The 11th Nikko International Symposium 2014

Inflammation, Cancer and Microenvironment

Opening Remark

Ryozo Nagai
President,Jichi Medical University

Introduction

Yusuke Furukawa
Director, Center for Molecular Medicine, Jichi Medical University

Speakers

Kazuhiro Nakayama, Jichi Medical University
Asuka Sakata, Jichi Medical University
Yasushi Saga, Jichi Medical University
Jiro Kikuchi, Jichi Medical University
Yoshiyuki Morishita, Jichi Medical University
Takanori Komada, Jichi Medical University

Yoshiro Maru, Tokyo Women's Medical University
Kensuke Miyake, Institute of Medical Science, University of Tokyo
Osamu Nureki, University of Tokyo

Orson Moe, University of Texas
James D. Griffin, Harvard Medical School

Closing Remark

Seiji Minota
Vice President, Jichi Medical University

Date: Thursday October 30, 2014
Venue: JMU Information and In-service Training Center

Abstract Book

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Sponsored by **Center for Molecular Medicine, Jichi Medical University**

The 11th Nikko International Symposium 2014
Inflammation, Cancer and Microenvironment

Date: Thursday October 30, 2014

Venue: JMU Information and In-service Training Center

10:00～10:05　Opening Remark　Ryozo　Nagai, Jichi Medical University

10:05～10:10　Introduction　　Yusuke Furukawa, Jichi Medical University

First Session: Therapeutic targeting of microenvironment

Chairpersons: Satoshi Nishimura, Kiyoshi Kawakami

10:10～10:25　Kazuhiro Nakayama, Jichi Medical University

10:25～10:40　Asuka Sakata, Jichi Medical University

Chairpersons: Tatsushi Onaka, Hiroaki Mizukami

10:40～10:55　Yasushi Saga, Jichi Medical University

10:55～11:10　Jiro Kikuchi, Jichi Medical University

Chairpersons: Yutaka Hanazono, Hironori Yamamoto

11:10～11:55　Yoshiro Maru, Tokyo Women's Medical University

12:00～13:00　Lunch Time

Second Session: Inflammation and disease

Chairpersons: Masafumi Takahashi, Makoto Kuro-o

13:00～13:15　Yoshiyuki Morishita, Jichi Medical University

13:15～14:00　Kensuke Miyake, Institute of Medical Science, University of Tokyo

14:00～14:15　Takanori Komada, Jichi Medical University

14:15～15:00　Orson Moe, University of Texas

15:00～15:15　Photo Session and Coffee Break

Third Session: Cancer treatment based on molecular and structural biology

Chairpersons: Naoya Shibayama, Yusuke Furukawa

15:15～16:00　Osamu Nureki, University of Tokyo

16:00～16:45　James D. Griffin, Harvard Medical School

16:45～16:50　Closing Remark　Seiji Minota, Jichi Medical University

17:00～　　Buffet-style dinner party

LIST OF SPEAKERS

Invited Speakers

Orson Moe

James D. Griffin

Yoshiro Maru

Kensuke Miyake

Osamu Nureki

Speakers of Jichi Medical University

Kazuhiro Nakayama

Asuka Sakata

Yasushi Saga

Jiro Kikuchi

Yoshiyuki Morishita

Takanori Komada

CURRICULA VITAE
&
ABSTRACTS

CURRICULUM VITAE

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Professional Experience

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Education

1995-1999 B. Eng., Tokai University
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2001-2004 Ph.D., Graduate School of Science, The University of Tokyo

Recent Publications

1. Ishizuka Y, Nakayama K, et al. TRIB1 downregulates hepatic lipogenesis and glycolysis via multiple molecular interactions. J Mol Endocrinol. 52:145-158. 2014
2. Nakayama K et al. Positive natural selection of TRIB2, a novel gene that influences visceral fat accumulation, in East Asia. Hum Genet. 132:201-217. 2013
3. Naka I, Hikami K, Nakayama K et al. A functional SNP upstream of the beta-2 adrenergic receptor gene (ADRB2) is associated with obesity in Oceanic populations. Int J Obes 37 1204-1210, 2013
4. Nakayama K et al. Seasonal effects of UCP1 gene polymorphism on visceral fat accumulation in Japanese adults. PLOS ONE 8:e74720, 2013
5. Nakayama K et al. High prevalence of an anti-hypertriglyceridemic variant of the MLXIPL gene in Central Asia. J Hum Genet. 56:828-833. 2011

