2012
2. Ehara T, Kamei Y, Takahashi M, Yuan X, Kanai S, Tamura E, Tanaka M, Yamazaki T, Miura S, Ezaki O, Suganami T, Okano M, Ogawa Y. Role of DNA methylation in the regulation of lipogenic glycerol-3-phosphate acyltransferase 1 gene expression in the mouse neonatal liver. **Diabetes** 61: 2442-2450, 2012
3. Satoh-Asahara N, Shimatsu A, Sasaki Y, Nakaoka H, Himeno A, Tochiya M, Kono S, Takaya T, Ono K, Wada H, Suganami T, Hasegawa K, Ogawa Y. Highly purified eicosapentaenoic acid increases interleukin-10 levels of peripheral blood monocytes in obese patients with dyslipidemia. **Diabetes Care** 35: 2631-2639, 2012

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) fatty liver; 6) aquaporin-2 trafficking.

Publications

1. Takumi Irie, Koji Ito, Hisashi Ozasa, Yumi Noda, Satoru Ikeda, Shinji Tanaka, Shigeki Arii, Saburo Horikawa. Splenic artery ligation: A protection against hepatic ischemia/reperfusion injury in partially hepatectomized rats. Hepatology Research 2012; 42(8): 819-827.

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.

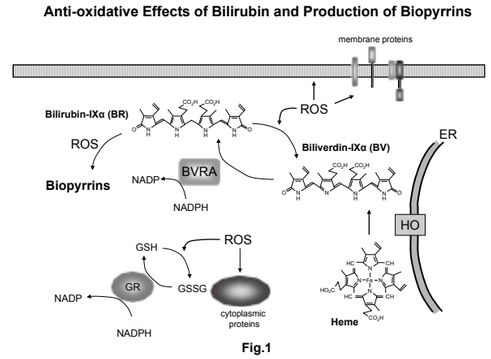


Fig.1

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. Determination of the epitope of anti-bilirubin monoclonal antibody 24G7 by kinetic analysis. Takuya Iwabuchi, Makoto Suematsu, Akiko Sugimoto, Tokio Yamaguchi. In submission (**Biochem Biophys Res Commun**)
2. 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.
3. 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.
4. 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.

Medical Genomics

Associate Professor　Michinori Kubota

Auditory induction is a continuity illusion in which missing sounds are perceived. To elucidate the neural mechanisms underlying auditory induction, neural responses were examined in the auditory cortex using real time optical imaging with a voltage-sensitive dye. Tone stimuli interrupted by broad-band noise, considered to cause auditory induction, considerably reduced responses to the tone following the noise. This reduction was stronger in field DC and belt fields compared to AI. Tone stimuli interrupted by notched noise, considered to decrease strength of auditory induction, partially restored the second responses. The results indicate that field DC is the first area in which these changes emerge, suggesting that it may be an important region for auditory induction of simple sounds.

Publications

Kubota M, Miyamoto A, Hosokawa Y, Sugimoto S, Horikawa J. Spatiotemporal dynamics of neural activity related to auditory induction in the core and belt fields of guinea pig auditory cortex. Neuroreport. 23(8): 474-478 (2012).
Ojima H, Taira M, Kubota M, Horikawa J. Recognition of Non-Harmonic Natural Sounds by Small Mammals Using Competitive Training. PLoS ONE 7(12): e51318 (2012).

Laboratory of Gene Expression

Associate Professor　Hidehito KUROYANAGI
Project Assistant Professor　Mariko KIMURA

Scope: We have been working to decipher "splicing codes" of precursor messenger RNAs (pre-mRNAs) from protein-coding genes. We have developed a transgenic fluorescence alternative splicing reporter system that visualizes alternative splicing patterns at a single cell resolution in living organisms. With the reporter system, we have elucidated expression profiles and regulation mechanisms of alternative splicing *in vivo*.

