Donation Laboratories

Department of Molecular and Developmental Biology
再生基礎医科学寄付研究部門(SBI, トミー, ロート製薬, 慈照会)

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Our long-term goal is to understand the molecular mechanisms which coordinately regulate growth and differentiation of stem cells as well as differentiated cells with emphasis on intracellular signal transduction. For this purpose we are using models ranging from iPS and various culture cells, zebrafish, mouse, to monkey. Based on our research background on the area of cytokine signals, we now focus on the analysis of development and regeneration of neural retina.
The neural retina is a part of the central nervous system (CNS), and regeneration of the retina from retinal stem cells or other sources by transplantation is a critical issue from both clinical and neurobiological points of view. Although reports of successful regeneration of the CNS from neural stem cells (NSC) have appeared in the literature, such has not been the case for the vertebrate neural retina. Furthermore, the nature of retinal stem cells has not been clarified, making it difficult to attempt regeneration of the retina. Based on the techniques and knowledge that have been accumulated through work on of haematopoietic systems in our laboratory, we attempt to identify mammalian retinal stem cells and following developmental processes by revealing the expression pattern of cell surface proteins. We found that various CD antigens mark spatiotemporally distinct populations of retinal cells, and genes specifically expressed in such populations has been revealed by microarray analyses. Various signaling molecules and transcriptional factors are under investigation for their roles on retinal development. For developmental biological analyses, we use zebrafish in addition to mouse as model animals. We also continue to work on haematological projects, and bidirectional cooperative progress between neurological and haematological works is one of unique features of our laboratory. Projects, which gave major findings during 2013 are as follows.

1. A transposon screen for medulloblastoma cancer genes reveals new genes that regulate SHH signaling and proliferation of granule neuron precursors

Hideto Koso, Asano Tsuhako, Eli Lyons, Nancy A. Jenkins[1], Neal G. Gopeland[1], Sumiko Watanabe: [1]Division of Genetics and Genomics, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore

Medulloblastoma (MB) is the most common malignant childhood brain tumor. These tumors are thought to arise within the cerebellum, with ～25% originating from granule neuron precursor cells (GNPs) after aberrant activation of the Sonic Hedgehog (SHH) pathway. To identify novel driver genes for MB, we performed a transposon mutagenesis screen in the developing brain of wild-type and *Trp53* mutant mice. These mice are not predisposed to MBs, however mobilized transposons in-

duced MBs in ~50% of the mice. Sequencing of the transposon insertion sites from 44 tumors identified 26 candidate MB genes, including well-known MB-associated genes such as *Gli1* and *Crebbp*, with 38% of the genes involved in transcription. *Foxr2*, the most frequently mutated gene identified in the screen, is upregulated by transposon insertions, and is overexpressed in a small subset of human MBs of the SHH subtype. Two putative novel oncogenes identified in the screen, *Tgif2* and *Alx4*, are strongly expressed in the SHH subtype of human MB. Mutations in these two genes tend to co-occur and be mutually exclusive with mutations in *Gli1*, suggesting potential involvement in the SHH pathway. Consistent with this, Foxr2, *Tgif2* and *Alx4* activated the Gli-binding site in cooperation with *Gli 1*, providing evidence that these genes function in SHH signaling. We also show that *Foxr2* has transforming activity in NIH3T3 cells and promotes proliferation of GNPs. Co-transduction of *Tgif2* and *Alx4* also promoted proliferation of GNPs. Taken together, these data provide the first forward genetic and functional evidence associating *Foxr2*, *Tgif 2* and *Alx4* with MB.

2. Critical roles of timed Jmjd3 expression for the development of retinal bipolar cell-subsets

Atsumi Iida, Toshiro Iwagawa, Hiroshi Kuribayashi, Yukihiro Baba, Hiromitsu Nakauchi[2], Akira Murakami[3], Sumiko Watanabe: [2]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo, [3]Department of Ophthalmology, Juntendo University School of Medicine

Di- and tri-methylation of lysine 27 on histone H 3 (H3K27me2/3) is an important gene repression mechanism. The H3K27me2/3-specific demethylase, Jmjd3, was expressed in the inner nuclear layer during late retinal development. In contrast, H3K27 methyltransferase, Ezh2, was highly expressed in embryonic retina, but its expression decreased rapidly after birth. Jmjd3 loss-of-function in the developing retina resulted in failed differentiation of PKC-positive bipolar cell subsets (rod-ON-bipolar) and reduced Bhlhb4 expression, which is critical for the differentiation of rod-ON-bipolar cells. Expression of *Bhlhb4*, but not other bipolar cell-related genes such as *Neurod* and *Chx10*, in Jmjd3-knockdown retina rescued loss of PKC-positive bipolar cells. Populations of other retinal cell subsets were not significantly affected. In addition, proliferation activity and apoptotic cell number during retinal development was not affected by the loss of Jmjd3. Levels of the *Bhlhb4* locus H3K27me3 were lower in Islet-1-positive bipolar cells and amacrine cells than in the Islet-1-negative cell fraction, which consisted mainly of photoreceptors, suggestive of lineage-specific de-methylation of H3K27me3 in the *Bhlhb4* locus. In contrast, knockout of the H3K27 methylase, Ezh2, resulted in microphthalmia and a higher proportion of PKC positive bipolar cells. Based on these results, we propose that lineage-specific H3K 27me3 demethylation of critical gene loci by spatio-temporal-specific Jmjd3 expression is required for appropriate maturation of retinal cells.

3. Profiling of MicroRNA in Human and Mouse ES and iPS Cells Reveals Overlapping but Distinct MicroRNA Expression Patterns

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Using quantitative PCR-based miRNA arrays, we comprehensively analyzed the expression profiles of miRNAs in human and mouse embryonic stem (ES), induced pluripotent stem (iPS), and somatic cells. Immature pluripotent cells were purified using SSEA-1 or SSEA-4 and were used for miRNA profiling. Hierarchical clustering and consensus clustering by nonnegative matrix factorization showed two major clusters, human ES/iPS cells and other cell groups, as previously reported. Principal components analysis (PCA) to identify miRNAs that segregate in these two groups identified miR-187, 299-3p, 499-5p, 628-5p, and 888 as new miRNAs that specifically characterize human ES/iPS cells. Detailed direct comparisons of miRNA expression levels in human ES and iPS cells showed that several miRNAs included in the chromosome 19 miRNA cluster were more strongly expressed in iPS cells than in ES cells. Similar analysis was conducted with mouse ES/iPS cells and somatic cells, and several miRNAs that had not been reported to be expressed in mouse ES/iPS cells were suggested to be ES/iPS cell-specific miRNAs by PCA. Comparison of the average expression levels of miRNAs in ES/iPS cells in humans and mice showed quite similar expression patterns of human/mouse miRNAs. However, several mouse- or human-specific miRNAs are ranked as high expressers. Time course tracing of miRNA levels during embryoid body formation revealed drastic and different patterns of changes in their levels. In summary, our miRNA expression profiling encompassing human and mouse ES and iPS cells gave various perspectives in understanding the miRNA core regulatory networks regulating pluripotent cells characteristics.