Genetic variation of the Tribbles family and metabolic syndrome

Kazuhiro Nakayama1, Hiroshi Miyashita2, Yasuo Kagawa3, and Sadahiko Iwamoto1
1Division of Human Genetics, Jichi Medical University, 2Jichi Medical University Health Care Center, 3Kagawa Nutrition University

Tribbles pseudokinase family (TRIB) consists of three paralogous proteins (TRIB1~3) that participated in the regulation of various intra-cellular signaling pathways and transcription. Our large scale genetic association studies showed that single nucleotide polymorphism (SNP) s near the gene encoding TRIB1 strongly influenced plasma triglycerides, low density lipoprotein-cholesterol levels, and susceptibility to non-alcohol fatty liver disease in Japanese. Further molecular genetic analyses revealed that TRIB1 regulates hepatic lipogenesis and glycogenesis by multiple molecular interactions. We also discovered that a functional SNP in 3' untranslated region of the TRIB2 gene was associated with visceral fat area in Japanese. Further studies of TRIBs and the interacting partner molecules will shed light on the mechanisms of lipid metabolism dysregulation in multiple organs/tissues.

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Professional Experience

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Education

2000-2006 M.D., Akita University
2009-2014 Ph.D., Jichi Medical University

Recent Publications

1. Sakata A, Ohmori T, Nishimura S, Suzuki H, Madoiwa S, Mimuro J, Kario K, SakataY. Paxillin is an intrinsic negative regulator of platelet activation in mice. *Thromb J*. 2014 Jan 2;12(1):1. [Epub ahead of print]
2. Koyama K, Madoiwa S, Tanaka S, Koinuma T, Wada M, Sakata A, Ohmori T,Mimuro J, Nunomiya S, Sakata Y. Evaluation of hemostatic biomarker abnormalities that precede platelet count decline in critically ill patients with sepsis.
J Crit Care. 2013 Oct;28(5):556-63.
3. Mimuro J, Mizukami H, Hishikawa S, Ikemoto T, Ishiwata A, Sakata A, Ohmori T, Madoiwa S, Ono F, Ozawa K, Sakata Y. Minimizing the inhibitory effect of neutralizing antibody for efficient gene expression in the liver with adeno-associated virus 8 vectors. *Mol Ther*. 2013 Feb;21(2):318-23.
4. Kashiwakura Y, Mimuro J, Onishi A, Iwamoto M, Madoiwa S, Fuchimoto D, Suzuki S, Suzuki M, Sembon S, Ishiwata A, Yasumoto A, Sakata A, Ohmori T, Hashimoto M, Yazaki S, Sakata Y. Porcine model of hemophilia A. *PLoS One*. 2012;7(11):e49450.
5. Kashiwakura Y, Ohmori T, Mimuro J, Yasumoto A, Ishiwata A, Sakata A, Madoiwa S, Inoue M, Hasegawa M, Ozawa K, Sakata Y. Intra-articular injection of mesenchymal stem cells expressing coagulation factor ameliorates hemophilic arthropathy in factor VIII-deficient mice. *J Thromb Haemost*. 2012 Sep;10(9):1802-13.

Establishment of new evaluation methods for treatments of thrombosis with in vivo animal models.

