Department of Cancer Biology

Division of Molecular Pathology
人癌病因遺伝子分野

Professor	Yoshinori Murakami, M.D., Ph.D.	教 授	医学博士	村 上 善 則
Senior Assistant Professor	Daisuke Matsubara, M.D., Ph.D.	講 師	医学博士	松 原 大 祐
Assistant Professor	Takeharu Sakamoto, Ph.D.	助 教	医学博士	坂 本 毅 治
Assistant Professor	Takeshi Ito, Ph.D.	助 教	医学博士	伊 東 剛

Human cancers develop and progress toward malignancy through accumulation of multiple genetic and epigenetic alterations. Elucidation of these alterations is essential to provide molecular targets for prevention, diagnosis, and treatment of cancer. Our current interest is to understand the roles of cell adhesion in cancer invasion and metastasis. Genetic and epigenetic abnormalities involved in human tumors, including adult T-cell leukemia, cholangiocarcinoma, lung, breast, head and neck and urological cancers, are also being investigated.

1. The biological functions of cell adhesion in human tumor

Takeshi Ito, Yumi Tsuboi, Anri Saito, Shuejen Shiu, Yuki Kumagai, Hiroyuki Kogai, Ken Akashi, Atsushi Kanatani, Takehiro Tsuchiya, Masanori Muroi, Masaru Kasai, Tomoko Maruyama, Hiromi Ichihara, Mika Sakurai-Yageta, Shigefumi Murakami, Takahiro Mimae, Akihiko Ito[1] and Yoshinori Murakami; [1]Department of Pathology, Kinki University School of Medicine

Disruption of cell adhesion is a critical step to invasion and metastasis of human cancer. CADM1/TSLC1 is an immunoglobulin superfamily cell adhesion molecule and acts as a tumor suppressor in various cancers. In order to understand the intracellular signaling pathways activated by CADM1-mediated cell adhesion, we established a cell-based spreading assay to identify the signaling pathway specifically activated by the *trans*-homophilic interaction of CADM1. Using this assay, we found that a PI3K inhibitor, LY294002, inhibited cell spreading caused by *trans*-homophilic interaction of CADM1. Furthermore, we identified a cascade, CADM1-MPP3-Dlg-p85PI3K-AKT or RAC1, suggesting that *trans*-homophilic interaction mediated by CADM1 activates the PI3K pathway to reorganize the actin cytoskeleton, form epithelial cell structure and suppress tumor formation (1). We have also examined the dynamic regulation of CADM1, 4.1B and MPP3 proteins on the cell membrane using photo-bleaching assay in combination with double-exponential fitting analysis and have demonstrated that CADM1 is expressed stably on the membrane with time constant (tau) of around 960 sec, whereas 4.1B and MPP3 are turned over more rapidly with tau of 40-60 sec. Our analyses reveal a central role of CADM1 in adhesion complex and provide a novel approach to understand the dynamics of protein complex (2). Molecular mechanisms of tumor suppressor activity of CADM1 in comparison with other cell adhesion molecules are also investigated using gene-deficient mice model and 3-dimensional model of cultured cells in collaborations with others (3, 4, 5).

2. Analysis of oncogenic function of CADM1 in adult T-cell leukemia (ATL) and small cell lung cancer (SCLC)

Mika Sakurai-Yageta, Takeshi Ito, Yumi Tsuboi,

Hiroyuki Kogai, Siew-Pei Gan, Misaki Noguchi, Tetsuya Tabuchi, Tomoko Maruyama, Hiromi Ichihara, Motoi Oba[2], Akiteru Goto[3] and Yoshinori Murakami; [2]Research Institute of Molecular and Cell Biology of Cancer, Showa University, [3]Department of Pathology, Akita University Graduate School of Medicine.

In contrast to a tumor suppressor function of CADM1 in epithelial cancers, CADM1 is overexpressed in adult T-cell leukemia (ATL), conferring an invasive phenotype characteristic to ATL. We also reported expression of a splicing variant of CADM1, v8/9, which is specific to small cell lung cancer (SCLC), in comparison with an epithelial isoform of v8 or a neuronal isoform of v8(-). To understand the oncogenic functions of CADM1 in ATL and SCLC that are distinct from tumor suppressor functions in many epithelial cancers, structural and functional features of *N*-linked and *O*-linked glycosylation of CADM1 protein are being examined using mass spectrometry in collaboration with Shimadzu Co. Ltd.. To establish sensitive diagnostic tools of ATL through detecting CADM1, specific antibodies against the fragment of CADM1 unique to ATL are being generated. These antibodies would be useful to improve the FACS system to identify and characterize malignant ATL cells, which has been established in the IMSUT Hospital. Systems of whole exome sequencing or whole genome sequencing in combination with banking protocol of ATL materials are also being constructed in collaboration with a number of clinical scientists in ATL.

3. Analyses of genetic and epigenetic alterations of human cholangiocarcinoma and head and neck, lung, breast, and urological cancers.

Takeshi Ito, Fumi Taira, Ken Akashi, Atsushi Kanatani, Ayaka Sato, Hasaya Dokdang, Daisuke Matsubara, Akiteru Goto[3], Kaoru Kiguchi[4] and Yoshinori Murakami; [4]Department of Pediatrics, MD Anderson Cancer Center, USA.

To unveil the molecular mechanisms of multistage carcinogenesis, genetic and epigenetic alterations in key molecules in human tumorigenesis were examined in various cancers. The genetic susceptibility to individual cancers is also being investigated by genome-wide analysis of structural alterations. In the analysis of cholangiocarcinoma (CCA) that was developed on the basis of chronic infection of liver fluke (*Opisthorchis viverrini: Ov*) in Thailand, microarray analysis showed enhanced expression of the genes involved in chronic inflammatory responses, including NFKB. Moreover, Ov-associated CCA shows frequent mutations of the *KRAS, TP53, SMAD4*, and *CDKN2A* genes and preferential nucleotide substitutions from C:G to T:A, suggesting that mutation characteristics are similar to those of pancreatic ductal carcinoma but distinct from those of hepatocellular carcinoma (6, 7). Analysis of head and neck squamous cell carcinoma (HNSCC) revealed that the incidence of null-type mutations, which could not be detected as abnormal by conventional immunohistochemical (IHC) studies, was significantly higher (40%) in Japanese HNSCC than that of HNSCC reported in other countries. These results suggest that the sequencing analysis of the *TP53* mutation rather than IHC analysis of p53 provides a potentially useful marker to predict the response of HNSCC to chemotherapy or radiotherapy (8).

4. Molecular pathological analyses of human lung cancer

Daisuke Matsubara, Ibrahim Reem, Osmal Reem, Kana Makiya, Toshiro Niki[5] and Yoshinori Murakami; [5]Department of Integrative Pathology, Jichi Medical University.