4. Expression of Sox4 and Sox11 is regulated by multiple mechanisms during retinal development

Ayumi Usui[3], Yujin Mochizuki[3], Atsumi Iida, Akira Murakami[3], Sumiko Watanabe

Sox11 and Sox4 play critical roles in retinal development, during which they display specific and unique expression patterns. The expression of Sox 11 and Sox4 is temporally sequential, albeit spatially overlapping in some retinal subtypes. Gain-of-function and loss-of-function analyses suggested that Notch signaling suppresses Sox11 expression in the early developing retina but not during the later period of development. The levels of histone H3-acetylation and H3-lysine 4 tri-methylation at the *Sox11* locus declined during development, as did the levels of Sox11. A similar but less marked change was seen for *Sox4*. For both genes, histone H3-lysine 27 methylation was low during development and increased markedly in the adult.

5. Temporal regulation of cre activity in neural stem cells and rod photoreceptors

Hideto Koso, Asano Tsuhako, Sumiko Watanabe

Retinitis pigmentosa is one of the most common inherited diseases of the retina, which is characterized by progressive degeneration of rod photoreceptor cells. More than thirty disease-causing genes have been identified, but there is still no effective treatment to stop or cure this disease. To model rod photoreceptor degeneration in mouse, we developed a novel model, in which expression of diphtheria toxin fragment a (dta) is induced in rod photoreceptor cells upon tamoxifen administration (dta mice), thereby causing rod photoreceptor cell death. Compared to the existing models of rod photoreceptor degeneration such as *rd*/*rd* mice and light injury, this model allows photoreceptor injury in a temporally regulated and cell type specific manner. Histological examination of the retina of dta mice revealed decreased thickness of the outer nuclear layer at several weeks after tamoxifen administration. Consistent with this, scotopic erg showed decreased amplitude of a-wave responses in dta mice. Regardless of the primary causes, photoreceptor injury almost invariably leads to the activation of two types of glia: Mueller glia and microglia. We are currently investigating molecular mechanisms regulating the activation of glial cells in the damaged retina.

References

1. Mochizuki, Y., Iida, A., Lyons, E., Kageyama, R., Nakauchi, H., Murakami, A., Watanabe, S. (2013) Use of cell type-specific transcriptome to identify genes specifically involved in Müller glia differentiation during retinal development, Dev Neurobiol, Nov 4, in press
2. Usui, A., Mochizuki, Y., Iida, A., Miyauchi, E., Satoh, S., Sock, E., Nakauchi, H., Aburatani, H., Murakami, A., Wegner, M., Watanabe, S. (2013) The early retinal progenitor-expressed gene Sox 11 regulates the timing of the differentiation of retinal cells. Development, 140, 740-750
3. Okada, H., Ishibashi, H., Hayashita-Kinoh, H., Chiyo, T., Nitahara-Kasahara, Y., Baba, Y., Watanabe, S., Takeda, S., and Okada, T. (2013) Robust long-term transduction of common marmoset neuromuscular tissue with rAAV1 and rAAV9. Molecular Therapy-Nucleic Acids, 2, e95
4. Iwagawa, T, Tanaka, Y, Iida, A., Itoh, T., Watanabe, S. (2013) Enhancer/promoter activities of the long/middle wavelength-sensitive opsins of vertebrates mediated by thyroid hormone receptor β2 and COUP-TFII, Plos One, 8, e72065
5. Abdul Razak, S.R., Ueno, K., Takayama, T, Nariai, N, Nagasaki, M, Saito, R., Koso, H., Lai, C.-Y., Murakami, M, Tsuji, K., Michiue, T., Nakauchi, H, Otsu, M, Watanabe, S. (2013) Profiling of MicroRNA in Human and Mouse ES and iPS Cells Reveals Overlapping but Distinct MicroRNA Expression Patterns. Plos One, 8(9), e 73532
6. Iida, A., Iwagawa, T., Kuribayashi, H., Satoh. S., Mochizuki, Y., Baba, Y., Nakauchi, H., Furukawa, T, Koseki, H, Murakami, A., and Watanabe, S. (2014) Jmjd3 is required for the development of subsets of retinal bipolar cells, Proc.Natl.Acad.Sci., USA, 111, 3751-3756
7. Koso, H., Tsuhako, A., Lyons, E., Ward, J.M., Rust, A.G., Adams, D.J., Jenkins, N.A., Copeland, N.G., and Watanabe, S. (2014) Transposon mutagenesis identifies Foxr2 as a putative oncogene in medulloblastoma, Can Res, in press
8. 「網膜とマップキナーゼ」, Clinical Neuroscience vol. 31−6 MAPキナーゼの基礎, p 673−675, 中外医学社, 2013
9. 「2013年はiPS細胞の臨床応用の元年, 課題多い治療への道」, 週間東洋経済「2013大予測」2013年12月29日号, 東洋経済新報社, 2013

Donation Laboratories

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Tumors contain a small population of putative cancer stem cells (CSC), which possess unique self-renewal properties, and survive in a quiescent state for many years after remission and result in later relapse and metastasis. Therefore, it is conceivable that targeting CSCs will eradicate tumor-initiating cells, whereas conventional chemotherapies will only eradicate the bulk of a tumor.
Cancer stem cells and normal tissue stem cells utilize the same self-renewal pathway. However, researchers characterize some of changes, which occur in cancer stem cells, not in normal tissue stem cells. The design of new therapeutic agents should be aimed at targeting these unique molecular changes.
We have currently focused on studying these unique molecular changes, which occur in cancer stem cells, not in normal tissue stem cells. This could be a new therapeutic target against solid tumors.

A) Zinc-finger-containing transcriptional factor, Kruppel-like factor 2 (KLF2)

The Kruppel-like factor (KLF) proteins are multitasked transcriptional regulators with an expanding tumor suppressor function. KLF2 is a member of the KLF family of zinc-finger transcription factors and is involved in maintaining T-cell quiescence, regulating preadipocyte differentiation, endothelial cell function, lung development and the self-renewal of ES cells. Furthermore, KLF2 is one of the prominent members of the family because of its diminished expression in malignancies and its growth-inhibitory, pro-apoptotic and anti-angiogenic roles.

We indicate that epigenetic silencing of KLF2 occurs in cancer cells through direct transcriptional repression mediated by the Polycomb group protein Enhancer of Zeste Homolog 2 (EZH2). Binding of EZH2 to the 5'-end of KLF2 is also associated with a gain of trimethylated lysine 27 histone H3 and a depletion of phosphorylated serine 2 of RNA polymerase.