Asuka Sakata, Tsukasa Ohmori, Satoshi Nishimura
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Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. Experimental models of thrombosis that mimic human vascular disease are essential to unravel the complex factors involved in pathologic thrombus formation. All reported ones, however, have both advantages and limitations. Using a confocal laser-scanning microscope, we developed “live thrombus imaging” technique that enables us to observe real time thrombus formation in detail. In our technique, thrombus is triggered by inducing reactive oxygen species production with a laser or by direct laser injury of endothelial cells. This is the first time thrombi have been visualized with sufficient resolution to identify individual platelets. We also use venous thrombus models to study factors involved in thrombus growth, stabilization, lysis, and effectiveness of anticoagulants. Venous thrombus is triggered by ligation of inferior vena cava. Using this venous thrombus model, we clarify that efficacy of NOACs for treating venous thrombosis is provided by secondary fibrinolysis. We now plan to image fibrinolysis using confocal laser-scanning microscope.

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Education

1987-1993 M.D., Jichi Medical University
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Recent Publications

1. Machida, S., Takei, Y., Yoshida, C., Takahashi, Y., Koyanagi, T., Sato, N., Taneichi, A., Saga, Y., Fujiwara, H., Suzuki, M.: Radiation therapy for chemotherapy-resistant recurrent epithelial ovarian cancer. Oncology. 86(4):232-238, 2014
2. Takahashi, K., Mizukami, H., Saga, Y., Takei, Y., Urabe, M., Kume, A., Machida, S., Fujiwara, H., Suzuki, M., Ozawa, K.: Suppression of lymph node and lung metastases of endometrial cancer by muscle-mediated expression of soluble vascular endothelial growth factor receptor-3. Cancer Sci. 104(8):1107-1111, 2013.
3. Fujiwara, H., Takei, Y., Ishikawa, Y., Saga, Y., Machida, S., Taneichi, A., Suzuki, M.: Community-based interventions to improve HPV vaccination coverage among 13- to 15-year-old females: measures implemented by local governments in Japan. PLoS One. 8(12):e84126, 2013.
4. Koyanagi, T., Suzuki, Y., Saga, Y., Machida, S., Takei, Y., Fujiwara, H., Suzuki, M., Sato, Y.: In vivo delivery of siRNA targeting vasohibin-2 decreases tumor angiogenesis and suppresses tumor growth in ovarian cancer. Cancer Sci. 104(12):1705-1710, 2013.
5. Uchibori, R., Tsukahara, T., Mizuguchi, H., Saga, Y., Urabe, M., Mizukami, H., Kume, A., Ozawa, K.: NF-κB activity regulates mesenchymal stem cell accumulation at tumor sites. Cancer Res. 73(1):364-372, 2013.

Robust treatment for cervical cancer targeting human papillomavirus E6/E7

Yasushi Saga1,2, Naoto Sato1,2, Hiroaki Mizukami2, Ryosuke Uchibori2, Tomonori Tsukahara2, Masashi Urabe2, Akihiro Kume2, Mitsuaki Suzuki1, Keiya Ozawa2,3

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Human papillomavirus (HPV) is the major causative agent of cervical cancer. The HPV oncoproteins E6 and E7 induce carcinogenesis by inactivating host tumor suppressor genes. Therefore, stable expression of specific inhibitors of E6 and E7 in cancer cells could provide effective treatment without affecting normal tissue. Here, we propose a novel therapeutic approach that uses an adeno-associated virus (AAV) vector encoding a short-hairpin (sh) RNA to target E6 and E7 (shE6E7) of HPV type 16 (HPV-16). Three different HPV-16-positive cervical cancer cell lines (BOKU, SiHa, and SKG-IIIa) were tested for gene transfer efficiency using AAV vectors of different serotypes. In all three cervical cancer cell lines, the highest gene transfer efficiency was obtained using AAV2 vectors. The proportions of GFP-positive cells at the dose of 1 × 105 vg/cell were 87.3%, 98.3%, and 87.9%, respectively. Transduction of cervical cancer cells by AAV2-shE6E7, an shRNA-encoding AAV vector that targets both E6 and E7, resulted in apoptosis, G1 arrest, and cell growth inhibition. In transduced cells, E6, E7, and p16 expression was reduced, whereas p53, p21, and pRb expression was enhanced. Next, AAV2-shE6E7 was directly injected into cervical cancer cell-derived subcutaneous tumors in mice. Tumor growth was markedly inhibited by a single AAV2-shE6E7 administration, and most tumors showed complete regression without any adverse effects. We are currently extending this strategy to target other HPVs, especially, type 18. These results suggest the utility of AAV2-shE6E7 as a novel therapeutic approach for cervical cancer.