Lung cancer is a leading cause of cancer death in Japan. Understanding the molecular pathological characteristics of human lung cancer is prerequisite to overcome this malignant cancer. We have been interested in the epithelial-mesenchymal transition (EMT) observed in malignant cancer cells with invasive and metastatic ability.

Protein arginine methyltransferase 5 (PRMT5) is a candidate histone methyltransferase gene whose expression is correlated with EMT by microarray analysis of 40 non-small cell lung carcinoma (NSCLC) cell lines. By immunohistochemical (IHC) analysis, we found that PRMT5 expression was frequently localized to the cytoplasms of E-cadherin-low and vimentin-high cancer cells. Moreover, cytoplasmic high expression of PMRT was frequently observed in tumors with high grade subtypes and in those with low cytokeratin7 and E-cadherin. High expression of PRMT5 in the cytoplasm was also significantly correlated with vessel invasion and poor prognosis, suggesting that cytoplasmic PRMT5 expression is involved in EMT of lung adenocarcinoma (9).

ZEB1, one of the master regulators of EMT, is another candidate gene involved in EMT of lung cancer. IHC analysis of 157 NSCLC revealed that 50% and 90% of poorly differentiated carcinoma showed high expression of ZEB1 and low expression of E-cadherin, respectively, whereas none of 93 adenocarcinomas and only 1 of 36 squamous cell carcinomas showed high expression of ZEB1. Overall, ZEB1 expression was inversely correlated with E-cadherin expression, suggesting that ZEB1 is rather correlated with undifferentiated and/or sar-

comatoid morphology that may occur in the late stage of EMT (10).

5. Pathophysiological analyses of cell growth regulation mediated by enzymatic digestion through the membrane protease, MT1-MMP

Takeharu Sakamoto, Seiko Yoshino, Hiroki Nakaoka, Akane Kanamori and Miho Ishiura.

Hypoxia inducible factor-1 (HIF-1) plays a key role in the cellular adaptation to hypoxia. Although HIF-1 is usually strongly suppressed by post-translational mechanisms during normoxia, HIF-1 is active and enhances tumorigenicity in malignant tumor cells that express the membrane protease MT1-MMP. The cytoplasmic tail of MT1-MMP, which can bind to a HIF-1 suppressor protein called factor inhibiting HIF-1 (FIH-1), promotes inhibition of FIH-1 by Mint3 during normoxia. To investigate novel roles of HIF-1 in malignant tumor cells, possible links between HIF-1 activation by MT1-MMP/Mint3 and tumor growth signals were explored by surveying a panel of 252 signalling inhibitors. The mTOR inhibitor rapamycin was identified as a possible modulator and it inhibited the mTOR-dependent phosphorylation of Mint3 that was required for FIH-1 inhibition. A mutant Mint3 that cannot be phosphorylated exhibited a reduced ability to inhibit FIH-1 and promoted tumor formation in mice. These data suggest a novel molecular link between the important hub proteins MT1-MMP and mTOR that contribute to tumor malignancy (11). On the other hand, since MMP-2 activity is an important marker of tumor malignancy, MMP-2 activity dependent anchoring probes was developed for a nuclear imaging of cancer cells in collaboration with others (12).

Publications

1. Murakami S, Sakurai-Yageta M, Maruyama T, Murakami Y. Intercellular adhesion of CADM1 activates PI3K by forming a complex with MAGuK-family proteins MPP3 and Dlg. *PLoS One*, 9: e82894, 2014. (DOI: 10.1371/journal.pone. 0082894.)
2. Sakurai-Yageta M, Maruyama T, Suzuki T, Ichikawa K, Murakami Y. Dynamic Regulation of a Cell Adhesion Protein Complex Including CADM1 by Combinatorial Analysis of FRAP with Exponential Curve-fitting. *PLoS ONE*, in press.
3. Wang F, Akashi K, Murakami Y, Inoue Y, Furuta T, Yamada H, Ohtomo K, Kiryu S. Detection of lung tumors in mice using a 1-Tesla compact magnetic resonance imaging system. *PLoS One*, 9, e94945, 2014. (doi: 10.1371/journal.pone. 0094945).
4. Cortez VS, Cervantes-Barragan L, Song C, Gilfillan S, McDonald KG, Edelson BT, Murakami Y, Newberry RD, Sibley LD, Colonna M. CRTAM controls residency of gut CD4 + CD8 + T cells in the steady state and maintenance of gut CD4 TH17 during parasitic infection. *J Exp. Med.*, 211, 623-633, 2014. (DOI: 10.1084/jem. 20130904).
5. Mimae T, Ito A, Hagiyama M, Nakanishi J, Okada M, Murakami Y, Kondo T. A novel approach to pseudopodia proteomics: excimer laser etching, two-dimensional difference gel electrophoresis, and confocal imaging. *Protocol Exchange,* 2014. (DOI: 10.1038/ protex.2014.007)
6. Murakami Y, Yamamoto M. International collaboration to control cholangiocarcinoma. *J Hepato-Biliary and Pancreatic Science,* 21, 297-298, 2014. (DOI: 10.1002/jhbp.89).
7. Journal name: Ito T, Sakurai M, Goto A, Pairojkul C, Yongvanit P, Murakami Y. Genomic and transcriptional alterations of cholangiocarcinoma. *J Hepato-Biliary and Pancreatic Science,* 21, 380-387, 2014. (DOI: 10.1002/jhbp.67).
8. Ebihara Y, Iwai M, Akashi K, Ito T, Omura G, Saito Y, Yoshida M, Ando M, Asakage T, Yamasoba T, Murakami Y. High incidence of null-type mutations of the TP53 gene in Japanese patients with head and neck squamous cell carcinoma. *Journal of Cancer Therapy*, 5, 664-671, 2014. (doi.org/10236/jct2014.57075)
9. Ibrahim R, Matsubara D, Osman W, Morikawa T, Goto A, Morita S, Ishikawa S, Aburatani H, Takai D, Nakajima J, Fukayama M, Niki T, Murakami Y. Expression of PRMT5 in lung adenocarcinoma and its significance in epithelial-mesenchymal transition. *Human Pathology*, 45, 1397-1405, 2014. (doi: 10.1016/j.humpath. 2014.02. 013).
10. Matsubara D, Murakami Y, Niki T. Immunohistochemical analysis of the expression of E-cadherin and ZEB1 in non-small cell lung cancer. *Pathology International*, in press
11. Sakamoto T, Weng JS, Hara T, Yoshino S, Kozuka-Hata H, Oyama M, Seiki M. Hypoxia-Inducible Factor 1 Regulation through Cross Talk between mTOR and MT1-MMP, *Mol Cell Biol*, 34, 30-42, 2014.
12. Temma T, Hanaoka H, Yonezawa A, Kondo N, Sano K, Sakamoto T, Seiki M, Ono M, Saji H. Investigation of a MMP-2 Activity-Dependent Anchoring Probe for Nuclear Imaging of Cancer, *PLoS One*, 9, e102180, 2014.