Upon depletion of EZH2 by RNA interference, short hairpin RNA or use of the small molecule 3-Deazaneplanocin A, the expression of KLF2 is restored. The transfection of KLF2 in cells with EZH2-associated silencing showed a significant anti-tumoral effect, both in culture and in xenografted nude mice.

In this last setting, KLF2 transfection was also associated with decreased dissemination and lower mortality rate. In EZH2-depleted cells, which characteristically have lower tumorigenicity, the induction of KLF2 depletion 'rescued' partially the oncogenic phenotype, suggesting that KLF2 repression has an important role in EZH2 oncogenesis.

Most importantly, the translation of the described results to human primary samples demonstrated that patients with prostate or breast tumors with

low levels of KLF2 and high expression of EZH2 had a shorter overall survival.

B) PR domain-containing protein, PRDM14

PRDM have been linked to human cancers. To explore the role of the PR domain family genes in breast carcinogenesis, we examined the expression profiles of 16 members of the PRDM gene family in a panel of breast cancer cell lines and primary breast cancer specimens using semiquantitative real-time PCR.

We found that PRDM14 mRNA is overexpressed in about two thirds of breast cancers. Moreover, immunohistochemical analysis showed that expression of PRDM14 protein is also up-regulated. PRDM14 are known as a key transcription factor required for the maintenance of hESC identity and the reacquisition of pluripotency in human somatic cells.

Introduction of PRDM14 into cancer cells reduced their sensitivity to chemotherapeutic drugs. Conversely, knockdown of PRDM14 by siRNA induced apoptosis in breast cancer cells and increased their sensitivity to chemotherapeutic drugs. Moreover, PRDM14 regulated cancer metastasis, angiogenesis, and stemness of cancer cells.

That little or no expression of PRDM14 is seen in noncancerous tissues suggests that PRDM14 could be an ideal therapeutic target for the treatment of breast cancer. Now, we also develop new methodology with nuclear acid medicine and modified antibody drug against PRDM14.

Publications

Project Associate Professor, Taniguchi Hiroaki:

1. Adachi Y, Ohashi H, Imsumran A, Yamamoto H, Matsunaga Y, Taniguchi H, Nosho K, Suzuki H, Sasaki Y, Arimura Y, Carbone DP, Imai K, Shinomura Y. The effect of IGF-I receptor blockade for human esophageal squamous cell carcinoma and adenocarcinoma. Tumour Biol, 35(2): 973-85, 2014.
2. Kato N, Yamamoto H, Adachi Y, Ohashi H, Taniguchi H, Suzuki H, Nakazawa M, Kaneto H, Sasaki S, Imai K, and Shinomura Y. Cancer detection by ubiquitin carboxyl-terminal esterase L1 methylation in pancreatobiliary fluids, World J Gastroenterol, 19(11): 1718-1727, 2013.

Professor, Kohzoh Imai:

Please refer to Center for Antibody and Vaccine Therapy.

Professor, Kouhei Tsumoto:

Please refer to Medical Proteomics Laboratory.

Project Assistant Professor, Takeharu Sakamoto:

1. Sakamoto T, Weng JS, Hara T, Yoshino S, Kozuka-Hata H, Oyama M, Seiki M.
   HIF-1 regulation through crosstalk between mTOR and MT1-MMP. Mol Cell Biol., 2014; 34 (1): 30-42.

Donation Laboratories

Division of Social Communication System for Advanced Clinical Research

先端医療社会コミュニケーションシステム社会連携研究部門

Project Professor　Masahiro Kami, M.D., Ph.D.

特任教授　医学博士　　上　　昌　広

The aim of our division is to establish and popularize state-of-art medicine and to promote translational research (TR). We investigate medical governance and the methodology to develop national consensus in health care by using media.
We also perform individual case studies on economic burden of health care on patients, medical support for disaster-stricken area by the Great East Japan Earthquake on March 11, 2011 and physician supply. In each case, we also study the system of management, information circulation, and network.

【Medical Governance】

Masaharu Tsubokura, Koichiro Yuji[1], Yasuhiro Mizuno[2], Tomoko Matsumura[3], Naoko Murashige[3], Yuko Kodama, Masahiro Kami, Tetsuya Tanimoto[3], Masayoshi Nagata[4], Haruka Nakada: [1]Center for Antibody and Vaccine Therapy, Institute of Medical Science, the University of Tokyo, [2]Maru Clinic, [3]Navitas Clinic, [4]National Center for Global Health and Medicine Center Hospital

We simulated the insufficiency of the surgeon and the pandemic of the flu, and we published the results in scientific journals (Yuji K, et al., Mizuno Y, et al., Nagata M, et al., Yamaguchi R, et al., Saito M, et al.).

【Medical support for disaster-stricken area】

Masaharu Tsubokura, Yukio Kanazawa[5], Tomoyoshi Oikawa[5], Akemi Takada[5], Tomoko Matsumura, Morihito Takita[6], Kazuhiko Kobayashi[7], Syuichi Iwamoto[3], Jinichi Mori[8], Masaki Miyasaka[9] Kenji Shibuya[10], Amina Sugimoto[10], Syuhei Nomura[10], Tetsuya Tanimoto[3], Takeaki Ishii[11], Shigeaki Kato[11], Sae Ochi[11], Giichiro Oiso[12], Yuko Kodama, Masahiro Kami: [5]Minamisoma Municipal General Hospital, [6]Baylor Research Institute, USA, [7]JR Tokyo General Hospital, [8]Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital, [9]Sendai Kousei Hospital, [10]Graduate school of Medicine, Department of Global Health Policy, The University of Tokyo, [11]Soma Central Hospital, [12]Hamamatsu University School of Medicine

Collaborating with physicians who work in disaster-stricken area and many support physicians, we conducted measurement of internal radiation exposure and gave medical guidance to the local people and publish the results in scientific journals (Tsubokura M, et al., Mori J, et al. Nomura S, et al., Tsubokura M, et al., Ishii T, et al., Tsubokura M, et al, Sugimoto A, et al). These results were widely published in news papers and popular magazines.

【Economic Burden of Health Care on Patients】

Yuko Kodama, Ryoko Morozumi[13], Akihiko Matsui[14], Masahiro Kami: [13]Faculty of Economics, University of Toyama, [14]Faculty of Economics, the University of Tokyo

We conducted a research about high medical expenses of long term patients with economists. This research will change the government's plan for the burden of high medical expenses of the patients. The economic burden on patients or the government with prevailing advanced medical care including anticancer drugs is an important issue, we continue further investigation.

