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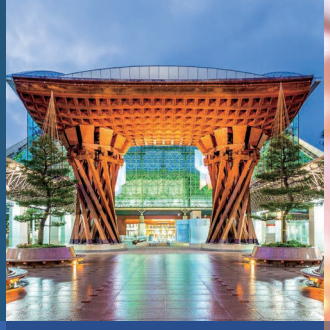
Cancer Research Institute
Kanazawa University



The 17th International Symposium of the Institute Network for Biomedical Sciences International Symposium on Tumor Biology in Kanazawa 2022
2022 CRI & DUKE-NUS Joint Symposium

Fundamental Biological Principles and Cancer

Cancer Research Institute of Kanazawa University

October 13-14, 2022
Kanazawa University, Japan

The 17th International Symposium of the Institute Network for Biomedical Sciences
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Fundamental Biological Principles and Cancer

Cancer Research Institute of Kanazawa University
October 13-14, 2022
Kanazawa University, Japan

Thursday, October 13, 2022

12:30-13:00 **Registration**

13:00-13:05 **Opening Remarks**
Kunio Matsumoto (Director of Cancer Research Institute, Vice president, Kanazawa University)

Session 1 (Chair Noriko Goto, Masaya Ueno)

13:05-13:30 S-01: **Tetsuro Yoshimaru** (Institute of Advanced Medical Sciences, Tokushima University) ・・・1
The plasma membrane BIG3-PHB2 complex contributes to the acquisition of trastuzumab-resistance in HER2-positive breast cancer

13:30-13:55 S-02: **Kozo Tanaka** (Institute of Development, Aging and Cancer, Tohoku University) ・・・3
Chromosomal instability induced by chromosome dynamics in cancer cells

13:55-14:07 Y-01: **Yutaka Kasai** (The Institute of Medical Science, The University of Tokyo) ・・・5
Trans-homophilic interaction of CADM1 promotes organ infiltration of T-cell lymphoma by adhesion to vascular endothelial cells

14:07-14:19 Y-02: **Shunya Tsuji** (Research Institute for Microbial Diseases, Osaka University) ・・・7
SARS-CoV-2 and cellular senescence

14:19-14:31 Y-03: **Taisho Yamada** (Institute for Genetic Medicine, Hokkaido University) ・・・9
RIG-I restrains SARS-CoV-2 replication in human lung cells without activation of innate immune signaling

14:31-14:45 **Break**

International Symposium (Chair Masanobu Oshima, Eishu Hirata)

14:45-14:50 **Opening Remarks**
Shinichi Nakamura (Trustee(Research, Social Co-creation, and Graduate School Support) ,Kanazawa University)

14:50-15:20 I-01: **Koji Itahana** (DUKE-NUS, Singapore) ・・・57
The potential role of multi-drug resistance protein ABCB1 in tumor suppression in bats

15:20-15:32 Y-04: **Yasuto Takeuchi** (Cancer Research Institute, Kanazawa University) ・・・59
The membrane-linked adaptor FRS2・ fashions a cytokine-rich microenvironment that promotes breast cancer carcinogenesis

15:32-16:02 I-02: **S. Tiong Ong** (DUKE-NUS, Singapore) ・・・61
Predicting primary resistance to cancer targeted therapies at diagnosis: lessons from chronic myeloid leukemia

16:02-16:10 **Break**

16:10-16:35 S-03: **Yoshikazu Johmura** (Cancer Research Institute, Kanazawa University) ・・・63
Identification and functional analysis of senescent cells in tumor microenvironment

16:35-17:05 I-03: **Enrico Petretto** (DUKE-NUS, Singapore) ・・・65
Systems Genetics identifies WWP2 as a new target for fibrotic disease in fibroblasts and macrophages

17:05-17:20 **Break**

Session 2 (Chair Atsushi Hirao)

17:20-17:45 S-04: **Kosuke Hashimoto** (Institute for Protein Research, Osaka University) ・・・11
Single-cell transcriptome analysis of human immune cells and early embryos

17:45-17:57 Y-05: **Ryoji Kawakami** (Institute for Life and Medical Sciences, Kyoto University) ・・・13
Coordinated activation of enhancer elements for thymic Treg development and immunological self-tolerance