Department of Cancer Biology

Division of Cellular and Molecular Biology
分子発癌分野

Professor	Jun-ichiro Inoue, Ph.D.	教　授	薬学博士　井　上　純一郎
Associate Professor	Taishin Akiyama, Ph.D.	准教授	薬学博士　秋　山　泰　身
Assistant Professor	Yuu Taguchi, Ph.D.	助　教	医学博士　田　口　　祐
Assistant Professor	Yuri Shibata, Ph.D.	助　教	生命科学博士　柴　田　佑　里

Gene expression is largely regulated by signal transduction triggered by various stimulations. Several lines of evidence indicate that genetic defects of molecules involved in the signal transduction or the gene expression lead to abnormal cell differentiation or tumor formation. Our goal is to understand the molecular mechanisms of disease pathogenesis and oncogenesis by elucidating normal regulation of intracellular signal transduction and gene expression involved in cell proliferation and differentiation. We have identified and been interested in Tumor necrosis factor receptor-associated factor 6 (TRAF6), which acts as an E3 ubiquitin ligase to generate Lys63-linked polyubiquitin chains that are crucial for transducing signals emanating from the TNFR superfamily or the TLR/IL-1R family leading to activation of transcription factor NF-κB and AP-1. By generating TRAF6-deficient mice, we found that TRAF6 is essential for osteoclastogenesis, immune self-tolerance, lymph node organogenesis and formation of skin appendices. We are currently focusing on molecular mechanisms underlying TRAF6-mediated activation of signal transduction pathways and how TRAF6 is involved in osteoclastogenesis and self-tolerance. In addition, NF-κB is constitutively activated in various cancer cells and this activation is likely involved in the malignancy of tumors. Thus, we are also investigating the molecular mechanisms of the constitutive activation of NF-κB and how this activation leads to the malignancy of breast cancers.

1. Molecular mechanism of the regulation of NF-κB transcription factor

Takao Seki, Mami Yamamoto, Noriyuki Ishibashi, Hironori Ohtsuki, Yuko Hata[1], Masaaki Oyama[1], Jin Gohda[2], Aya Kojima[3], Toshifumi Akizawa[4], Taishin Akiyama and Jun-ichiro Inoue: [1]Medical Proteomics Laboratory, and [2]Center for Asian Infectious Diseases, IMSUT; [3]Ritsumeikan University; [4]Setsunan University.

Transcription factor NF-κB binds specifically to a decameric motif of nucleotide, κB site, and activates transcription. The activation of NF-κB has been demonstrated to be carried out post-translationally upon extracellular stimuli through membrane receptors such as members of the TLR/IL-1R family and of TNFR superfamily. NF-κB forms a complex with regulatory protein, IκB, and is sequestered in the cytoplasm prior to stimulation. Upon stimulation, IκB is rapidly phosphorylated on two specific serine residues by IκB kinase (IKK) complex followed by lysine 48 (K48)-linked ubiquitination and proteasome-dependent degradation of IκB. NF-κB subsequently translocates to the nucleus to activate transcription of target genes. This project is to identify molecules that regulate signal from membrane receptors to NF-κB/IκB complex. We have previ-

ously identified upstream activators of NF-κB, tumor necrosis factor receptor-associated factor (TRAF) 6. TRAF6 contains RING domain in the N-terminus and acts as an E3 ubiquitin-ligase to catalyze the lysine 63 (K63)-linked polyubiquitination of several signaling molecules and TRAF6 itself. To understand the molecular mechanisms of TRAF6-mediated NF-κB activation, we try to identify proteins that are ubiquitinated by TRAF6 upon stimulation. We took advantage of using the peptide that specifically binds K63-linked polyubiquitin chain to purify such proteins. We have confirmed that the peptide-based affinity column is useful for specific concentration of recombinant K63-linked polyubiquitin chain, suggesting that it also works for purification of the proteins of our interest.

2. Analysis of the physiological role of p47

Xiao Han, Yuri Shibata, Masaaki Oyama[1], Hiroko Kozuka-Hata[1], Jin Gohda[2] and Jun-ichiro Inoue

p47 (also known as NSFL1C) is originally identified as a major cofactor of the cytosolic ATPase associated with various cellular activities p97, and the p47/p97 complex is required for the reassembly of Golgi stacks at the end of mitosis. We have previously reported that p47 targets polyubiquitinated NEMO for lysosomal degradation, thereby negatively regulating NF-κB activation. We also have shown that the expression of p47 is reduced in adult T-cell leukemia (ATL) patient-derived cell lines, in which NF-κB is constitutively activated. Our results suggest that the altered p47 expression may trigger development of various cancers. To further investigate the physiological significance of p47, we tried to generate p47 knockout mice. Heterozygous knockout mice were viable and fertile, while homozygous knockout mice exhibited embryonic lethality. Then, p47-loxP-flanked mice were crossed with LysM-Cre knock-in mice to disrupt p 47 specifically in macrophages. We obtained p47-deficient macrophages and are investigating the effect of p47 deficiency on NF-κB signaling pathway.

3. Molecular mechanism of HTLV-1 Tax-induced IKK activation

Yuri Shibata, Masaaki Oyama[1], Hiroko Kozuka-Hata[1] and Jun-ichiro Inoue

Activation of NF-κB by human T-cell leukemia virus type 1 (HTLV-1) Tax is thought to be crucial in T-cell transformation and the onset of adult T-cell leukemia (ATL). Although it is well known that Tax interacts with NEMO and induces IKK activation, the molecular mechanism of Tax-induced IKK activation remains largely unknown. To elucidate this mechanism, we established a cell-free assay system, in which the IKK complex can be activated by adding recombinant Tax to cytosolic extracts. Whereas recombinant Tax induced activation of the IKK complex in cytosolic extracts, it failed to activate the purified IKK complex. These results suggest that Tax requires intermediary molecules for IKK activation. Using cell-free assay system, we previously found that Tax requires Lys63-linked polyubiquitination to activate the IKK complex. We are currently tying to identify the E3 ligase involved in Lys63-linked polyubiquitination induced by Tax.