【Clinically Oriented Research】

Tomoko Matsumura[3], Tetsuya Tanimoto[3], Hiroto Narimatsu[15], Natsuko Watanabe[16], Jinichi Mori[8], Yukie Takahashi[8], Masaki Miyasaka[9], Kenji Tsuda[17], Tomohiro Morita[18]: [15]Graduate School of Medicine/Cohort Management Unit, Yamagata University, [16]Ito Hospital, [17]Teikyo University Chiba Medical Center, [18]Kameda Medical Center

We studied in prophylaxis and treatment in lymphoma, heat disorder, and so on. These results were published in scientific journals (Tanimoto T, et al., Miyasaka M, et al., Tsuda K, et al. Watanabe N, et al. Morita T, et al.).

Publications

1. Mizuno Y, Narimatsu H, Kodama Y, Matsumura T, Kami M. Mid-career changes in the occupation or specialty among general surgeons, from youth to middle age, have accelerated the shortage of general surgeons in Japan. Surg Today. 2013.
2. Nagata M, Takita M, Kishi Y, Kodama Y, Matsumura T, Murashige N, Homma Y, Kami M. Cancer articles in weekly magazines: useful media to deliver cancer information to the public? Jpn J Clin Oncol. 43: 426-430, 2013.
3. Nagata M, Matsumura T, Kami K. How do Weekly Magazines Provide Information on Urogenital Cancer to the Public in Aged Societies? Open Journal of Urology, 3: 246-247, 2013
4. Yamaguchi R, Imoto S, Kami M, Watanabe K, Miyano S, Yuji K. Does Twitter trigger bursts in signature collections? PLoS One. 8: e58252, 2013.
5. Saito MM, Imoto S, Yamaguchi R, Tsubokura M, Kami M, Nakada H, Sato H, Miyano S, Higuchi T. Enhancement of collective immunity in Tokyo metropolitan area by selective vaccination against an emerging influenza pandemic. PLoS One. 8: e72866, 2013
6. Saito MM, Imoto S, Yamaguchi R, Sato H, Nakada H, Kami M, Miyano S, Higuchi T. Extension and verification of the SEIR model on the 2009 influenza A(H1N1)pandemic in Japan. Math Biosci. 246: 47-54, 2013.
7. Tanimoto T, Tsubokura M, Mori J, Pietrek M, Ono S, Kami M: Differences in drug approval processes of 3 regulatory agencies: a case study of gemtuzumab ozogamicin. Invest New Drugs. 3: 473-478, 2013.
8. Tanimoto T, Kami M, Shibuya K. Research misconduct and scientific integrity: a call for a global forum. Lancet. 382: 940, 2013.
9. Gilmour S, Kanda M, Kusumi E, Tanimoto T, Kami M, Shibuya K. HPV vaccination programme in Japan. Lancet. 382: 768, 2013.
10. Tsubokura M, Takita M, Matsumura T, Hara K, Tanimoto T, Kobayashi K, Hamaki T, Oiso G, Kami M, Okawada T, Tachiya H. Changes in metabolic profiles after the Great East Japan Earthquake: a retrospective observational study. BMC Public Health. 13: 267, 2013.
11. Mori J, Tsubokura M, Sugimoto A, Tanimoto T, Kami M, Oikawa T, Kanazawa Y. Increased incidence of dog-bite injuries after the Fukushima nuclear accident. Prev Med. 57: 363-365, 2013.
12. Nomura S, Gilmour S, Tsubokura M, Yoneoka D, Sugimoto A, Oikawa T, Kami M, Shibuya K. Mortality Risk amongst Nursing Home Residents Evacuated after the Fukushima Nuclear Accident: A Retrospective Cohort Study. PLoS One. 8: e60192, 2013.
13. Tsubokura M, Shibuya K, Kato S, Oikawa T, Kanazawa Y. Acute intake of radionuclides immediately after the incident as the main contributor of the internal radiation exposure after fukushima daiichi nuclear disaster. JAMA pediatrics. 167: 1169-1170, 2013.
14. Ishii T, Ito K, Kato S, Tsubokura M, Ochi S, Iwamoto Y, Saito Y. A report from Fukushima: an assessment of bone health in an area affected by the Fukushima nuclear plant incident. J Bone Miner Metab. 31: 613-617, 2013.
15. Tsubokura M, Nihei M, Sato K, Masaki S, Sakuma Y, Kato S, Sugimoto A, Nomura S, Matsumura T, Miyazaki M, Hayano R, Shibuya K, Kami M, Sasaki T. Measurement of internal radiation exposure among decontamination workers in villages near the crippled Fukushima Daiichi Nuclear Power Plant. Health Physics Journal. 105: 379-381, 2013.
16. Sugimoto A, Nomura S, Tsubokura M, Matsumura T, Muto K, Sato M, Gilmour S. The relationship between media consumption and health-related anxieties after the Fukushima Daiichi nuclear disaster. PLoS One. 8: e65331: 1-7, 2013.
17. Tsubokura M, Kato S, Nihei M, Sakuma Y, Furutani T, Uehara K, Sugimoto A, Nomura S, Hayano R, Kami M, Watanobe H, Endo Y. Limited internal radiation exposure associated with