17:57-18:09 Y-06: **Akiko Satoh** (Institute of Development, Aging and Cancer, Tohoku University) ・・・15
The role of hypothalamic neurons in sleep, aging and longevity

Friday, October 14, 2022

Sesson 3 (Chair Dominic Voon, Kazuhiro Murakami)

9:00-9:25 S-05: **Kazuhiro Suzuki** (Research Institute for Microbial Diseases, Osaka University) ・・・17
Novel control mechanisms of lymphocyte trafficking

9:25-9:50 S-06: **Tetsuro Izumi** (Institute for Molecular and Cellular Regulation, Gunma University) ・・・19
Roles of the GDF3-ALK7 axis in adiposity

9:50-10:02 Y-07: **Junko Sasaki** (Medical Research Institute, Tokyo Medical and Dental University) ・・・21
Premature ovarian insufficiency in mice lacking phosphoinositide-metabolizing enzymes

10:02-10:14 Y-08: **Kou Motani** (Institute of Advanced Medical Sciences, Tokushima University) ・・・23
ACBD3 forms specialized ER-Golgi contact sites to drive the ER exit of STING

10:14-10:26 Y-09: **Macpherson Tom** (Institute for Protein Research, Osaka University) ・・・25
Cell type-specific control of reward learning in the brain

10:26-10:40 **Break**

Session 4 (Chair Toshinari Minamoto, Ryu Imamura)

10:40-11:05 S-07: **Nobuo Noda** (Institute for Genetic Medicine, Hokkaido University) ・・・27
Molecular mechanisms underlying autophagosome biogenesis

11:05-11:17 Y-10: **Eiji Miyauchi** (Institute for Molecular and Cellular Regulation, Gunma University) ・・・29
Gut microbiota modulates inflammation in the central nervous system

11:17-11:29 Y-11: **Minako Ito** (Medical Institute of Bioregulation, Kyushu University) ・・・31
Immune cell dynamics and role in central nervous system diseases

11:29-11:41 Y-12: **Jun Hatakeyama** (Institute of Molecular Embryology and Genetics, Kumamoto University) ・・・33
Strategies for the expansion of cerebral cortex in primates

11:41-13:00 **Lunch**

Poster Session & Coffee Break

13:00-14:00 P-01: **Yukako Nishimura** (Institute for Genetic Medicine, Hokkaido University) ・・・45
A cross-talk between microtubules and focal adhesions regulates dynamics of actin cytoskeleton

P-02: **Madoka Kawaguchi** (Institute of Development, Aging and Cancer, Tohoku University) ・・・45
Extracellular Domains I and II of CD44 mediate its trans-homophilic dimerization and tumor cluster aggregation

Session 5 (Chair Chiaki Takahashi)

15:15-15:25 **Break**

Session 6 (Chair Takashi Suda)

16:15-16:30 **Award Ceremony & Closing Remarks**
Kunio Matsumoto (Director of Cancer Research Institute, Vice president, Kanazawa University)

Session1

S - 0 1

The plasma membrane BIG3-PHB2 complex contributes to the acquisition of trastuzumab-resistance in HER2-positive breast cancer

Yoshimaru[1], Tetsuro; Matsushita[1], Yosuke; Kosako[2], Hidetaka; Sasa[3], Mitsunori; Miyoshi[4], Yasuo; Katagiri[1], Toyomasa

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[3] Department of Surgery, Tokushima Breast Care Clinic, Japan
[4] Division of Breast and Endocrine Surgery, Department of Surgery, Hyogo College of Medicine, Japan

The human epidermal growth factor receptor 2 (HER2) amplification is linked to aggressive tumor behavior and a poorer prognosis in breast cancer. Although the anti-HER2 antibody, trastuzumab, have greatly improved outcomes of HER2-positive breast cancer, resistance acquisition remains a severe clinical problem. Several resistance mechanisms against trastuzumab, such as proteolytic cleavage of the extracellular domain of HER2, alternative activation of downstream signaling, and EGFR overexpression, have been proposed, but a conclusive trastuzumab-resistant acquisition mechanism still has not been revealed. Therefore, the most urgent issue is to develop a novel therapeutic approach targeting resistance acquisition mechanisms.