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Professional Experience

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2011-present	Associate Professor, Division of Stem Cell Regulation, Center for Molecular Medicine, Jichi Medical University, Japan

Education

1985-1989	BSc., Science University of Tokyo
1993-2001	Ph.D., Jichi Medical School

Recent Publications

1. Koyama D, Kikuchi J, Hiraoka N, Wada T, Kurosawa H, Chiba S, and Furukawa Y. Proteasome inhibitors exert cytotoxicity and increase chemosensitivity via transcriptional repression of Notch1 in T-cell acute lymphoblastic leukemia. Leukemia. 28, 1216-1226, 2014.
2. Kikuchi J, Koyama D, Mukai H, and Furukawa Y. Suitable drug combination with bortezomib for multiple myeloma under stroma-free conditions and in contact with fibronectin or bone marrow stromal cells.
Int J Hematol. 99, 726-736, 2014.
3. Kikuchi J, Yamada S, Koyama D, Wada T, Nobuyoshi M, Izumi T, Akutsu M, Kano Y, and Furukawa Y. The novel orally active proteasome inhibitor K-7174 exerts anti-myeloma activity *in vitro* and *in vivo* by down-regulating the expression of class I histone deacetylases.
J Biol Chem, 288, 25593-25602, 2013
4. Kikuchi J, Wada T, Shimizu R, Izumi T, Akutsu M, Mitsunaga K, Noborio-Hatano K, Nobuyoshi M, Ozawa K, Kano Y, and Furukawa Y. Histone deacetylase is a critical target of bortezomib-induced cytotoxicity in multiple myeloma.
Blood, 116, 406-417, 2010.
5. Noborio-Hatano K, Kikuchi J, Takatoku M, Shimizu R, Wada T, Ueda M, Nobuyoshi M, Oh I, Sato K, Suzuki T, Ozaki K, Mori M, Nagai T, Muroi K, Kano Y, Furukawa Y, and Ozawa K. Bortezomib overcomes cell adhesion-mediated drug resistance via down-regulation of VLA-4 expression in multiple myeloma.
Oncogene, 28, 231-242, 2009.

Phosphorylation-mediated EZH2 Inactivation as a Principal Mechanism of Drug Resistance in Multiple Myeloma

Jiro Kikuchi and Yusuke Furukawa
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Multiple myeloma (MM) is one of the most intractable malignancies characterized by the infiltration and growth of malignant plasma cells in the bone marrow (BM). MM cells are much less sensitive to chemotherapeutic agents in the BM microenvironment depending on the interaction with bone marrow stromal cells . This type of drug resistance, termed cell adhesion-mediated drug resistance (CAM-DR), is a major obstacle in achieving cure in MM patients. Although epigenetics has critical roles in various aspects of MM biology, little is known about their specific roles in CAM-DR.In this study, we identified trimethylation of histone H3 at lysine-27 (H3K27me3) as a critical histone mark for CAM-DR in MM cells. Cell adhesion counteracted drug-induced hypermethylation of H3K27 via inactivating phosphorylation of EZH2, leading to sustained expression of anti-apoptotic genes including IGF-1, Bcl-2 and HIF-1a. Inhibition of the IGF-1R/PI3K/Akt pathway was able to reverse CAM-DR by promoting EZH2 dephosphorylation and H3K27 hypermethylation. Among them, the IGF-1R inhibitor OSI-906 appears to be particularly effective to overcome CAM-DR in vitro and in refractory murine MM models.These results suggest that phosphorylation-mediated EZH2 inactivation and subsequent H3K27 hypermethylation are crucial for CAM-DR. This is the first report of the epigenetic mechanism of CAM-DR involving a regulatory circuit from the membrane to the nucleus and provides a rationale for the inclusion of kinase inhibitors counteracting EZH2 phosphorylation. The IGF-1R inhibitor OSI-906 may be translated to the clinic to improve the treatment outcome of myeloma patients in combination with conventional anti-MM agents.