resettlements to a radiation-contaminated homeland after the fukushima daiichi nuclear disaster. PLoS One. 8: e81909, 2013.
18. Tsubokura M, Nomura S, Ishii T, Kami M, Kato S. Detection of 131I in a Patient With Thyroid Cancer by Internal Radiation Exposure Screening Using a Whole-Body Counter in Fukushima. Clin Nucl Med. 39: 281-282, 2014.
19. Tanimoto T, Oshima Y, Kami M. Eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 369: 1378-1379, 2013.
20. Nomura S, Gilmour S, Tsubokura M, Yoneoka D, Sugimoto A, Oikawa T, Kami M, Shibuya K. Mortality Risk amongst Nursing Home Residents Evacuated after the Fukushima Nuclear Accident: A Retrospective Cohort Study. PLoS One. 8: e60192, 2013.
21. Nishikawa Y, Okuda S, Takebayashi C, Tanimoto T, Kami M, Kobayashi K. A case of late onset erythropoietic protoporphyria associated with myelodysplastic syndrome treated by the combination of beta carotene and azacitidine. Ann Hematol. 92: 1415-1416, 2013.
22. Miyasaka M, Hamaki T, Kami M, Hamabe Y. A patient with heatstroke associated with consciousness disturbance secondary to hyponatremia: a case report. J Med Case Rep. 7: 62, 2013.
23. Tsuda K, Tanimoto T, Komatsu T. Treatment for high-risk smoldering myeloma. N Engl J Med. 369: 1763, 2013.
24. Watanabe N, Narimatsu H, Noh JY, Kunii Y, Mukasa K, Matsumoto M, Suzuki M, Sekiya K, Ohye H, Yoshihara A, Iwaku K, Kobayashi S, Kameyama K, Kobayashi K, Nishikawa Y, Kami M, Sugino K, Ito K.. Rituximab-including combined modality treatment for primary thyroid lymphoma: an effective regimen for elderly patients. Thyroid. 2014.
25. Morita T, Tanimoto T, Kaji S, Fukutake T. Post-stroke crowned dens syndrome. Spine J. 13: 1161-1162, 2013.
26. Tsuda K, Morita T, Tsubokura M, Matsumura T, Kami M. Therapeutic platelet transfusion for hypoproliferative thrombocytopenia. Lancet. 381: 723-724, 2013.
27. Takahashi Y, Nishikawa Y, Mori J, Hamaki T, Tanimoto T. Therapeutic platelet transfusion for hypoproliferative thrombocytopenia. Lancet. 381: 724, 2013.
28. Nishikawa Y, Tanimoto T, Tsubokura M, Kami M, Saito Y. Treatment for patients with indolent and mantle cell lymphoma. Lancet. 382: 1093-1094, 2013.
29. Yamaguchi T, Morita T, Takahashi Y, Tsuda K, Mori J. Treatment for patients with indolent and mantle cell lymphoma. Lancet. 382: 1094-1095, 2013.
30. Honda K, M Ando M, Tsubokura M, Yamashita T, Akiyama H, Sakamaki H. An autopsy case that manifested no convincing histological changes of severe renal failure after hematopoietic stem cell transplantation. CEN Case Rep. Japanese Society of Nephrology. 2013.
31. 古島智恵，井上範江，分島るり子，児玉有子，村田尚恵，高島利．不眠を訴える入院患者への足浴の効果 不眠の種類による検討．日本看護技術学会誌．12(1)：85−94，2013.
32. 永田政義，松村有子，本間之夫，上昌広．週刊誌のがん報道：週刊誌はがん情報を伝える有益な媒体である得るか？ 日本腎泌尿器疾患予防医学研究会誌．21(1)：76−78，2013.

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Interactome Medical Sciences Laboratory
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In our lab, the interactome (protein-protein interactions) is analyzed using technology developed in Japan and referred to as "puromycin technology". Toward the "personalized medicine" era, we will apply these tools to medical science. To develop the "interactome analysis pipeline", we are collaborating with the human genome center, where large amounts of sequence data are generated from next generation sequencers and analyzed using a supercomputer. In particular, our research focuses on "cancer", and we collaborate with cancer researchers. For a cooperative study involving society and academia, our research involves industrial partnerships.

Efficiency of puromycin-based technologies mediated by release factors and a ribosome recycling factor

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Two useful puromycin-based techniques, *in vitro* virus (IVV) and C-terminal labelling of proteins, were developed based on the observation that puromycin can bind to the C-terminus of a full-length protein. Puromycin technology is a useful tool for the detection of proteins and analysis of protein-protein interactions (PPIs); however, problems arise due to the existence of stop codons in the native mRNAs. Release factors (RFs) enter the A site of the ribosome at stop codons, which inevitably compete with the puromycin. To overcome this difficulty, we have used a highly controllable reconstituted cell-free system for puromycin-based techniques and observed efficient IVV formation and C-terminal labelling using templates possessing a stop codon. The optimal conditions of IVV formation using templates possessing a stop codon was RF (−), while that of C-terminal labelling was RF (−) and the ribosome recycling factor (RRF) (+). Thus, we have overcome the experimental limitations of conventional IVV. In addition, we discovered that RRF significantly increases the efficiency of C-terminal protein labelling, but not IVV formation. This approach overcomes the experimental limitations of conventional IVV. Thus, we believe that our results support the practical use of puromycin-based techniques for evolutionary protein engineering and comprehensive proteome research.

Towards Personalized Medicine Mediated by *in Vitro* Virus-based Interactome Approaches

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We have developed a simple *in vitro* virus (IVV) selection system based on cell-free co-translation, using a highly stable and efficient mRNA display method. The IVV system is applicable to the high-throughput and comprehensive analysis of proteins and protein-ligand interactions. Huge amounts of genomic sequence data have been generated over

the last decade. The accumulated genetic alterations and the interactome networks identified within cells represent a universal feature of a disease, and knowledge of these aspects can help to determine the optimal therapy for the disease. The concept of the 'integrome' has been developed as a means of integrating large amounts of data. We have developed an interactome analysis method aimed at providing individually-targeted health care. This will facilitate archiving of the interactome map of a whole-cell library at low cost. We suggest that IVV systems can provide an important contribution to our understanding of the interactome networks in cancer cells, and thus help in the development of pharmaceutical agents to treat currently intractable diseases.

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

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Virus-derived double-stranded RNAs (dsRNAs) are sensed in the cytosol by retinoic acid-inducible gene (RIG)-I-like receptors (RLRs). These induce the expression of type I IFN and proinflammatory cytokines through signaling pathways mediated by the mitochondrial antiviral signaling (MAVS) protein. TNF receptor-associated factor (TRAF) family proteins are reported to facilitate the RLR-dependent expression of type I IFN by interacting with MAVS. However, the precise regulatory mechanisms remain unclear. We investigated 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 first identified by "*in vitro* virus" selection and was subsequently confirmed with a coimmunoprecipitation assay in HEK293T cells. 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. Besides TRAF 6, we found that FKBP51 also interacts with TRAF3. The depletion of FKBP51 reduced the expression of type I IFN induced by dsRNA transfection or Newcastle disease virus infection in murine fibroblasts. Consistent with this, FKBP51 depletion delayed the phosphorylation of IRF3 induced by dsRNA stimulation. Interestingly, dsRNA stimulation promoted the accumulation of FKBP51 in the mitochondria. Moreover, the overexpression of FKBP51 inhibited RLR-dependent transcriptional activation, suggesting a scaffolding function for FKBP51 in the MAVS-mediated signaling pathway. Overall, we have demonstrated that FKBP51 interacts with TRAF proteins and facilitates the expression of type I IFN induced by cytosolic dsRNA. These findings suggest a novel role for FKBP51 in the innate immune response to viral infection.

The analysis of transcription factor networks using the IVV method

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We have developed a simple and totally *in vitro* selection procedure based on cell-free cotranslation using a highly stable and efficient *in vitro* virus (IVV). Cell-free cotranslation of tagged bait and prey proteins is advantageous for the formation of protein complexes and allows high-throughput analysis of protein-protein interactions (PPI) as a result of providing *in vitro* instead of *in vivo* preparation of bait proteins. The use of plural selection rounds and a two-step purification of the IVV selection, followed by *in vitro* post-selection, is advantageous for decreasing false positives. This simple IVV selection system based on cell-free cotranslation is applicable to high-throughput and comprehensive analysis of transcription factor networks.