We previously reported that the cancer-specific A-kinase anchoring protein BIG3 (brefeldin A-inhibited guanine nucleotide exchange protein 3) acts as a critical modulator of estrogen signaling in breast cancer cells by inhibiting the tumor-suppressive activity of prohibitin 2 (PHB2). Interestingly, RNA-seq data in public databases revealed high expression of the BIG3 in refractory HER2-positive breast cancer patients. Interestingly, BIG3-PHB2 complexes showed different intracellular localization between trastuzumab-sensitive and -resistant cells. Here, we report the pathophysiological significance of the BIG3-PHB2 complexes in HER2-positive breast cancer, especially in trastuzumab-resistant cells. BIG3 forms a complex of PHB2 in the trans-Golgi network (TGN) of trastuzumab-sensitive cells, suppressing the tumor-suppressive activity of PHB2 as well as estrogen receptor-positive breast cancer cells. Notably, BIG3-PHB2 complexes regulate secreting the extracellular proteins associated with HER2-positive breast cancer progression. Furthermore, we found that BIG3-PHB2 complexes are transported to the plasma membrane from the TGN by EGFR overexpression via its cargo-X protein, contributing to the HER2-EGFR heterodimerization involved in trastuzumab resistance.

More importantly, treatment with peptide inhibitor ERAP targeting the BIG3-PHB2 interaction led to the remarkable inhibition of transcriptional activity, extracellular protein secretion, and HER2-EGFR heterodimerization, resulting in suppression of NF-κB signaling and trastuzumab-sensitive/resistant cell proliferation. Furthermore, intravenous injection of this peptide inhibitor rapidly exhibited the long-term antitumor effect in vivo mice with HER2-positive breast cancer cells, especially trastuzumab-resistant cells. These findings suggest that the BIG3-PHB2 complexes have pivotal roles in the progression of HER2-positive breast cancer, especially in acquiring trastuzumab resistance.

References
(Related articles)

1. Cancer Science, 2021, 112, 4208–4219
2. Journal of Human Genetics, 2021, 66, 927–935
3. International Journal of Oncology, 2020, 56, 581–595
4. Biochemical and Biophysical Research Communications, 2019, 518, 183–189
5. Cancer Research, 2018, 78, 2233–2247, 2018
6. Nature Communications, 2017, 8, 15427
7. Scientific Reports, 2017, 7, 1821
8. Cancer Science, 2017, 108, 785–794
9. PLoS One, 2015, 10, e0127707
10. Cancer Science, 2015, 106, 550–558
11. Scientific Reports, 2014, 4, 7355
12. BMC Res Notes, 2014, 7, 435
13. Nature Communications, 2013, 4, 2443

CV

Education:

1997 - 2000 : Department of Food Sciences and Technology, Graduate School of Agriculture, Kyushu University, Awarded the degree of Ph.D (Agricultural science)

1995 - 1997 : Department of Food Sciences and Technology, Graduate School of Agriculture, Kyushu University, Awarded the degree of Master of Science

1990 - 1994 : Faculty of Agriculture, Kyushu University, Awarded the degree of Bachelor of Science

Professional carriers:

2019 Associate Professor, Tokushima University
2016 Lecturer, Tokushima University
2012 Assistant Professor, Tokushima University
2010 Designated Assistant Professor, Tokushima University
2009 Academical Researcher, Tokushima University
2000 - 2009 Post-doctoral Fellow, Advanced Medical Research Center, Graduate School of Medical Sciences Nihon University

Specialties:

Molecular oncology

Personality:

I can promote good working relationships with any persons due to my cheerful character and cooperative personality. I believe that “communication with people” is the best shortcut and solution to “research expansion”, that’s why I will grasp the objective of research progress through regular communication. In addition, I am always honest, enthusiastic, patient, and a very creative hard worker for scientific research.