4. Molecular mechanism of RANK signaling in osteoclastogenesis

Yuu Taguchi, Kazuaki Tsumura, Yo Yumiketa, Mizuki Yamaniha, Youko Hirayama, Masaaki Oyama[1], Hiroko Kozuka-Hata[1], Jin Gohda[2], and Jun-ichiro Inoue

Osteoclasts are differentiated from hematopoietic stem cells, and play a crucial role in bone homeostasis in concert with osteoblasts. Excess formation or activation of osteoclasts leads to pathological bone resorption as observed in postmenopausal osteoporosis and bone destruction in rheumatoid arthritis. Therefore, it is essential to elucidate precisely the molecular mechanisms of osteoclastogenesis for understanding such bone diseases. Activation of signal transduction pathway emanating from receptor activator of nuclear factor-κB (RANK) is essential for osteoclastogenesis. This RANK signaling activates NF-κB and AP-1 through the E3 ubiquitin ligase TRAF6, and induces PLCγ2-mediated Ca^{2+} oscillation. Co-operation of these signals leads to the induction of NFATc1, a master transcriptional factor in osteoclastogenesis. We have previously identified a unique functional domain in the cytoplasmic region of RANK, named Highly Conserved domain in RANK (HCR), which does not share any significant homology with other proteins. The HCR functions as a platform for formation of signal complex including TRAF6, PLCγ2, and Gab2, and emanates sustained RANK signaling, which is essential for the NFATc1 induction and osteoclastogenesis. To elucidate the other functions and the precise molecular mechanism of HCR, we tried to identify the binding protein to HCR by using proteomics approaches and yeast two-hybrid methods. Some proteins were identified as candidates binding to HCR, and we are investigating the functional relationship between these candidates and osteoclastogenesis. In addition, we tried to identify the novel negative regulator of osteoclastogenesis based on microarray analyses, and also tried to elucidate the role of TRAF6 at the subsequent step of NFATc1 induction in osteoclastogenesis. We are now investigating whether candidate

proteins repress osteoclastogenesis *in vivo*, and also elucidating molecular mechanisms of such proteins and TRAF6 for developing drugs to treat bone diseases.

5. Roles of NF-κB in breast cancer development

Taku Ito-Kureha[5], Sakura Wakinaga, Kota Sakane, Naohiko Koshikawa[6], Mizuki Yamamoto[7], Kentaro Semba[7], Noritaka Yamaguchi[8], Tadashi Yamamoto[5], Motoharu Seiki[9], and Jun-ichiro Inoue: [5]Cell Signal Unit, Okinawa Institute of Science and Technology; [6]Kanagawa Cancer Center Research Institute; [7]Department of Life Science and Medical Bio-science, Waseda University; [8]Department of Molecular Cell Biology, Graduate School of Pharmaceutical Sciences, Chiba University and [9]Graduate School of Medicine, Kochi University, Kochi.

Breast tumors that express estrogen receptors (ERs), progesterone receptors (PRs), or erythroblastic leukemia viral oncogene homolog 2 (ERBB2) can be treated with selective estrogen receptor modulators (SERMs) or trastuzumab, an anti-ERBB2 antibody. However, triple-negative (ER^-, PR^-, $ERBB2^-$) breast cancers (TNBCs), including basal-like and claudin-low subtypes, lack effective molecular targets even though TNBCs show aggressive clinical behavior. We have previously demonstrated that NF-κB activation in TNBCs is significantly higher than in other subtypes. In addition, several lines of evidence indicate that NF-κB activation is involved in the malignant phenotypes of breast cancers. These previous results suggest that proteins derived from NF-κB target genes might be molecular targets for cancer therapy when such proteins are involved in the malignant phenotypes. In this study, to search for novel NF-κB inducible proteins that are likely to be therapeutic targets for TNBCs, we performed a microarray analysis using the claudin-low subtype cell line MDA-MB-436, and identified tropomodulin 1 (TMOD1) as a candidate. TMOD1 expression is directly regulated by NF-κB and is significantly higher in TNBC than in other subtypes. TMOD1 was associated with enhanced tumor growth in a mouse xenograft model and in a type I collagen three-dimensional culture. TMOD1-dependent tumor growth was likely due to the MMP 13 induction, which is mediated by the TMOD1-dependent accumulation of β-catenin. Taken together, our study identified the TMOD1-mediated novel link between NF-κB activation and MMP13 induction, which accounts in part for the NF-κB-dependent malignant phenotypes of TNBCs.

6. Limitation of immune tolerance-inducing thymic epithelial cell development by Spi-B-mediated negative feedback regulation

Nobuko Akiyama, Maki Miyauchi, Ryosuke Tateishi, Jun-ichiro Inoue and Taishin Akiyama

Mature mTECs express high levels of MHC class II (MHC II) and co-stimulatory molecules CD80 and CD86 and play as thymic self-antigen presenting cells by uniquely expressing a wide variety of tissue-specific endogenous antigens (TSAs). The autoimmune regulator Aire, in which dysfunctional mutations provokes human autoimmune diseases, enhances the diversity of TSAs. Consequently, Aire-expressing mature mTECs promote clonal deletion and regulatory T cell (Tregs) conversion of potentially TSA-reactive T cells; therefore, are critical for preventing the onset of autoimmunity. Furthermore, recent studies show that Aire deficiency inhibits tumor growth and Treg accumulation in tumors. Thus, precise regulation of mTEC-mediated tolerance may be critical for balancing prevention of autoimmunity with induction of tumor immunity. We previously reported that TNF family cytokine RANKL triggers mTEC differentiation by activating NF-κB pathways. However, the molecular events that connect RANKL-NF-κB signaling with expression of mature mTEC molecules are currently unknown. In this study, we found that Spi-B links RANKL-NF-κB signaling with up-regulation of several molecules expressed in mature mTECs, including CD80, CD86, some TSAs and osteoprotegerin (OPG), the natural inhibitor of RANKL. OPG-mediated negative regulation attenuates thymic generation of regulatory T cells and tumour development *in vivo*. Thus, our data suggest that the negative RANKL-Spi-B-OPG feedback mechanism finely tunes mTEC development and function and may optimise the trade-off between prevention of autoimmunity and induction of anti-tumour immunity.

7. Mitochondria-nucleus shuttling FK506-binding protein 51 interacts with TRAF proteins and facilitates the RIG-I-like receptor-mediated expression of type I IFN

Taishin Akiyama, Takuma Shiraishi, Junwen Qin, Hiroyasu Konno, Nobuko Akiyama, Miho Shinzawa, Maki Miyauchi, Nobukazu Takizawa, Hiromi Yanai, Hiroyuki Ohashi[10], Etsuko Miyamoto-Sato[10], and Jun-Ichiro Inoue: [10]Division of Interactome Medical Sciences, IMSUT

Virus-derived double-stranded RNAs (dsRNAs) are sensed in the cytosol by retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), thereby inducing the expression of type I IFN and proinflammatory cytokines through signaling pathways promoted by the mitochondrial antiviral signaling (MAVS) protein. TNF receptor-associated factor (TRAF) family proteins reportedly facilitate the RLR-dependent expression of type I IFN by binding with MAVS.

However, the precise regulatory mechanisms remain elusive. We found the role of FK506-binding protein 51 (FKBP51) in regulating the dsRNA-dependent expression of type I IFN. The binding of FKBP51 to TRAF6 was identified by "*in vitro* virus" selection and was subsequently confirmed with a coimmunoprecipitation assay. The TRAF-C domain of TRAF6 is required for its interaction, although FKBP51 does not contain the consensus motif for interaction with the TRAF-C domain. Moreover, we identified the binding of FKBP51 to TRAF3. The depletion of FKBP51 reduced the expression of type I IFN induced by dsRNA transfection or Newcastle disease virus infection. Consistent with this, the FKBP51 depletion attenuated dsRNA-mediated phosphorylations of IRF3 and JNK and nuclear translocation of RelA. Interestingly, dsRNA stimulation promoted the accumulation of FKBP51 in the mitochondria. Overall, we concluded that FKBP51 interacts with TRAF proteins and facilitates the expression of type I IFN induced by cytosolic dsRNA.