The Dynamic Whole-Cell Omics Analyses to Understand and Control Cancer Stem Cells

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Recent scientific discoveries resulted from the next-generation sequencing (NGS) highlight the striking impact of massively parallel sequencing data on genetics. Thus, the interest of basic research in the medical care has changed from the conventional post-genomic to the personal genomic research, especially for cancer therapy. To understand the individuality of cancer, we have to conduct various dynamic omics analyses and comprehend

the individual biomolecular networks for each cell type. Our research focuses on diversity of cancer stem cells (CSCs), and is aimed at discerning and regulation of CSCs by the collaboration of researchers, who have the experiment system for studying CSCs, the technology for omics analysis, and the network analysis technique. We have model CSCs derived from bone-marrow stromal cells of Ink4a/Arf KO mice (Shimizu et al., *Oncogene*. 2010). To compare those CSCs having distinct characters, we attempt to conduct "dynamic whole-cell omics"; analyses, especially for interactome sequencing. To collect comprehensive protein level's data from NGS, we have developed IVV-HiTSeq (Fujimori et al., Scientific Reports 2012) standing for the *In Vitro* Virus method (Miyamoto-Sato et al., PLoS ONE 2010) coupled with High-Throughput Sequencing. IVV-HiTSeq can obtain reliable interactome data suitable for the medical field due to low false positives. Moreover, we attempt to develop the bait-free IVV method as IVV square (IVV^2) to obtain not partial but a whole-cell?interactome. IVV^2 also detects 'interacting regions (IR)', allowing us to analyze relations between interactome and aberrance (e.g., DNA mutation) observed in cancer. Accordingly, we have developed the database to investigate such relations.

A whole-cell Interactome Mapping using IVV Square toward Personal Genomics

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In the last decade, huge amount of genomic sequence data have been determined and gathered. The Human genome project provided the human genome sequence. Understanding the genetic sequences of individual patients is becoming a central feature of medical care. It is very important that the accumulation of genetic alterations and the interactome networks in cancer cells represents a universal feature of the disease. The knowledge can contribute to determining optimal therapy for the disease. Since protein-protein interactions (PPIs) are at the core of the biomolecule network, we have developed *in vitro virus* (IVV) system, which is a mRNA display, to detect PPIs toward personal genomics. We have succeeded to obtain significant results of interactome analysis by using the system. On the other hand, the system has a limitation in the high-throughput screening and identification of interaction pairs of proteins, due to the time consuming preparation of bait proteins and the low ability of the conventional sequencing method. To overcome the problem, we are trying to develop "a bait-free IVV", termed IVV Square (IVV^2), which enables genes encoding interacting protein pairs to be linked. This will facilitate archiving of the interactome mapping of a whole-cell library. IVV^2 libraries will be subjected to the high-throughput sequencing with the next-generation sequencers to generate interactome information. We believe that the new system will contribute to understand the interactome networks in cancer cells, and to develop pharmaceutical drugs to treat intractable diseases.

Publications

Hiroyuki Ohashi, Masamichi Ishizaka, Naoya Hirai, Etsuko Miyamoto-Sato: Efficiency of puromycin-based technologies mediated by release factors and a ribosome recycling factor. Protein Engineering, Design and Selection, 26(8): 533-537 (2013)

Hiroyuki Ohashi, and Etsuko Miyamoto-Sato: Towards Personalized Medicine Mediated by *in Vitro* Virus-based Interactome Approaches. International Journal of Molecular Sciences: (2014), *in press*

Hiroyuki Ohashi, Shigeo Fujimori, Naoya Hirai, Hiroshi Yanagawa, Etsuko Miyamoto-Sato: The analysis of transcription factor networks using the IVV method. Methods in Molecular Biology: chapter 2. (2014), *in press*

Etsuko Miyamoto-Sato: Next-generation sequencing coupled with a cell-free display technology for reliable interactome of translational factors. Methods in Molecular Biology: chapter 3. (2014), *in press*

Etsuko Miyamoto-Sato et al. (eds.): Transcription Factor Regulatory Networks - Methods and Protocols. *Methods in Molecular Biology, Springer*, (2014) *in press.*

宮本悦子，西川純一：インタラクトームが拓くパーソナル医療への展開．実験医学．31：93–99（増刊）2013.

Etsuko Miyamoto, Toru Tsuji, Shigeo Fujimori, Masamichi Ishizaka: CONSTITUTION OF TOOL FOR ANALYZING BIOMOLECULAR INTERACTION AND ANALYSIS METHOD USING SAME. PCT/ European Patent /2554681 (2013)

Etsuko Miyamoto, Toru Tsuji, Shigeo Fujimori, Masamichi Ishizaka: CONSTITUTION OF TOOL FOR ANALYZING BIOMOLECULAR INTERACTION AND ANALYSIS METHOD USING SAME. PCT/ US Patent/ 2013/0196331 (2013)

宮本悦子，辻融，藤森茂雄，石坂正道：生体分子相互作用解析ツールの構成とそれを用いた解析方法，

国立大学法人東京大学（JP02），日本，公開番号：WO2011/125833（2013）
宮本悦子，藤森茂雄：標的物質と相互作用するタンパク質の検出方法，国立大学法人東京大学（WO01），世界知的所有権機関（WIPO），公開番号：WO2012/047785（2013）
宮本悦子，藤森茂雄：標的物質と相互作用するタンパク質の検出方法，国立大学法人東京大学（JP01），日本，公開番号：2013070680（2013）

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RNA no longer stands behind DNA or protein but stands in front of DNA and protein. Recent achievements and discovery in biological science clearly emphasize the importance of RNA in life; the discovery of RNA interference, molecular mimicry between protein and RNA, ribosome structure at atomic resolution, and RNA/polypeptide quality control triggered by aberrant mRNAs. Moreover, the completed human genome project revealed, to our great surprise, the existence of a large amount of protein-noncoding RNAs (ncRNAs). These ncRNAs can be classified into two types: one, like antisense and microRNA, those function with the sequence complementarity to the target mRNA or DNA, while the other, like aptamer, those function independent of the sequence complementarity. In our laboratory, we aim to: 1) uncover the molecular mechanism underlying the mRNA surveillance and aberrant product clearance; and 2) create artificial aptamers to target proteins of therapeutic interest. Additionally, molecular biology of the 'prion' nature associated with yeast translation factor Sup35 is under investigation in this laboratory.