S - 0 2

Chromosomal instability induced by chromosome dynamics in cancer cells

Iemura[1], Kenji; Tanaka[1], Kozo

[1]Department of Molecular Oncology, Institute of Development, Aging and Cancer, Tohoku University, Japan

Most cancer cells show chromosomal instability, a condition in which chromosome missegregation occurs at high rates. Among the causes of chromosomal instability, insufficient correction of erroneous kinetochore-microtubule attachments plays pivotal roles in various situations. We recently found the previously unappreciated role of chromosome oscillation, an iterative chromosome motion during metaphase, in the correction of erroneous kinetochore-microtubule attachments. Chromosome oscillation facilitates the metaphase phosphorylation of Hec1, a kinetochore protein that binds to microtubules, by Aurora A kinase on the spindle. The phosphorylation of Hec1 reduces its affinity to microtubules, which promotes the correction of erroneous kinetochore-microtubule attachments. Our data showed that the Aurora A–dependent Hec1 phosphorylation and the chromosome oscillation amplitude facilitate each other. Importantly, chromosome oscillation is attenuated in cancer cell lines compared with non-transformed cell lines. The Hec1 phosphorylation in metaphase was reduced in cancer cells, which was restored by enhancement of chromosome oscillation. Enhancement of chromosome oscillation in cancer cells also reduced the number of erroneous kinetochore–microtubule attachments and chromosome missegregation, whereas inhibition of Aurora A during metaphase increased such errors. We propose that Aurora A–mediated metaphase Hec1 phosphorylation through chromosome oscillation ensures mitotic fidelity by eliminating erroneous kinetochore-microtubule attachments. Attenuated chromosome oscillation and the resulting reduced Hec1 phosphorylation may be a cause of chromosomal instability in cancer cells.

References
(Related articles)

1. Iemura K, Natsume T, Maehara K, Kanemaki MT, Tanaka K. Chromosome oscillation promotes Aurora A-dependent Hec1 phosphorylation and mitotic fidelity. ***J Cell Biol*** (2021) 220, e202006116.
2. Campos Medina M, Iemura K, Kimura A, Tanaka K. A mathematical model of kinetochore-microtubule attachment regulated by Aurora A activity gradient describes chromosome oscillation and correction of erroneous attachments. ***Biomed Res*** (2021) 42, 203-219.
3. Iemura K, Yoshizaki Y, Kuniyasu K, Tanaka K. Attenuated chromosome oscillation as a cause of chromosomal instability in cancer cells. ***Cancers*** (2021) 13, 4531.

CV

Education

1985.4~1991.3 Faculty of Medicine, University of Tokyo
1993.4~1997.3 Graduate School of Medicine, University of Tokyo

Appointments

1991.6~1992.5 Resident, Internal Medicine, The University of Tokyo Hospital
1992.6~1993.5 Junior Resident, Internal Medicine, Jichi Medical School Hospital
1997.4~1998.3 Senior Resident, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo
1998.4~2001.12 Research Associate, Department of Molecular Pathology, Research Institute for Radiation Biology and Medicine, Hiroshima University
2002.1~2007.2 Postdoctoral Research Assistant, School of Life Sciences, University of Dundee
2007.3~2011.2 Assistant Professor, Institute of Development, Aging and Cancer, Tohoku University
2011.3~ Professor, Institute of Development, Aging and Cancer, Tohoku University

Y - 0 1

Trans-homophilic interaction of CADM1 promotes organ infiltration of T-cell lymphoma by adhesion to vascular endothelial cells

Kasai[1], Yutaka; Funaki[1], Toko; Kumagai[1], Yuki; Tominaga[1], Mizuki; Matsubara[1,2], Daisuke; Ito[1], Takeshi; Murakami[1], Yoshinori

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[2]Department of Diagnostic Pathology, University of Tsukuba, Tsukuba, Japan

The initial step of organ infiltration of malignant cells is the interaction with host vascular endothelial cells, which is often mediated by specific combinations of cell adhesion molecules. Cell adhesion molecule 1 (CADM1) is a single-pass membranous protein of immunoglobulin superfamily (IgSF) and participates in the formation of various types of cell adhesion, including epithelial cell- and synaptic cell-adhesion through homophilic and heterophilic interactions1. CADM1 is overexpressed in adult T-cell leukemia/lymphoma (ATL) and provides a cell-surface diagnostic marker, and also promotes the adhesion of ATL cells to vascular endothelial cells and multiple organ infiltration in mice. However, its binding partner on host cells has not yet been identified. In this study, we show that CADM1 promotes transendothelial migration of ATL cells in addition to the adhesion to vascular endothelial cells. Moreover, CADM1 enhances liver infiltration of mouse T-cell lymphoma cells, EL4, after tail vein injection, whereas a CADM1 mutant lacking adhesive activity did not. Among the known CADM1-binding proteins expressed in primary endothelial cells, only CADM1 and CADM4 could induce morphological extension of ATL cells when plated onto glass coated with these proteins. Furthermore, CADM1-mediated liver infiltration of EL4 cells was canceled in conventional Cadm1 knockout mice, whereas it was not canceled in Cadm4 knockout mice. Finally, CADM1-mediated liver infiltration was cancelled in Tie2-Cre; Cadm1fl/fl mice, vascular endothelium-specific Cadm1 knockout mice2. These results suggest that CADM1 on host vascular endothelial cells is required for organ infiltration of ATL and other T-cell lymphomas expressing CADM1. Possible involvement of CADM1 in metastasis of small cell lung cancercancer, another tumor expressing high amount of CADM1, will be also discussed.