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Professional Experience

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1989-1993	Associate, Howard Hughes Medical Institute, University of California, Los Angeles
1993-1996	Assistant Professor, Department of Genetics, Institute of Medical Science, University of Tokyo
1996-2002	Associate Professor, Department of Genetics, Institute of Medical Science, University of Tokyo
2002-present	Professor and Chairman, Department of Pharmacology, Tokyo Women's Medical University

Education

1977-1983	M.D., The University of Tokyo
1985-1989	Ph.D., The University of Tokyo

Recent Publications

1. Tomita T., Ieguchi K., Coin F., Kato Y., Kikuchi H., Oshima Y., Kurata S., and Maru Y. ZFC3H1, a zinc finger protein, modulates IL-8 transcription by binding with Celastramycin A, a potential immune suppressor. PLOS One, 2014, in press.
2. Ieguchi K, Tomita T, Omori T, Komatsu A, Deguchi A, Masuda J, Duffy S, Coulthard M, Boyd A, Maru Y. ADAM12-cleaved ephrin-A1 contributes to lung metastasis. Oncogene. 33,2179-2190, 2014
3. Hiratsuka S, Ishibashi S, Tomita T, Watanabe A, Tamura SA, Murakami M, Kijima H, Miyake K, Aburatani H, Maru Y. Primary tumours modulate innate immune signalling to create pre-metastatic vascular hyperpermeability foci. Nature communications. 4, 1853, 2013.
4. Deguchi A, Tomita T, Omori T, Komatsu A, Ohto U, Takahashi S, Tanimura N, Akashi-Takamura S, Miyake K, Maru Y. Serum amyloid A3 binds MD-2 to activate p38 and NF-kappaB pathways in a MyD88-dependent manner. Journal of immunology 191,1856-1864, 2013.
5. Maru Y. Molecular biology of chronic myeloid leukemia. Cancer Sci. 103,1601-1610, 2012.

How pre-metastatic milieu is established

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Circulating tumor cells in advanced cancer are never guaranteed for their growth in organs distinct from the original site of carcinogenesis. Innate immune responses to tumor are both anti-metastatic and pro-metastatic in conditions where tumor cells exist in the beginning. However, primary tumor tissues can alter microenvironments, whether physically and functionally, in the organs that are distant from the primary site. This remote control cultivates so-called soil before the actual arrival of tumor cells as seed from the primary site. The disseminated tumor cells may die in circulation or after reaching tissues separate from the primary site or stay alive in a quiescent state before acquisition of signals for re-growth. Irrespective of where the primary tumor cells remain alive in the body, establishment of metastatic soil is required for metastatic progression. Information on substances produced from the tumor cells are accumulating, which include actively secreted molecules upon stimulation by growth factors or upon senescence-associated secretory phenotype, and passively released molecules by cell death caused by chemotherapy and irradiation. Those substances can stimulate immune cells directly or indirectly via non-immune cells such as endothelial cells and resident epithelial cells. The major biological roles are played by pattern recognition receptors (PRRs) that include TLR and inflammasomes. Here I discuss on molecular mechanisms of pre-metastatic lungs in which TLR4 is clearly engaged.

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Professional Experience

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2007-2009	Research Fellow in Immune Disease Institute, Harvard Medical School, USA
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Education

1990-1996	M.D., Jichi Medical University
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Recent Publications

1. Morishita, Y., Yoshizawa, H., Watanabe, M., Ishibashi, K., Muto, S., Kusano, E., Nagata, D.: siRNAs targeted to Smad4 prevent renal fibrosis in vivo. Sci Rep, 2014 (in press)
2. Morishita, Y., Watanabe, M., Nakazawa, E., Ishibashi, K., Kusano, E.: The interaction of LFA-1 on mononuclear cells and ICAM-1 on tubular epithelial cells accelerates TGF-β1-induced renal epithelial-mesenchymal transition. PLoS One. 6: e23267, 2011
3. Morishita, Y., Ohnishi, A., Watanabe, M., Ishibashi, K., Kusano, E.: Establishment of acute kidney injury mouse model by 0.75% adenine ingestion. Ren Fail. 33: 1013-1018, 2011
4. Peer, D., Park, EJ., Morishita, Y., Carman, CV., Shimaoka, M.: Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. Science. 319: 627-630, 2008
5. Morishita, Y., Uenaka, A., Kaya, S., Sato, S., Aji, T., Nakayama, E: HLA-DRB1*0410-restricted recognition of XAGE-1b37-48 peptide by CD4 T cells. Microbiol Immunol. 51: 755-62, 2007