1. Nonstop mRNA and Protein degradation in *Drosophila melanogaster* cells

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Nonstop mRNA decay (NSD) is quality-control mechanism that detects and degrades mRNAs lacking stop codons. Nonstop protein degradation (NSPD) eliminates aberrant proteins derived from nonstop mRNA. The previous studies in yeast indicate that the Dom34:Hbs1 complex, which are paralogues of mammalian eRF1:eRF3 complex, promotes peptidyle-tRNA release and stalled ribosome dissociation by binding to the empty A site of the stalled ribosome. This enables access of the Ski: Exosome exonuclease complex at 3' end of nonstop mRNAs for subsequent NSD. The E3 ubiquitin ligase Ltn1 is responsible for proteasome-dependent aberrant nonstop polypeptides degradation (NSPD) following the dissociation of ribosome. Here we show that Pelota (Dom34 paralog) and Hbs1 are involved in the degradation of nonstop mRNAs in shared as well as distinct manners compared with yeast, and Ltn1 is essential for NSPD via proteasome pathway in *Drosophila melanogaster* cells.

2. Therapeutic Aptamer Discovery

a. Selection of RNA aptamer against fibroblast growth factor 2 and its therapeutic application

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Fibroblast growth factor is a class of heparin

binding proteins that mediate a variety of cellular responses during embryonic development and in the adult organism. Fibroblast growth factor 2 (FGF2 or bFGF for basic fibroblast growth factor) has an important role in proliferation, migration, angiogenesis and morphogenesis. Secreted FGF2 protein activates several cell signaling such as JNK, MAPK, via through FGF receptors on the cell surface. Recent reports suggested that the expression level of FGF2 protein is enhanced expression in rheumatoid arthritis (RA) patients, and also elevated in rat joints of adjuvant-induced arthritis (AIA).

By screening of a large library of nuclease-resistant RNA oligonucleotides by SELEX, we selected an RNA aptamer that bind human and mouse FGF2 proteins with high affinity and specificity. The selected aptamer binds strongly to FGF2, but not to FGF1, and inhibits the interaction between FGF2 and its receptor when examined by the surface plasmon resonance (SPR) analysis. Consistently, the aptamer prevented efficient phosphorylation of FGF2 signaling factors, FRS2 and MAPK, in the cell-based assay with NIH3T3. Furthermore, the aptamer inhibited the FGF2-dependent repression of osteoprotegerin (OPG) secretion in HFLS-RA (Human Fibroblast-Like Synoviocytes cells from RA patient).

We then demonstrated that *in vivo* efficacy of the aptamer using GPI-induced rheumatoid arthritis mice model. When administered immediately after immunization with GPI, the aptamer inhibited the development of arthritic symptoms in a dose-dependent manner. Significantly, the aptamer slowed the progression of arthritis when administered after the onset of GPI induced arthritis. Our findings indicate that the chemically processed anti-FGF2 aptamer inhibits FGF2 action and the development of RA in mouse models. These results offer, for the first time an aptamer-based therapeutic approach for FGF2 related disorders.

b. An RNA aptamer targeting the growth factor midkine suppresses the tumorigenesis of neuroblastoma

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Midkine is a heparin-binding growth factor highly expressed in various cancers, including neuroblastoma, the most common extracranial pediatric solid tumor. Prognosis of patients with neuroblastoma in which MYCN is amplified remains particularly poor. In this study, we used a MYCN transgenic model for neuroblastoma in which midkine is highly expressed in precancerous lesions of sympathetic ganglia. Genetic ablation of midkine in this model delayed tumor formation and reduced tumor incidence. Furthermore, an RNA aptamer that specifically bound midkine suppressed the growth of neuroblastoma cells in vitro and in vivo in tumor xenografts. In precancerous lesions, midkine-deficient MYCN transgenic mice exhibited defects in activation of Notch2, a candidate midkine receptor, and expression of the Notch target gene HES1. Similarly, RNAaptamer-treated tumor xenografts also showed attenuation of Notch2-HES1 signaling. Our findings establish a critical role for the midkine-Notch2 signaling axis in neuroblastoma tumorigenesis, which implicates new strategies to treat neuroblastoma.

3. Molecular Biology of Yeast Prions.

a. Clearance of yeast prions by misfolded multi-transmembrane proteins

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Accumulation of misfolded proteins in the endoplasmic reticulum (ER) induces the stress response to protect cells against toxicity by the unfolded protein response (UPR), heat shock response (HSR), and ER-associated degradation pathways. Here, we found that over-production of C-terminally truncated multi-transmembrane (MTM) mutant proteins triggers HSR, but not UPR, and clearance of yeast prions [*PSI*$^+$] and [*URE3*]. One of the mutant MTM proteins, Dip5ΔC-v82, produces a disabled amino-acid permease. Fluorescence microscopy analysis revealed abnormal accumulation of Dip5ΔC-v82 in the ER. Importantly, the mutant defective in the GET pathway, which functions for ER membrane insertion of tail-anchored proteins, failed to translocate Dip5ΔC-v82 to the ER and disabled Dip5ΔC-v 82-mediated prion clearance. These findings suggest that the GET pathway plays a pivotal role in quality assurance of MTM proteins, and entraps misfolded MTM proteins into ER compartments, leading to loss-of-prion through a yet undefined mechanism.

b. Clearance of yeast eRF-3 prion [PSI$^+$] by amyloid enlargement due to the imbalance between chaperone Ssa1 and cochaperone Sgt2

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The cytoplasmic [*PSI*$^+$] element of budding yeast represents the prion conformation of translation release factor eRF-3 (Sup35). Prions are transmissible agents caused by self-seeded highly ordered aggregates (amyloids). Much interest lies in understanding how prions are developed and transmitted. However, the cellular mechanism involved in the prion clearance is unknown. Recently we have found that excess misfolded multi-transmembrane protein, Dip5ΔC-v82, eliminates yeast prion [*PSI*$^+$]. In this study, we showed that the prion loss was caused by enlargement of prion amyloids, unsuitable for transmission, and its efficiency was affected by the cellular balance between the chaperone Hsp70-Ssa1 and Sgt2, a small cochaperone known as a regulator of chaperone targeting to different types of aggregation-prone proteins. The present findings suggest that Sgt2 is titrated by excess Dip5ΔC-v82, and the shortage of Sgt2 led to non-productive binding of Ssa1 on [*PSI*$^+$] amyloids. Clearance of prion [*PSI*$^+$] by the imbalance between Ssa1 and Sgt2 might provide a novel array to regulate the release factor function in yeast.