References
(Related articles)

1. Ito T†, Kasai Y†, Kumagai Y, Suzuki D, Ochiai-Noguchi M, Irikura D, Miyake S and Murakami Y. Quantitative analysis of interaction between CADM1 and its binding cell-surface proteins using surface plasmon resonance imaging. ***Front Cell Dev Biol***, 2018;6:86.

2. Kasai Y, Gan SP, Funaki T, Ohashi-Kumagai Y, Tominaga M, Shiu SJ, Suzuki D, Matsubara D, Sakamoto T, Sakurai-Yageta M, Ito T and Murakami Y. Trans-homophilic interaction of CADM1 promotes organ infiltration of T-cell lymphoma by adhesion to vascular endothelium. ***Cancer Sci***, 2022;113(5):1669-1678.

CV

Education:

2014 BSc, Faculty of Science and Technology, Tokyo University of Science
2016 MSc, Graduate School of Frontier Sciences, the University of Tokyo
2019 PhD, Graduate School of Frontier Sciences, the University of Tokyo

Position:

2019-2021 Postdoctoral Fellow, Division of Molecular Pathology, The Institute of Medical Science, The University of Tokyo
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Y - 0 2

SARS-CoV-2 and cellular senescence

Tsuji[1], Shunya; Minami[2], Shohei; Hashimoto[3], Rina; Suzuki[4], Tatsuya; Sasai[5], Miwa; Torii[6], Shiho; Ono[6], Chikako; Shichinohe[7], Shintaro; Sato[2, 8,] Shintaro; Yamamoto[5, 9, 10], Masahiro; Watanabe[7, 10], Tokiko; Matsuura[6, 10], Yoshiharu; Takayama[3], Kazuo; Kobayashi[2, 10], Takeshi; Okamoto[4], Toru; Hara[1, 9, 10], Eiji;

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Reports of post-acute COVID-19 syndrome, in which the inflammatory response persists even after SARS-CoV-2 has no longer detected, are increasing1, but the underlying mechanisms of post-acute COVID-19 syndrome remain unknown. Here, we show that SARS-CoV-2-infected cells trigger senescence-like cell-cycle arrest2,3 in neighboring uninfected cells in a paracrine manner via virus-induced cytokine production. In cultured human cells or bronchial organoids, these SASR-CoV-2 infection-induced senescent cells express high levels of a series of inflammatory factors known as senescence-associated secretory phenotypes (SASPs)4 in a sustained manner, even after SARS-CoV-2 is no longer detectable. We also show that the expression of the senescence marker CDKN2A (refs. 5,6) and various SASP factor4 genes is increased in the pulmonary cells of patients with severe post-acute COVID-19 syndrome. Furthermore, we find that mice exposed to a mouse-adapted strain of SARS-CoV-2 exhibit prolonged signs of cellular senescence and SASP in the lung at 14 days after infection when the virus was undetectable, which could be substantially reduced by the administration of senolytic drugs7. The sustained infection-induced paracrine senescence described here may be involved in the long-term inflammation caused by SARS-CoV-2 infection.