Publications

1. Kuroyanagi H, Watanabe Y, Hagiwara M. (2013) PLoS Genetics. 9: e1003337.
2. Kuroyanagi H, Watanabe Y, Suzuki Y, Hagiwara M. (2013) Nucleic Acids Research. 41: 4015-4025.
3. Ohno G, Ono K, Togo M, Watanabe Y, Ono S, Hagiwara M, Kuroyanagi H. (2012) PLoS Genetics. 8: e1002991.

Medical Genomics
Project Research Unit

Assistant Professor　Shuji Sassa

It participated in two students to do the graduation research from the Department of Clinical Laboratory Medicine, Faculty of Health Science Technology, Bunkyo Gakuin University. Thymidylate synthase is known to catalyze the methylation of deoxyuridine monophosphate in the *de novo* synthesis of deoxythymidine monophosphate, and thymidine kinase catalyzes the phosphorylation of thymidine in the salvage synthesis of deoxythymidine monophosphate *via* the pyrimidine pathway. In the present study, we investigated thymidylate synthase and thymidine kinase activities in human breast cancer, and relationship between the enzyme activities and the histopathological features of mammary carcinomas, as well as the clinical classification of patients. Thymidylate synthase and thymidine kinase activities of mammary carcinomas showed a roughly positive correlation, were found to be elevated with increasing cellular dedifferentiation, and rose with worsening of the clinicopathological stage and malignant invasion. Hence, the clinicopathological stage, as well as the invasiveness of the tumor, may depend on the *de novo* synthesis of DNA within human mammary carcinomas.

Publications

1. Sassa S, Suzuki S, Kudo H, Okabe H, Kikuchi H, Sakamoto S. DNA-synthesizing Enzyme Activities in Human Mammary Tumors can Predict Their Prognosis. Bunkyo J Health Sci Technol 4, 1-6, 2011
2. Sassa S, Kikuchi H, Okabe H, Suzuki S, Kudo H, Sakamoto S. Effect of Ipriflavone on Mammary Carcinogenesis, Uterine Adenomyosis and Bone Mineral Density of Tibia Mice. Bunkyo J Health Sci Technol 5, 51-56, 2012
3. Kudo H, Suzuki S, Okabe H, Kikuchi H, Sassa S, Sakamoto S. Gender Difference in Bone Loss Induced by Iron Overload in Rats. Bunkyo J Health Sci Technol 5, 57-65, 2012

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 2012.

1. Sequencing analyses

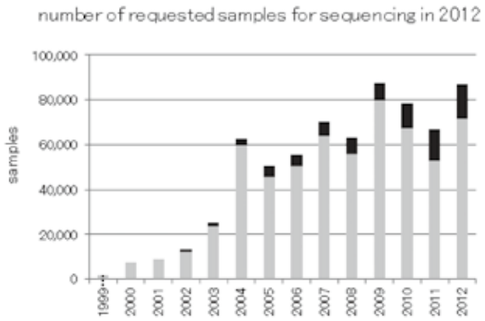
A total of 87,607 samples from 3,859 researchers were sequenced in the year of 2012. Among them 15,377 (17%) samples were requested by researchers outside the medical Research Institute (see below).

2. Equipment under the management of the Genome Laboratory.

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

3. Introductory seminars

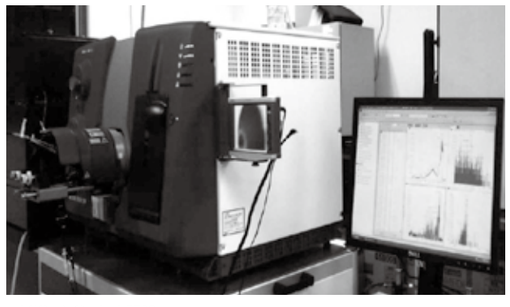
Introductory seminars were done for next generation sequencing method (3 times) and for manipulation of flow cytometer (1 time).