Publications

1. Arai, C., Kurahashi, H., Ishiwata, M., Oishi, K., Nakamura, Y.: Clearance of yeast prions by misfolded multi-transmembrane proteins. Biochimie, 95: 1223-1232 (2013)
2. Oishi, K., Kurahashi, H., Pack, C-G., Sako, Y., Nakamura, Y.: A bipolar functionality of Q/N-rich proteins: Lsm4 amyloid causes clearance of yeast prions. MicrobiologyOpen, 2: 415-430 (2013).
3. Kishida, S., Ping, M., Miyakawa, S., Fujiwara, M., Abe, T., Sakamoto, K., Onishi, A., Nakamura, Y., Kadomatsu, K.: Midkine promotes neuroblastoma through Notch2 signaling. Cancer Res., 73(4): 1318-1327 (2013).
4. Ohuchi, S., Shibuya, M., Nakamura, Y.: The RNA aptamer inhibiting human vesicular endothelial growth factor receptor 1 without affecting the cytokine binding. Biochemistry, 52: 2274-2279 (2013)
5. Fukunaga, J., Nomura, Y., Tanaka, Y., Tanaka, T., Nakamura, Y., Kawai, G., Sakamoto, T., Kozu, T.: The Runt domain of AML1 (RUNX1) binds a sequence-conserved RNA motif that mimics a DNA element. RNA, 19: 927-936 originally published online May 24, 2013 (2013)
6. Soeno, Y., Fujita, K., Kudo, T., Asagiri, M., Kakuta, S., Taya, Y., Shimazu, Y., Sato, K., Kubo, S., Iwakura, Y., Nakamura, Y., Aoba, T.: Generation of a mouse model with down-regulated U50 snoRNA (SNORD50) expression and its organ-specific phenotypic modulation. PLoS ONE, 8: e72105 (2013)
7. Arai, C., Kurahashi, H., Pack, C.-G., Sako, Y., Nakamura, Y.: Clearance of yeast eRF-3 prion [*PSI*$^+$] by amyloid enlargement due to the imbalance between chaperone Ssa1 and cochaperone Sgt2. Translation, 1:2, e26574 (2013).
8. Nomura, Y., Tanaka, Y., Fukunaga, J., Fujiwara, K., Chiba, M., Iibuchi, H., Tanaka, T., Nakamura, Y., Kawai, G., Kozu, T., Sakamoto, T.: Solution structure of a DNA mimicking motif of an RNA aptamer against transcription factor AML1 Runt domain. J Biochem., 154: 513-9 (2013)

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Division of Bacterial Infection Biology

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Many pathogenic bacteria, including Shigella, enteropathogenic Escherichia coli (EPEC), and enterohemorrhagic E. coli (EHEC), are associated with diarrheal diseases and are an important cause of death in many countries. Our current interest is to understand the complex interactions among pathogenic bacteria, the gastrointestinal epithelium and microbiota during pathogenic bacteria infection. The main goal of our research is to develop new therapeutic tools or vaccines that will target these bacterial infections.

1. Structural basis for the recognition of Ubc13 by the *Shigella flexneri* effector OspI.

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Ubc13 is a ubiquitin-conjugating enzyme that plays a key role in the nuclear factor-κB signal transduction pathway in human diseases. The *Shigella flexneri* effector OspI affects inflammatory responses by catalyzing the deamidation of a specific glutamine residue at position 100 in Ubc13 during infection. This modification prevents the activation of the TNF (tumor necrosis factor) receptor-associated factor 6, leading to modulation of the diacylglycerol-CBM (CARD-Bcl10-Malt1) complex-TNF receptor-associated factor 6-nuclear factor-κB signaling pathway. To elucidate the structural basis of OspI function, we determined the crystal structures of the catalytically inert OspI C62A mutant and its complex with Ubc13 at resolutions of 3.0 and 2.96 Å, respectively. The structure of the OspI-Ubc13 complex revealed that the interacting surfaces between OspI and Ubc13 are a hydrophobic surface and a complementary charged surface. Furthermore, we predict that the complementary charged surface of OspI plays a key role in substrate specificity determination.

2. The *Shigella* OspC3 effector inhibits caspase-4, antagonizes inflammatory cell death, and promotes epithelial infection.

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Caspase-mediated inflammatory cell death acts as an intrinsic defense mechanism against infection. Bacterial pathogens deploy countermeasures against inflammatory cell death, but the mechanisms by which they do this remain largely unclear. In a screen for *Shigella flexneri* effectors that regulate cell death during infection, we discovered that *Shigella* infection induced acute inflammatory, caspase-4-dependent epithelial cell death, which is counteracted by the bacterial OspC3 effector. OspC3 interacts with the caspase-4-p19 subunit and inhibits its activation by preventing caspase-4-p19 and caspase-4-p 10 heterodimerization by depositing the conserved OspC3 X1-Y-X_2-D-X_3 motif at the putative catalytic pocket of caspase-4. Infection of guinea pigs with a *Shigella* ospC3-deficient mutant resulted in enhanced inflammatory cell death and associated symptoms, correlating with decreased bacterial burdens. *Salmonella Typhimurium* and *enteropathogenic Escherichia coli* infection also induced caspase-4-dependent epithelial death. These findings highlight the importance of caspase-4-dependent innate immune responses and demonstrate that Shigella delivers a caspase-4-specific inhibitor to delay epithelial cell death and promote infection.

3. *Shigella* IpaH0722 E3 ubiquitin ligase effector targets TRAF2 to inhibit PKC-NF-κB activity in invaded epithelial cells.

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NF-κB plays a central role in modulating innate immune responses to bacterial infections. Therefore, many bacterial pathogens deploy multiple mechanisms to counteract NF-κB activation. The invasion of and subsequent replication of *Shigella* within epithelial cells is recognized by various pathogen recognition receptors as pathogen-associated molecular patterns. These receptors trigger innate defense mechanisms via the activation of the NF-κB signaling pathway. Here, we show the inhibition of the NF-κB activation by the delivery of the IpaH E3 ubiquitin ligase family member IpaH0722 using *Shigella*'s type III secretion system. IpaH0722 dampens the acute inflammatory response by preferentially inhibiting the PKC-mediated activation of NF-κB by ubiquitinating TRAF2, a molecule downstream of PKC, and by promoting its proteasome-dependent degradation.

Publications

Nishide A, Kim M, Takagi K, Himeno A, Sanada T, Sasakawa C, Mizushima T. Structural basis for the recognition of Ubc13 by the *Shigella flexneri* effector OspI. *J Mol Biol*, 425, 2623-2631, 2013.

Kobayashi T, Ogawa M, Sanada T, Mimuro H, Kim M, Ashida H, Akakura R, Yoshida M, Kawalec M, Reichhart JM, Mizushima T, Sasakawa C. The *Shigella* OspC3 effector inhibits caspase-4, antagonizes inflammatory cell death, and promotes epithelial infection. *Cell Host and Microbe*, 13, 570-583, 2013

Ashida H, Nakano H, Sasakawa C. *Shigella* IpaH 0722 E3 ubiquitin ligase effector targets TRAF2 to inhibit PKC-NF-κB activity in invaded epithelial cells. *PLos Pathog* 9, e1003409, 2013