References
(Related articles)

1. Nalbandian, A. et al. Post-acute COVID-19 syndrome. Nat. Med. 27, 601–615 (2021).
2. He, S. & Sharpless, N. E. Senescence in health and disease. Cell 169, 1000–1011 (2017).
3. Gorgoulis, V. et al. Cellular senescence: defining a path forward. Cell 179, 813–827 (2019).
4. Coppé, J.-P., Desprez, P.-Y., Krtolica, A. & Campisi, J. The senescence- associated secretory phenotype: the dark side of tumor suppression. Annu. Rev. Pathol. Mech. Dis. 5, 99–118 (2010).
5. Serrano, M., Hannon, G. J. & Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366, 704–707 (1993).
6. Hara, E. et al. Regulation of p16 expression and its implications for cell immortalization and senescence. Mol. Cell. Biol. 16, 859–867 (1996).
7. Di Micco, R., Krizhanovsky, V., Baker, D. & d'Adda di Fagagna, F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat. Rev. Mol. Cell Biol. 22, 75–95 (2021).

CV

Y - 0 3

RIG-I restrains SARS-CoV-2 replication in human lung cells without activation of innate immune signaling

Yamada[1,2], Taisho; Sato[1,2], Seiichi; Orba[3,4], Yasuko; Sawa,[3,4,5], Hirofumi; Sasaki[3,4], Michihito; Takaoka[1,2], Akinori

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COVID-19, caused by SARS-CoV-2, which is a positive-sense, single-stranded RNA betacoronavirus, remains an ongoing global pandemic. Although most people infected with SARS-CoV-2 show a mild and self-limited course, there are also a few severe and critical patients with exacerbated inflammatory response. The wide spectrum of clinical manifestations of COVID-19 suggests that individual immune responses to the underlying pathogen may play some crucial roles in determining the clinical course. However, the innate immune responses against SARS-CoV-2 remains poorly understood. Here we show that retinoic acid-inducible gene-I (RIG-I) but not MDA5 or TLR3 sufficiently restrains SARS-CoV-2 replication in human lung cells in a type I/III interferon (IFN)-independent manner.

RIG-I inhibits viral RNA-dependent RNA polymerase (RdRp)-mediated first step of replication through the preferential binding of RIG-I helicase domain to the 3- untranslated region of the viral genomic positive-sense RNA. This new mode of RIG-I recognition fails to activate the conventional downstream MAVS/IPS-1-dependent signaling, which is in accordance with lack of cytokine induction after SARS-CoV-2 infection. Consistently, genetic ablation of RIG-I expression allows lung cells to produce viral particles. On the other hand, SARS-CoV-2 can replicate with innate cytokine induction in cells with low levels of RIG-I expression. In such situation where negative-sense RNA initiates to be transcribed, MDA5 in turn senses the negative-sense RNA to induce type I/III IFNs and other cytokines.

Our data have defined RIG-I expression levels as one of the intrinsic determinants for the defense in human lung cells at least during the initial process of SARS-CoV-2 infection. It has been reported that nearly 40-45% people infected with SARS-CoV-2 show asymptomatic with no robust upregulation of innate cytokines. In this respect, the balance between RIG-I expression levels and the amount of invading virus would regulate the fate of viral replication, which might determine cytokine responses and the related patient outcomes.

References
(Related articles)

Yamada T., Sato S., Sotoyama Y., Orba Y., Sawa H., Yamauchi H., Sasaki M., and Takaoka A. RIG-I triggers a signaling-abortive anti-SARS-CoV-2 defense in human lung cells. *Nat. Immunol.*, 22, 820-828, 2021.

Takaoka A. and Yamada T. Regulation of signaling mediated by nucleic acid sensors for innate interferon-mediated responses during viral infection. *Int. Immunol*., 31, 477-488, 2019.

Yamada T., Horimoto H., Kameyama T., Hayakawa S., Yamato H., Dazai M., Takada A., Kida H., Bott D., Zhou AC., Hutin D., Watts TH., Asaka M., Matthews J. and Takaoka A. Constitutive aryl hydrocarbon receptor signaling constrains type I interferon-mediated antiviral innate defense. *Nat. Immunol.*, 17, 687-694, 2016.