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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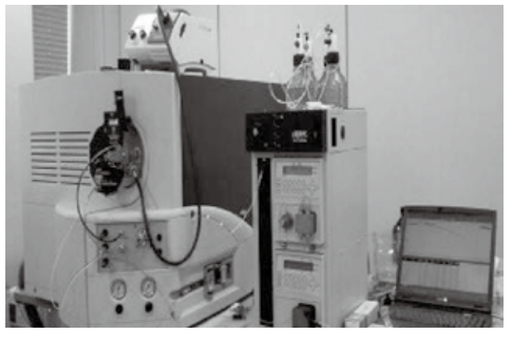
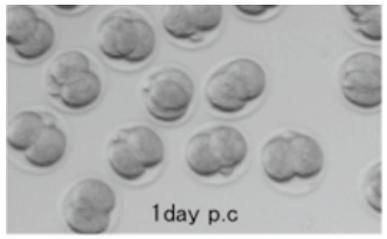


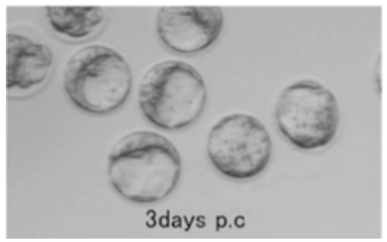
Q-Tofmicro

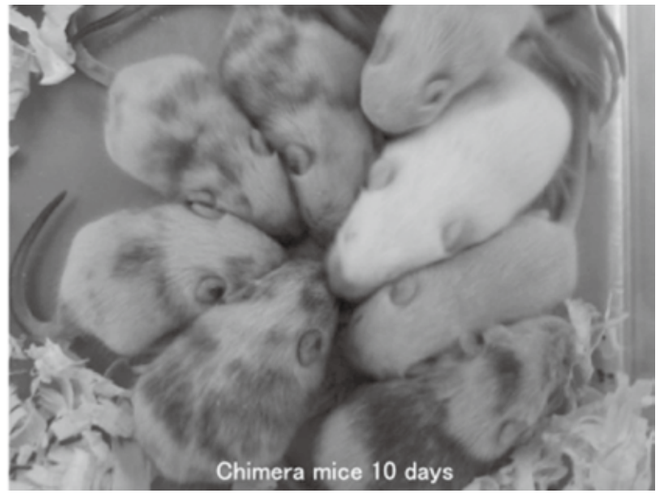
Laboratory of Recombinant Animals

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. This laboratory also supports the practical course on Genetic Engineering in Biomedical Science PhD Program.







Laboratory of Anatomy and Cell Function

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

<Common equipment>

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

Laboratory for Structure Analysis

The Laboratory for Structure Analysis is a small member of the Facilities and equipped with a high-brilliancy X-ray generator and an image plate X-ray detector. The Laboratory also has a dynamic light scattering (DLS) instrument, 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. The laboratory joined the Joint Usage/Research Program of the Institute this year and is now open for users from the outside of the university.