Publications

Akiyama, T., Shiraishi, T., Qin, J., Konno, H., Akiyama, N., Shinzawa, M., Miyauchi, M., Takizawa, N., Yanai, H., Ohashi, H., Miyamoto-Sato, E., Yanagawa, H., Yong, W., Shou, W. and Inoue, J. Mitochondria-nucleus shuttling FK506-binding protein 51 interacts with TRAF proteins and facilitates the RIG-I-like receptor-mediated expression of type I IFN. *PLoS ONE* 9(5): e95992. doi: 10.1371/journal.pone.0095992 (2014).

Akiyama, T., Qin, J., Ohshima, D. and Inoue, J. Identification of transcription factors activated in thymic epithelial cells during embryonic thymus development. *Methods Mol. Biol.* 1164, 163-170 (2014).

Akiyama, N., Shinzawa, M., Miyauchi, M., Yanai, H., Tateishi, R., Shimo, Y., Ohshima, D., Matsuo, M., Sasaki. I., Hoshino, K., Wu, G., Yagi, S., Inoue, J., Kaisho, T. and Akiyama, T. Limitation of immune tolerance-inducing thymic epithelial cell development by Spi-B-mediated negative feedback regulation. *J. Exp. Med.* 211, 2425-2438 (2014).

Blackburn, J., Kawasaki, K., Porntaveetus, T., Kawasaki, M., Otsuka-Tanaka, Y., Miake, Y., Ota, M.S., Watanabe, M., Hishinuma, M., Nomoto, T., Oommen, S., Ghafoor, S., Harada, F., Nozawa-Inoue, K., Maeda, T., Peterková, R., Lesot, H., Inoue, J., Akiyama, T., Schmidt-Ullrich, R., Liu, B., Hu, Y., Page, A., Ramírez, A., Sharpe, P.T. and Ohazama, A. Excess NF-κB induces ectopic odontogenesis in embryonic incisor epithelium. *J. Dent. Res.* 94, 121-128 (2015).

Ito-Kureha, T., Koshikawa, N., Yamamoto, M., Semba, K., Yamaguchi, N., Yamamoto, T., Seiki, M. and Inoue, J. Tropomodulin 1 expression driven by NF-κB enhances breast cancer growth. *Cancer Res.* 75, 62-72 (2015).

Sato, Y., Goto, E., Shibata, Y., Kubota, Y., Yamagata, A., Goto-Ito, S., Kubota, K., Inoue, J., Takekawa, M., Tokunaga, F. and Fukai, S. Structures of CYLD USP with Met1- or Lys63-linked diubiquitin reveal mechanisms for dual specificity. *Nat. Struct. Mol. Biol.* (2015). In press

Gohda, J., Ma, Y., Huang, Y., Zhang, Y., Gu, L., Han, Y., Li, T., Gao, G.F., Inoue, J., Iwamoto, A. and Ishida, T. HIV-1 replicates in human osteoclasts and enhances their differentiation in vitro. *Retrovirology* (2015). In press

Department of Cancer Biology

Division of Genetics
腫瘍抑制分野

Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士	山　梨　裕　司
Assistant Professor	Tohru Tezuka, Ph.D.	助　教	理学博士	手　塚　　徹
Assistant Professor	Ryo Ueta, Ph.D.	助　教	博士(生命科学)	植　田　　亮
Assistant Professor	Sumimasa Arimura, Ph.D.	助　教	医学博士	有　村　純　暢

The major interest of this division is in molecular signals that regulate a variety of cellular activities. Our aim is to address how dysregulated cellular signals give rise to neoplastic, immune, neural, metabolic, or developmental disorders. Our goal is to understand the molecular bases of tumorigenesis and the development of other intractable diseases as a path toward uncovering therapeutic targets. Currently, we are investigating regulatory mechanisms in protein-tyrosine kinase (PTK)-mediated signaling pathways and their pathophysiological roles in tumorigenesis, metastasis, inflammation, and myasthenia.

1. Activation of the receptor tyrosine kinase MuSK by the cytoplasmic protein Dok-7 in neuromuscular synaptogenesis.

Tezuka, T., Inoue-Yamauchi, A.[1], Weatherbee, SD.[2], Izawa, Y., Ueta, R., and Yamanashi, Y.: [1]Memorial Sloan Kettering Cancer Center. [2]Department of Genetics, Yale University.

Protein-tyrosine kinases (PTKs) play crucial roles in a variety of signaling pathways that regulate proliferation, differentiation, motility, and other activities of cells. Therefore, deregulated PTK signals give rise to a wide range of diseases such as neoplastic disorders. To understand the molecular bases of PTK-mediated signaling pathways, we identified Dok-1 as a common substrate of many PTKs in 1997. Since then, the Dok-family has been expanded to seven members, Dok-1 to Dok-7, which share structural similarities characterized by NH_2-terminal pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains, followed by Src homology 2 (SH2) target motifs in the COOH-terminal moiety, suggesting an adaptor function. Indeed, as described below, Dok-1 and Dok-2 recruit p120 rasGAP upon tyrosine phosphorylation to suppress Ras-Erk signaling. However, we found that Dok-7 acts as an essential cytoplasmic activator of the muscle-specific receptor tyrosine kinase (RTK) MuSK in the formation of the neuromuscular junction (NMJ), providing a new insight into RTK-mediated signaling. It now seems possible that local levels of cytoplasmic activators, like Dok-7, control the activity of RTKs in concert with their extracellular ligands.

The NMJ is a synapse between a motor neuron and skeletal muscle, where the motor nerve terminal is apposed to the endplate (the region of synaptic specialization on the muscle). The contraction of skeletal muscle is controlled by the neurotransmitter acetylcholine (ACh), which is released from the presynaptic motor nerve terminal. To achieve efficient neuromuscular transmission, acetylcholine receptors (AChRs) must be densely clustered on the postsynaptic muscle membrane of the NMJ. Failure of AChR clustering is associated with disorders of neuromuscular transmission such as congenital myasthenic syndromes and myasthenia gravis, which are characterized by fatigable muscle weakness. The formation of NMJs is orchestrated by MuSK and by

neural agrin, an extracellular activator of MuSK. However, experimentally when motor nerves are ablated, AChRs form clusters in the correct, central region of muscle during embryogenesis in a MuSK-dependent process known as prepatterning of the receptors. In addition, in vivo overexpression of MuSK causes neuromuscular synapse formation in the absence of agrin, suggesting that muscle-intrinsic, cell-autonomous activation of MuSK may be adequate to trigger presynaptic and postsynaptic differentiation in vivo. However, the mechanisms by which MuSK is activated independently of nerve and agrin had long been unclear.