CV

2006-2010	Bachelor of Medical Sciences, College of Nursing and Medical Technology, School of Medicine and Health Sciences, University of Tsukuba
2010-2012	Master of Medical Sciences, Graduate School of Medicine, Hokkaido University
2012-2016	Doctor of Medical Sciences, Graduate School of Medicine, Hokkaido University
2016-present	Assistant Professor, Institute for Genetic Medicine, Hokkaido University

Awards

2016	ICI-FIMSA Travel Award (Australia)
2017	Frate Reseach Award, Hokkaido University Medical School Alumni Association
2017	Reseach Award, The Hokkaido Medical Society
2021	Reseach Award, Japanese Society of Interferon & Cytokine Research

Session2

S - 0 4

Single-cell transcriptome analysis of human immune cells and early embryos

Hashimoto[1], Kosuke

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Aging has a profound impact on the immune system. Supercentenarians, people who have reached 110 y of age, are a great model of healthy aging. Their characteristics of delayed onset of age-related diseases and compression of morbidity imply that their immune system remains functional. In the first part of my talk, I present our single-cell transcriptome analysis of peripheral blood mononuclear cells (PBMCs), derived from 7 supercentenarians and 5 younger controls. In this study, we identified a marked increase of cytotoxic CD4 T cells (CD4 cytotoxic T lymphocytes [CTLs]) as a signature of supercentenarians. Furthermore, single-cell T cell receptor sequencing of 2 supercentenarians revealed that CD4 CTLs had accumulated through massive clonal expansion, with the most frequent clonotypes accounting for 15 to 35% of the entire CD4 T cell population.

In the second part, I present the single-cell analysis of human embryos. Long terminal repeat (LTR) retrotransposons are widely distributed across the human genome. They have accumulated through retroviral integration into germline DNA and are latent genetic modules. Active LTR promoters are observed in germline cells; however, little is known about the mechanisms underlying their active transcription in somatic tissues. We revealed that the LTR families MLT2A1 and MLT2A2 are primarily expressed in human four-cell and eight-cell embryos and are also activated in some adult somatic tissues, particularly pineal gland.

References
(Related articles)

1. **Hashimoto K**, Jouhilahti EM, Töhönen V, Carninci P, Kere J, Katayama S. Embryonic LTR retrotransposons supply promoter modules to somatic tissues. ***Genome Res***. 31, 1983-1993 (2021)

2. **Hashimoto K**, Kouno T, Ikawa T, Hayatsu N, Miyajima Y, Yabukami H, Terooatea T, Sasaki T, Suzuki T, Valentine M, Pascarella G, Okazaki Y, Suzuki H, Shin JW, Minoda A, Taniuchi I, Okano H, Arai Y, Hirose N, Carninci P. Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. ***Proc Natl Acad Sci USA***. 116, 24242-24251 (2019)

CV

Aug. 2020 – Present	Associate Professor, Osaka University
Apr. 2016 – July 2020	Senior Research Scientist, RIKEN, Japan
Oct. 2011 – Mar. 2016	Research Scientist, RIKEN, Japan
Apr. 2009 – Sep. 2011	Postdoctoral Visiting Fellow, NCBI, NIH, USA
Mar. 2009	Ph.D. Bioinformatics Center, Kyoto University, Japan

Y - 0 5

Coordinated activation of enhancer elements for thymic Treg development and immunological self-tolerance

Kawakami, Ryoji[1]; Sakaguchi, Shimon[1]

[1]Institute for Life and Medical Sciences, Kyoto University, Japan

CD4+Foxp3+ regulatory T cells (Tregs) are one of the most significant cells in the maintenance of immune tolerance. Treg cells work to maintain homeostasis of the body in various situations such as prevention of autoimmune diseases, termination of immune responses, and damaging tissue repairing, by braking excessive immune responses.

Here, we would like to discuss the differentiation mechanism of Treg cells. Many Tregs are generated from a portion of self-antigen-stimulated CD4+ T cells during the thymic negative selection process. However, the detailed mechanism, especially “How Treg-type epigenetic landscape is installed in immature T cells under selection”, is largely unknown. The research questions are;

(i) The detailed mechanisms of Treg generation in the epigenetic and physical three-dimensional chromatin contexts

(ii) Contribution/Significance of non-coding enhancer elements for Treg differentiation e.g. at the transcription factor Foxp3 gene locus

(iii) Kinetics of Treg differentiation in the thymic selection and identification of epigenetically-poised Treg-precursor cells

To elucidate these, we first described genome-wide DNA epigenetic landscape and their physical 3D interactions during thymic T cell differentiation (from DN-CD4SP-Treg) using ATAC-seq, anti-histone ChIP-seq, and H3K27ac-HiChIP. This analysis revealed the T cell-specific activation of Treg-type enhancer elements at the initial stages of T cell differentiation, prior to induction of Foxp3 expression. Using CRISPR-Cas9 technology in mice, we found that distinct two non-coding enhancer elements at the Foxp3 locus are indispensable for thymic Treg generation and immunological self-tolerance. These results demonstrate the significance of coordinated activation of enhancer elements in an epigenetic context for the establishment and maintenance of immunological self-tolerance.