The delivery of miR-146 with polyethylenimine nanoparticle inhibits renal fibrosis in vivo

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Renal fibrosis is the final common pathway leading to decreased renal function. No therapy has been established to prevent it. Recently, the roles of micro RNAs (miR) for the development of renal fibrosis have been reported; however, few studies have focused the effects of exogenous miR delivery in an in vivo therapeutic setting for the real fibrosis. In the present study, we investigated the effects of the delivery of miR-146 mimic with polyethylamine nanoparticle (PEI-NPs) for renal fibrosis in vivo. miR-146 mimic oligo / PEI-NPs complex (N/P ratio: 6) were injected into each renal fibrosis mice induced by unilateral ureteral obstruction by systemic tail vein injection three times. Non-targeted oligo (Control oliogo) / PEI-NPs were injected in the same way for a control group. miR-146 mimic-PEI-NPs significantly increased miR-146 expression (>20 fold) in the kidney compared with those of control group. They inhibited kidney fibrosis area and the expression of α-SMA, collagen type1 and fibroblastic specific protein 1. They also inhibited Samd4 expression. Control oligo-PEI-NPs did not show these effects. The results of this study suggest that the delivery of miR-146 mimic with PEI-NPs attenuated renal fibrosis and it may be one of the therapeutic options for the prevention of renal fibrosis in vivo.

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Professional Experience

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Education

Undergraduate	1978-1984	Okayama University Medical School
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Recent Publications

1. Miyake K, Kaisho T. Homeostatic inflammation in innate immunity. *Curr Opin Immunol.* 2014 doi: 10.1016/j.coi.2014.08.003.
2. Huh JW, Shibata T, Hwang M, Kwon EH, Jang MS, Fukui R, Kanno K, Jung DJ, Jang MH, *Miyake K, and *Kim YM (*: co-corresponding author) UNC93B1 is essential for the plasma membrane localization and signaling of Toll-like receptor 5. *Proc. Natl. Acad. Sci. USA* 2014, 111:7072-7077. doi:10.1073/pnas.1322838111.
3. Onji M, Kanno A, Saitoh S, Fukui R, Motoi Y, Shibata T, Matsumoto F, Lamichhane A, Sato S, Kiyono H, Yamamoto K, Miyake K. An essential role for the N-terminal fragment of Toll-like receptor 9 in DNA sensing. *Nat. Commun.* 2013, 4: 1949, doi:10.1038/ncomms2949
4. Fukui R, Saitoh S-I, Kanno A, Onji M, Shibata T, Ito A, Onji M, Matsumoto M, Akira S, Yoshida N, Miyake K. Unc93B1 restricts systemic lethal inflammation by orchestrating TLR7- and TLR9-trafficking. *Immunity*, 2011, 35:69-81.
5. Fukui R, Saitoh S-I, Matsumoto F, Kozuka-Hara H, Oyama M, Tabeta K, Beutler B, and Miyake K. Unc93B1 biases Toll-like receptor responses to nucleic acid in dendritic cells towards DNA- but against RNA-sensing. *J. Exp. Med.*, 2009, 206:1339-1350