Because both MuSK and the adaptor-like cytoplasmic protein Dok-7 are localized to the postsynaptic region of NMJs, we previously examined their interaction and found that Dok-7 is an essential cytoplasmic activator of MuSK. In addition, we found that Dok-7 directly interacts with the cytoplasmic portion of MuSK and activates the RTK, and that neural agrin requires Dok-7 in order to activate MuSK. Indeed, in vivo overexpression of Dok-7 increased MuSK activation and promoted NMJ formation. Conversely, mice lacking Dok-7 formed neither NMJs nor AChR clusters. Interestingly, mice lacking Lrp4, which forms a complex with MuSK and acts as an essential agrin-binding module, do not show MuSK-dependent AChR prepatterning or NMJ formation. This suggests that Lrp4 is required for MuSK activation under physiological conditions, in contrast to our observation that Dok-7 can activate MuSK in the absence of Lrp4 or its ligand agrin, at least in vitro. Thus, we examined the effects of forced expression of Dok-7 in skeletal muscle on NMJ formation in the absence of Lrp4 and found that it indeed induces MuSK activation in mice lacking Lrp4. However, the activation level of MuSK was significantly lower in the absence than in the presence of Lrp4. Together, these data indicate that Lrp4 is required for efficient activation of MuSK by Dok-7 in the muscle. We are investigating mechanisms of this apparent cooperation between Lrp4 and Dok-7 in MuSK activation in vivo.

2. Agrin's role separately from MuSK activation in the postnatal maintenance of NMJs.

Tezuka, T., Hoshi, T., Burgess, RW.[1], Ueta, R., and Yamanashi, Y.: [1]The Jackson Laboratory.

Although NMJ formation requires agrin under physiological conditions, it is dispensable for NMJ formation experimentally in the absence of the neurotransmitter acetylcholine, which inhibits postsynaptic specialization. Thus, it was hypothesized that MuSK needs agrin together with Lrp4 and Dok-7 to achieve sufficient activation to surmount inhibition by acetylcholine. To test this hypothesis, we examined the effects of forced expression of Dok-7 in skeletal muscle on NMJ formation in the absence of agrin and found that it indeed restores NMJ formation in agrin-deficient embryos. However, these NMJs rapidly disappeared after birth, whereas exogenous Dok-7-mediated MuSK activation was maintained. These findings indicate that the MuSK activator agrin plays another role essential for the postnatal maintenance, but not for embryonic formation, of NMJs. We are investigating the role that agrin plays in the postnatal maintenance of NMJs, apart from its role in MuSK activation.

3. Pathophysiological mechanisms underlying *DOK7* myasthenia.

Arimura, S., Tezuka, T., Hoshi, T., Yoshimura, T.[1], Motomura, M.[2], Yoshida, N.[3], Beeson, D.[4], and Yamanashi, Y.: [1]Department of Occupational Therapy, Nagasaki University School of Health Sciences. [2]Department of Electrical and Electronics Engineering, Faculty of Engineering, Nagasaki Institute of Applied Science. [3]Laboratory of Developmental Genetics, IMSUT. [4]Weatherall Institute of Molecular Medicine, University of Oxford.

As mentioned above, impaired clustering of AChRs could underlie NMJ disorders, be they autoimmune (MuSK antibody-positive myasthenia gravis) or genetic (congenital myasthenic syndromes (CMS)) in origin. Therefore, our findings that Dok-7 activates MuSK to cluster AChRs and to form NMJs suggested *DOK7* as a candidate gene for mutations associated with CMS. Indeed, we previously demonstrated that biallelic mutations in *DOK7* underlie a major subgroup of CMS with predominantly proximal muscle weakness that did not show tubular aggregates on muscle biopsy but were found to have normal AChR function despite abnormally small and simplified NMJs. We further demonstrated that several mutations, including one associated with the majority of patients with the disease, impaired Dok-7's ability to activate MuSK. This new disease entity is termed "*DOK7* myasthenia."

To investigate pathophysiological mechanisms underlying *DOK7* myasthenia, we established knock-in mice (Dok-7 KI mice) that have a mutation associated with the majority of patients with *DOK7* myasthenia. As expected, Dok-7 KI mice showed characteristic features of severe muscle weakness and died between postnatal day 13 and 20. Furthermore, they showed abnormally small NMJs lacking postsynaptic folding, a pathological feature seen in patients with *DOK7* myasthenia. Consistent with this, Dok-7 KI mice exhibited decreased MuSK activity in skeletal muscle, indicating that the Dok-7 KI mice develop defects similar to those found in patients with *DOK7* myasthenia, although the mice exhibit a more severe phenotype. We are investigating other defects in NMJ function

in the Dok-7 KI mice.

4. *DOK7* gene therapy that enlarges NMJs.

Arimura, S., Okada, T.[1], Miyoshi, S., Tezuka, T., Yukimura, R., Kito, M., Chiyo, T.[2], Kasahara, Y.[2], Yoshimura, T.[3], Motomura, M.[4], Yoshida, N.[5], Beeson, D.[6], Takeda, S.[2], and Yamanashi, Y.: [1]Department of Biochemistry and Molecular Biology, Nippon Medical School. [2]Department of Molecular Therapy, National Institute of Neuroscience. [3]Department of Occupational Therapy, Nagasaki University School of Health Sciences. [4]Department of Electrical and Electronics Engineering, Faculty of Engineering, Nagasaki Institute of Applied Science. [5]Laboratory of Developmental Genetics, IMSUT. [6]Weatherall Institute of Molecular Medicine, University of Oxford.

As mentioned above, *DOK7* myasthenia is associated with impaired NMJ formation due to decreased ability of Dok-7 to activate MuSK in myotubes at least in part. Interestingly, in vivo overexpression of Dok-7 increased MuSK activation and promoted NMJ formation in the correct, central region of the skeletal muscle. Because these genetically manipulated mice did not show obvious defects in motor activity, overexpression of Dok-7 in the skeletal muscle of patients with *DOK7* myasthenia might ameliorate NMJ formation and muscle weakness. To test this possibility, we generated an Adeno-associated virus-based vector (AAV-D7), which strongly expressed human Dok-7 in myotubes and induced AChR cluster formation. Indeed, therapeutic administration of AAV-D7 to Dok-7 KI mice described above resulted in enlargement of NMJs and substantial increases in muscle strength and life span. Furthermore, when applied to model mice of another neuromuscular disorder, autosomal dominant Emery-Dreifuss muscular dystrophy, therapeutic administration of AAV-D7 likewise resulted in enlargement of NMJs as well as positive effects on motor activity and life span. These results suggest that therapies aimed at enlarging the NMJ may be useful for a range of neuromuscular disorders. We are investigating the effects of AAV-D7 administration on other types of neuromuscular disease models.

5. Lrp4 antibodies in patients with myasthenia gravis.

Tezuka, T., Motomura, M.[1], Hoshi, T., and Yamanashi, Y.: [1]Department of Electrical and Electronics Engineering, Faculty of Engineering, Nagasaki Institute of Applied Science.