Stem Cell Laboratory

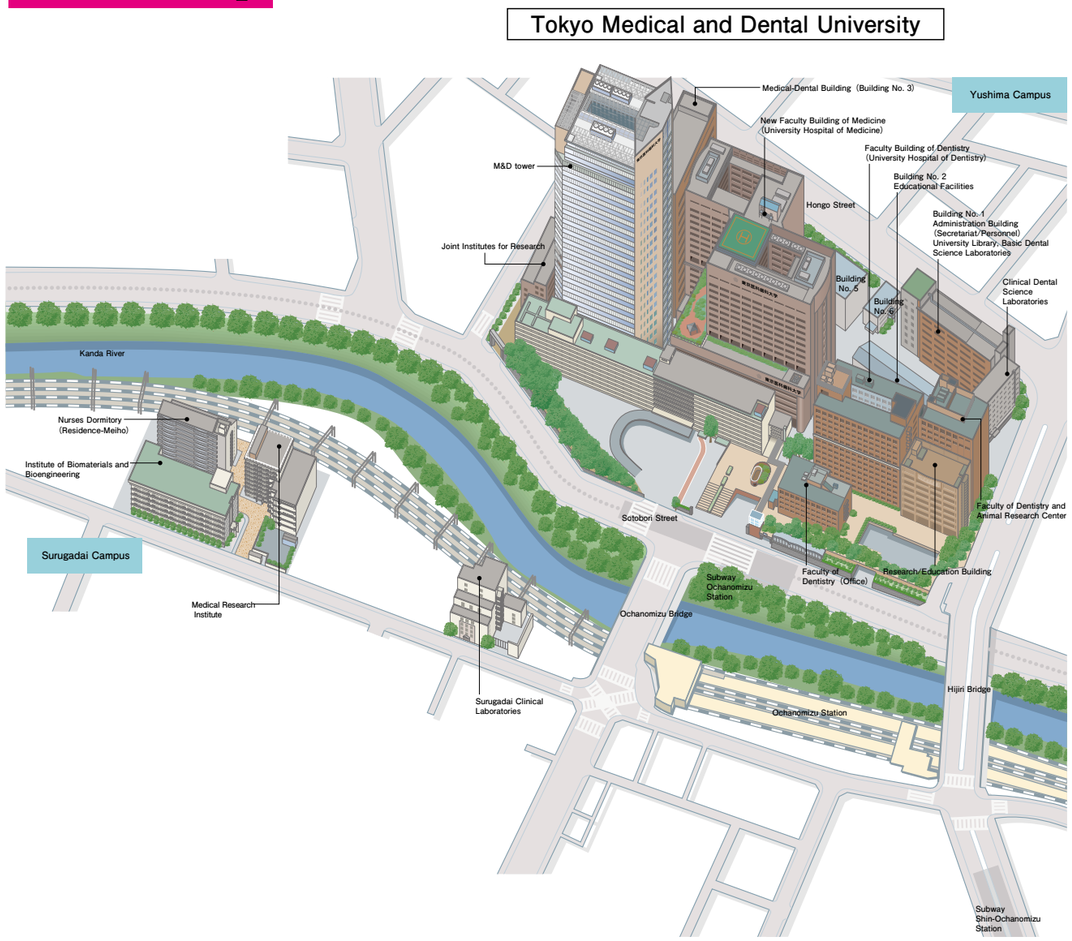
In order to assist researchers working on stem cell-related subjects across Medical Research Institute, Stem Cell Laboratory was established as of December, 2009, and thereafter expanded in the year of 2010 and 2011. This Laboratory used to have two rooms in Surugadai Area (1st floor) and M&D Tower (21st floor) until early March 2012. Equipments in these two rooms were moved to a new room on the 24th floor in M&D Tower, and were announced to be available again in March 15, 2012. Now the room is equipped with basic and state-of-the-art research facilities. For instance, we have high-speed cell sorters (MoFlo Legacy and MoFlo XDP), time-lapse confocal laser scanning microscope, sonicator, and hybridization oven.

This Laboratory is managed by the Operating Committee composed of five Professors and three Associate Professors in the Institute, and the services are provided by one Technical Staff who was stationed on April 1, 2010. A technical assistant was appointed to this Laboratory as of August 1, 2012. The Operating Committee was held 10 times in 2012 to discuss the way to smoothly set up the Laboratory and efficiently provide the services. In 2012, as part of activities of this Laboratory, we held technical courses for the equipment: e.g. once for MoFlo XDP, and three times for time-lapse confocal laser scanning microscope.

Advisory Committee Members

GO Mitiko	External Executive Director Research Organization of Information and Systems
GOJOBORI Takashi	Director and Professor Center for Information Biology and DNA Data Bank of Japan (DDBJ) National Institute of Genetics
KANAZAWA Ichiro	Professor International University of Health and Welfare
MURAMATSU Masami	The Former Director and Professor Research Center for Genome Medicine Saitama Medical University
NAGANO Tetsuo	Professor Graduate School of Pharmaceutical Sciences The University of Tokyo
NAKAJIMA Terumi	The Former President Hoshi University
SASAZUKI Takehiko	Professor Emeritus Kyushu University
TANIGUCHI Masaru	Director RIKEN, Research Center for Allergy and Immunology

Access Map



Nearest Stations

JR Chuo line : Ochanomizu station
JR Sobu line : Ochanomizu station
Marunouchi line : Ochanomizu station
Chiyoda line : Shin-ochanomizu station

ANNUAL REPORT 2013

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