Mechanisms regulating nucleic acid-sensing Toll-like receptors

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The Toll family of receptors has critical roles in microbial recognition and activation of defense responses. Cell surface Toll-like receptors (TLRs) including TLR4/MD-2, TLR1/TLR2, TLR2/TLR6, or TLR5 recognize microbial membrane lipids or flagellin, whereas TLR3, 7, 8, and 9 reside in intracellular organelle and sense nucleic acids. Recent progresses have revealed that self-pathogen discrimination by TLRs is error prone and TLRs have been implicated in a variety of autoimmune diseases. Although the ligand specificity and downstream signaling pathways of each TLR have been extensively studied, much less is known as to how innate immune responses to self-products are controlled. Nucleic acid-sensing TLRs, TLR7, 8 and 9, have a risk of responding to self-derived nucleic acids. To prevent autoimmune responses, these TLRs are thought to be controlled by restricting nucleic acid sensing in endolysosomes, not the cell surface. Extracellular self-nucleic acids are instantly degraded and do not get to endolysosomes, whereas microbial nucleic acids are protected by microbial membranes and get to the endolysosomes. To limit nucleic acid sensing in endolysosomes, trafficking of nucleic acid-sensing TLRs from ER to endolysosomes is tightly controlled. The subcellular distribution of TLR7 and 9 are dependent on the TLR transporter Unc93B1. Unc93B1 is associated with TLR7 and 9 and transports them from ER to the endolysosomes. Interestingly, Unc93B1 is not just the TLR transporter but also has a role in controlling the balance between TLR7 and TLR9. Unc93B1-dependent control of TLR7 and 9 balance prevents TLR7-dependent autoimmunity. TLR7 has a unique risk of autoimmunity. TLR7 is a promising target for therapeutic intervention in autoimmune diseases.

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Professional Experience

2002~2004: Junior Resident, Mie Prefectural Hospital
2004~2006: Medical Staff in Internal Medicine, Kinan Hospital
2006~2007: Senior Resident in Department of Nephrology, Jichi Medical University Saitama Medical Center
2007~2008: Medical Staff in Internal Medicine, Minamiise Municipal Hospital
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2011~Present :Graduate School of Medicine, Jichi Medical University

Education

2002: M.D., Jichi Medical University

Recent Publications

1. Komada T, Usui F, Shirasuna K, Kawashima A, Kimura H, Karasawa T, Nishimura S, Sagara J, Noda T, Taniguchi S, Muto S, Nagata D, Kusano E, Takahashi M: ASC in Renal Collecting Duct Epithelial Cells Contributes to Inflammation and Injury after Unilateral Ureteral Obstruction. *Am. J. Pathol*. 2014
2. Komada T, Morishita Y, Kitamura M, Iwazu K, Numata A, Kobayashi T, Yamamoto H, Akimoto T, Saito O, Ando Y, Takemoto F, Muto S, Yumura W, Kusano E: Acute kidney injury in a patient with nephrotic syndrome due to focal segmental glomerular nephritis induced by a single oral administration of high-dose bisphosphonate (minodronate). *Intern Med* 52: 1383–1387, 2013
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Inflammasome Activation in Renal Collecting Duct Epithelial Cells: The Potential Role of Non-Immune cells in Renal Inflammation and Fibrosis

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Inflammation plays a crucial role in the pathophysiological characteristics of chronic kidney disease; however, the inflammatory mechanisms underlying the chronic kidney disease process remain unclear. Recent evidence indicates that sterile inflammation triggered by tissue injury is mediated through a multiprotein complex called the inflammasome. Therefore, we investigated the role of the inflammasome in the development of chronic kidney disease using a murine unilateral ureteral obstruction (UUO) model. Inflammasome-related molecules were up-regulated in the kidney after UUO. Apoptosis- associated speck-like protein containing a caspase recruitment domain deficiency significantly reduced inflammatory responses, such as inflammatory cell infiltration and cytokine expression, and improved subsequent renal injury and fibrosis. Furthermore, apoptosis-associated speck-like protein containing a caspase recruitment domain was specifically up-regulated in collecting duct (CD) epithelial cells of the UUO-treated kidney. In vitro experiments showed that extracellular adenosine triphosphate (ATP) induced inflammasome activation in CD epithelial cells through P2X7-potassium efflux and reactive oxygen species-dependent pathways. These results demonstrate the molecular basis for the inflammatory response in the process of chronic kidney disease and suggest the CD inflammasome as a potential therapeutic target for preventing chronic kidney disease progression.