Myasthenia gravis (MG) is an autoimmune disease of the NMJ. About 80% of patients with generalized MG have AChR antibodies, the presence of which is a causative factor for the disease, and a variable proportion of the remaining patients (0-50% throughout the world) have MuSK antibodies. However, diagnosis and clinical management remain complicated for patients who are negative for MuSK and AChR antibodies. Given the essential roles and postsynaptic localization of Lrp4 in the NMJ, we hypothesized that Lrp4 autoantibodies might be a pathogenic factor in MG. To test this hypothesis, we developed a luminescence-based method to efficiently detect serum autoantibodies to Lrp4 in patients, and found that 9 patients were positive for antibodies to the extracellular portion of Lrp4 from a cohort of 300 patients with AChR antibody-negative MG. 6 of these 9 patients with Lrp4 antibody-positive MG were also negative for MuSK antibodies, and generalized MG was diagnosed in all 9 patients, who showed severe limb muscle weakness or progressive bulbar palsy or both. Thymoma was not observed in any of these patients, unlike the situation in patients with AChR antibody-positive MG. Furthermore, we confirmed that serum antibodies to Lrp4 recognize its native form and inhibit binding of Agrin to Lrp4, which is crucial for NMJs. Also, we found that Lrp4 autoantibodies were predominantly comprised of IgG1, a complement activator, suggesting the potential for these antibodies to cause complement-mediated impairment of NMJs. Together, our findings indicate the involvement of Lrp4 antibodies in the pathogenesis of AChR antibody-negative MG. Following this study, two groups in Germany and USA reported respectively that about 50% and 10% of MG patients, who were negative for both MuSK and AChR antibodies, were positive for antibodies to Lrp4 (*J. Neurol*., 259: 427-435, 2012; *Arch. Neurol.*, 69: 445-451, 2012). Consistent with our observation, these groups reported that some Lrp4 antibodies from patients suppressed Agrin-induced AChR cluster formation in cultured myotubes. Recently, it was also reported that antibodies to Lrp4 induced MG in model animals (*J. Clin. Invest.*, 123: 5190-5202, 2013). We are further investigating the etiology and pathology of Lrp4 antibody-positive MG.

6. Roles of Dok-1 to Dok-6.

Arimura, S., Waseda, M., Kajikawa, S., Hoshi, T., Kanno, T., Jozawa, H., Oda, H.[1], Nakae, S.[2], Ezura, Y.[3], Taguchi, Y.[4], Inoue, J.[4], Noda, M.[3], and Yamanashi, Y.: [1]Department of Pathology, Tokyo Women's Medical University. [2]Laboratory of Systems Biology, IMSUT. [3]Department of Molecular Pharmacology, Medical Research Institute, Tokyo Medical & Dental University. [4]Division of Cellular and Molecular Biology, IMSUT.

Dok-family proteins can be classified into three

subgroups based on their structural similarities and expression patterns; namely, 1) Dok-1, -2, and -3, which are preferentially expressed in hematopoietic cells, 2) Dok-4, -5, and -6, which are preferentially expressed in non-hematopoietic cells, and 3) Dok-7, which is preferentially expressed in muscle cells. As mentioned above, Dok-1 and its closest paralog, Dok-2, recruit p120 rasGAP upon tyrosine phosphorylation to suppress Ras-Erk signaling. Although Dok-3 does not bind with p120 rasGAP, it also inhibits Ras-Erk signaling. Consistently, we previously demonstrated that Dok-1, Dok-2 and Dok-3 are key negative regulators of hematopoietic growth and survival signaling. For example, Dok-1, Dok-2, and Dok-3 cooperatively inhibit macrophage proliferation and *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ mice develop histiocytic sarcoma, an aggressive malignancy of macrophages. In addition, we found that these Dok proteins cooperatively play critical anti-inflammatory roles in lung homeostasis. We are further investigating roles of Dok-1 to Dok-6, including those in tumor malignancy.

7. Role of Mesdc2 in postsynaptic specialization in myotubes.

Hoshi, T., Tezuka, T., Iemura, S.[1], Natsume, T.[2], and Yamanashi, Y.: [1]Translational Research Center, Fukushima Medical University. [2]National Institute of Advanced Science and Technology, Biological Information Research Center.

To gain insights into signaling mechanisms underlying NMJ formation, we have performed mass spectrometric analysis of Lrp4-binding proteins and found the chaperon Mesdc2 as a candidate. We confirmed their binding in cells, and revealed that Mesdc2 bind selectively to the lower molecular mass form of Lrp4 (lower Lrp4) but not to the upper, more glycosylated form (upper Lrp4). Although the Mesdc2 binds to lower Lrp4, forced expression of Mesdc2 increased upper Lrp4, implying a role for Mesdc2 in the Lrp4 glycosylation, which might facilitate the receptor's cell surface expression. Indeed, we found that down regulation of Mesdc2 expression in cultured myotubes suppressed cell-surface expression of Lrp4, or upper Lrp4 more specifically. Furthermore, down regulation of Mesdc2 also inhibited agrin-induced postsynaptic specialization in myotubes, which requires binding of Lrp4 to its extracellular ligand, the neural agrin. Together, these findings demonstrated that Mesdc2 plays a key role in Lrp4-dependent postsynaptic specialization probably by promoting glycosylation and cell-surface expression of Lrp4 in myotubes. We are investigating roles for glycosylation of Lrp4 in its pleiotropic activities.

8. Proteomic and genomic analyses.

Tezuka, T., Eguchi, T., Honda, M., Ueta, R., Arimura, S., Iemura, S.[1], Natsume, T.[2], Oyama, M.[3], Sagara, H.[3], and Yamanashi, Y.: [1]Translational Research Center, Fukushima Medical University. [2]National Institute of Advanced Science and Technology, Biological Information Research Center. [3]Medical Proteomics Laboratory, IMSUT.

To gain insights into signaling mechanisms underlying a variety of physiological and pathophysiological events, including NMJ formation, tumorigenesis, and tumor metastasis, we have performed proteomic and genomic analyses. We are investigating the roles of candidate proteins and genes that appear to be involved in each of these biological events.

9. Screening of chemical compound libraries.

Ueta, R., Yukimura R., Waseda, M., Honda, M., Kito M., Sasaki, Y., Nagatoishi, S.[1], Tsumoto, K.[1], and Yamanashi, Y.: [1]Medical Proteomics Laboratory, IMSUT.

In addition to the omics analyses described above, we performed high throughput screenings of chemical libraries, aiming to intervene in pathogenic signals or to gain insights into signaling mechanisms underlying a variety of biological events. We continue the ongoing screenings to collect appropriate hit compounds.

Publications

Tezuka, T., Inoue, A., Hoshi, T, Weatherbee, SD., Burgess, RW., Ueta, R. and Yamanashi Y. The MuSK activator agrin has a separate role essential for postnatal maintenance of neuromuscular synapses. Proc. Natl. Acad. Sci. USA, 111: 16556-16561, 2014

Arimura, S., Okada, T., Tezuka, T., Chiyo, T., Kasahara, Y., Yoshimura, T., Motomura, M., Yoshida, N., Beeson, D., Takeda, S. and Yamanashi, Y. *DOK7* gene therapy benefits mouse models of diseases characterized by defects in the neuromuscular junction. Science, 345: 1505-1508, 2014

Hughan, SC., Spring, CM., Schoenwaelder, SM., Sturgeon, S., Alwis, I., Yuan, Y., McFadyen, JD., Westein, E., Goddard, D., Ono, A., Yamanashi, Y., Nesbitt, WS. and Jackson, SP. Dok-2 regulates the shear-dependent adhesive function of platelet

integrin αIIbβ3 in mice. J. Biol. Chem., 289: 5051-5060, 2014

Doi, R., Endo, M., Yamakoshi, K., Yamanashi, Y., Nishita, M., Fukada, S. and Minami, Y. Critical role of Frizzled1 in age-related alterations of Wnt/β-catenin signal in myogenic cells during differentiation. Genes Cells, 19: 287-296, 2014

Ueta, R. and Yamanashi, Y. Molecular signaling and its pathogenic alterations in neuromuscular junction formation and maintenance. In "Protein modifications in pathogenic dysregulation of signaling". (Springer), in press.

Department of Cancer Biology

Division of Mathematical Oncology
腫瘍数理分野

Project Professor Kazuhisa Ichikawa

特任教授 理学博士 市 川 一 寿

Intracellular space is crowded with spatio-temporally organized organelles, and huge number of proteins are interacting in this complex space. Events arising from this complex system are the basis for the cellular functions. Dysregulation of spatio-temporally organized organelles and protein-protein interactions causes various diseases. Our goal is to elucidate underlying mechanisms for cellular functions by a method of computing spatio-temporal system of a cell. We are focusing on the following topics: regulation of transcription factor NF-κB, the initial step of cancer invasion, and stochastic simulation of stress granule assembly.

1. Roles of Spatial Parameters on the Oscillation of Nuclear NF-κB: Computer Simulations of a 3D Spherical Cell

Daisuke Ohshima and Kazuhisa Ichikawa

The transcription factor NF-κB shuttles between the cytoplasm and the nucleus, and nuclear NF-κB is known to oscillate with a cycle of 1.5-2.5 h following the application of external stimuli. Oscillation pattern of NF-κB is implicated in regulation of the gene expression profile. In a previous report, we found that the oscillation pattern of nuclear NF-κB in a computational 3D spherical cell was regulated by spatial parameters such as nuclear to cytoplasmic volume ratio, nuclear transport, locus of protein synthesis, and diffusion coefficient. Here we report analyses and a biological implication for the regulation of oscillation pattern by diffusion coefficient. Our analyses show that the "reset" of nuclear NF-κB, defined as the return of nuclear NF-κB to the initial level or lower, was crucial for the oscillation; this was confirmed by the flux analysis. In addition, we found that the distant cytoplasmic location from the nucleus acted as a "reservoir" for storing newly synthesized IκBα. When the diffusion coefficient of proteins was large ($\geq 10^{-11}$ m^2/s), a larger amount of IκBα was stored in the "reservoir" with a large flux by diffusion. Subsequently, stored IκBα diffused back to the nucleus, where nuclear NF-κB was "reset" to the initial state. This initiated the next oscillation cycle. When the diffusion coefficient was small ($\leq 10^{-13}$ m^2/s), oscillation of nuclear NF-κB was not observed because a smaller amount of IκBα was stored in the "reservoir" and there was incomplete "reset" of nuclear NF-κB. If the diffusion coefficient for IκBα was increased to 10^{-11} m^2/s keeping other proteins at 10^{-13} m^2/s, the oscillation was rescued confirming the "reset" and "reservoir" hypothesis. Finally, we showed altered effective value of diffusion coefficient by diffusion obstacles. Thus, organelle crowding seen in stressed cells possibly changes the oscillation pattern by controlling the effective diffusion coefficient.

2. Computer Simulation Revealed Mechanisms of Stress Granule Assembly

Daisuke Ohshima, Kyoko Arimoto-Matsuzaki[1], Taichiro Tomida[1], Mutsuhiro Takekawa[2], and Kazuhisa Ichikawa: [1]Division of Molecular Cell Signaling, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Division of Cell Signaling and Molecular Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Stress granules (SGs) are non-membranous cytoplasmic aggregates of mRNAs and related proteins, assembled in response to environmental stresses such as heat shock, hypoxia, endoplasmic reticulum (ER) stress, chemicals (e.g. arsenite), and viral infections. SGs are hypothesized as a loci of mRNA triage and/or maintenance of proper translation capacity ratio to the pool of mRNAs. In brain ischemia, hippocampal CA3 neurons, which are resilient to ischemia, assemble SGs. In contrast, CA1 neurons, which are vulnerable to ischemia, do not assemble SGs. These results suggest a critical role SG plays in regards to cell fate decisions. Thus SG assembly along with its dynamics should determine the cell fate. However, the process that exactly determines the SG assembly dynamics is largely unknown. In this paper, analyses of experimental data and computer simulations were used to approach this problem. SGs were assembled as a result of applying arsenite to HeLa cells. The number of SGs increased after a short latent period, reached a maximum, then decreased during the application of arsenite. At the same time, the size of SGs grew larger and became localized at the perinuclear region. A minimal mathematical model was constructed, and stochastic simulations were run to test the modeling. Since SGs are discrete entities as there are only several tens of them in a cell, commonly used deterministic simulations could not be employed. The stochastic simulations replicated observed dynamics of SG assembly. In addition, these stochastic simulations predicted a gamma distribution relative to the size of SGs. This same distribution was also found in our experimental data suggesting the existence of multiple fusion steps in the SG assembly. Furthermore, we found that the initial steps in the SG assembly process were critical to the dynamics. Thus our experiments and stochastic simulations presented a possible mechanism regulating SG assembly.

Publications

1. Ohshima, D. and Ichikawa, K., "Regulation of nuclear NF-κB oscillation by a diffusion coefficient and its biological implications", PLoS ONE, 9 (2014), e109895, doi:10.1371/journal.pone.0109895.
2. Sakurai-Yageta, M., Maruyama,T., Suzuki, T., Ichikawa, K., and Murakami, Y., "Dynamic Regulation of a Cell Adhesion Protein Complex Including CADM1 by Combinatorial Analysis of FRAP with Exponential Curve-fitting", PLoS ONE, accepted.
3. Ichikawa, K., Ohshima, D., and Sagara, H., "Regulation of signal transduction by spatial parameters: a case in NF-κB oscillation", IET Systems Biology, 1-11, (2014), doi:10.1049/iet-syb.2013.0020.