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Education and Employment

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1994.4-1995.4	Special Researcher at Crystallography Laboratory in RIKEN
1995.4-2003.4	Assistant Professor at the University of Tokyo
2002.4-2003.4	Associate Professor at the University of Tokyo
2003.5-	Professor at Department of Biological Information, Graduate School of Biosciences and Biotechnology, Tokyo Institute of Technology
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Awards

JSPS PRIZE in 2008 by JSPS, Mochida Memorial Research Award in 2009, Kihara Memorial Research Award in 2010, Inoue Prize for Science in 2011, Uehara Award in 2014, Takeda Prize for Medical Science in 2014

Recent publications.

1. "Structural basis for Sec-independent membrane protein insertion by YidC" K. Kumazaki, S. Chiba, M. Takemoto, A. Furukawa, K. Nishiyama, Y. Sugano, T. Mori, N. Dohmae, K. Hirata, Y. Nakada-Nakura, A. D. Maturana, Y. Tanaka, H. Mori, Y. Sugita, F. Arisaka, K. Ito, R. Ishitani, T. Tsukazaki and O. Nureki
Nature 509, 516-520 (2014).
2. "Crystal Structure of Cas9 in Complex with Guide RNA and Target DNA" H. Nishimasu, F. A. Ran, P. D. Hsu, S. Konermann, S. I. Shehata, N. Dohmae, R. Ishitani, F. Zhang F and O. Nureki.
Cell 156, 935-949 (2014).
3. "Structural basis for the counter-transport mechanism of a H^+/Ca^{2+} exchanger" T. Nishizawa, S. Kita, A. D. Maturana, N. Furuya, K. Hirata, G. Kasuya, S. Ogasawara, N. Dohmae, T. Iwamoto, R. Ishitani, O. Nureki *Science* 341, 168-172 (2013).
4. "Structural basis for the novel mechanism of drug extrusion by a MATE multidrug transporter." Y. Tanaka, C. J. Hipolito, A. D. Maturana, K. Ito, T. Kuroda, T. Higuchi, T. Katoh, H. E. Kato, M. Hattori, K. Kumazaki, T. Tsukazaki, R. Ishitani, H. Suga and O. Nureki
Nature 496, 247–251 (2013)
5. "Crystal structure of the channelrhodopsin light-gated cation channel" H. E. Kato, F. Zhang, O. Yizhar, C. Ramakrishnan, T. Nishizawa, K. Hirata, J. Ito, Y. Aita, T. Tsukazaki, S. Hayashi, P. Hegemann, A. D. Maturana, R. Ishitani, K. Deisseroth and O. Nureki
Nature 428, 369-374 (2012).

Crystal structure of Cas9 complexed with guide RNA and target DNA

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The CRISPR-associated endonuclease Cas9 can be targeted to specific genomic loci by single guide RNAs (sgRNAs). Here, we solved the crystal structure of Streptococcus pyrogenes Cas9 in complex with sgRNA and its target DNA at 2.5 A resolution. The structure revealed a bilobed architecture consisting of target recognition and nuclease lobes (Rec and Nuc lobes, respectively), accommodating the sgRNA:DNA heteroduplex in a positively-charged groove at their interface. While Rec lobe is essential for binding sgRNA and DNA, Nuc lobe contains the HNH and RuvC nuclease domains, which are properly located for cleavage of the complementary and noncomplemantary strands of the target DNA, respectively. Nuc lobe also contains a C-terminal domain responsible for the recognition of the protospacer adjacent motif (PAM). This high-resolution structure combined with functional analyses have revealed the molecular mechanism of RNA-guided DNA targeting by Cas9, thus paving the way for rational design of new, versatile genome-editing technologies.

<Reference>

“Crystal Structure of Cas9 in Complex with Guide RNA and Target DNA” H. Nishimasu, F. A. Ran, P. D. Hsu, S. Konermann, S. I. Shehata, N. Dohmae, R. Ishitani, F. Zhang F and O. Nureki.
Cell 156, 935-949 (2014).

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