References
(Related articles)

R. Kawakami et al. Immunity. 2021 May 11;54(5):947-961.e8.
doi: 10.1016/j.immuni.2021.04.005.

CV

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[Research Interest]
Immune tolerance, Regulatory T cells, Foxp3, Autoimmunity, Control of autoimmune diseases/allergy/cancer
Epigenetics, Differentiation of T cells, Translation of Dry into Wet

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- Jun, 2020 – Present Kyoto University
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- Apr, 2019 - May, 2020 Researcher, INFRONT, Kyoto University

[Education]
- Apr, 2015 - Mar, 2019 Majored in Medical Science, Department of Medicine, Osaka University
- Apr, 2013 - Mar, 2015 Department of Pharmaceutical Science, Osaka University

Y - 0 6

The role of hypothalamic neurons in sleep, aging and longevity

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It has been reported that the hypothalamus plays an important role in the regulation of aging and longevity in mammals. However, detailed mechanisms are not fully elucidated. Aging has significant impacts on sleep, resulting in increases in sleep fragmentation and sleep onset latency, and a decrease in sleep quality in mammals. We have found that aged mice also display severe sleepiness during sleep deprivation. Intriguingly, diet restriction (DR), a dietary regimen well known to delay the aging process and to extend lifespan in a wide variety of organisms including mammals, can dramatically attenuate some of age-associated sleep alterations. For example, DR significantly reduces the levels of age-associated sleep fragmentation and sleep attempt during sleep deprivation. Therefore, DR may delay the aging process and extend lifespan by critically altering sleep control in aged animals. To further elucidate mechanisms by which DR ameliorates age-associated sleep alterations, we focus on the gene called PR-domain containing factor 13 (Prdm13). Prdm13 is a Sirt1 downstream gene and is exclusively expressed in the dorsomedial hypothalamus (DMH). The DMH has been shown to control age-associated physiological changes and longevity through Sirt1. We have recently found that conditional DMH-specific Prdm13-knockout mice mimic age-associated sleep alterations including sleep fragmentation and severe sleepiness during sleep deprivation. We are currently testing whether Prdm13 is necessary to promote DR-induced sleep phenotypes. In this talk, I will discuss about a role of DMH neurons on age-associated sleep alterations. I will also propose how poor sleep quality promotes the age-associated pathophysiology in peripheral organs/tissues and ultimately affects longevity.

Tsuji S, Brace CS, Yao R, Tanie Y, Tokizane K, Tada H, Rensing N, Mizuno S, Almunia J, Kong Y, Nakamura K, Ogiso N, Toyokuni, S, Takahashi S, Wong M, Imai S, Satoh A#, Prdm13-expressing neurons in the dorsomedial hypothalamus regulate sleep-wake patterns during aging in mice, under revision

Satoh A#, Central Mechanisms linking age-associated physiological changes to health span through the hypothalamus, Aging Mechanisms II (Springer), pp289-306, 2022

Satoh A, Imai S, Guarente LP: The brain, ageing, and sirtuins. Nat. Rev. Neurosci. 18:362–374, 2017

Satoh A#, Brace CS, Rensing N, Imai S#: Prdm13, a newly identified dorsomedial hypothalamus-enriched gene, affects age-associated pathophysiology. Aging Cell 14:209–218, 2015

Satoh A, Brace CS, Rensing N, Clifton P, Wozniak DM, Herzog ED, Yamada KA, Imai S: Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab. 18:416–430, 2013

Akiko Satoh is an Associate Professor in the Department of Integrative Physiology at Institute of Development, Aging, and Cancer, Tohoku University, Japan (2021-present) (also an Associate Professor at the National Center for Geriatrics and Gerontology, Japan). She obtained her PhD in Pharmaceutical Science from the University of Toyama in 2005. She had her postdoctoral training at Washington University School of Medicine in St. Louis. Her current research aims to understand the role of hypothalamic sleep regulation in mammalian aging and longevity control.

Session3

S - 0 5

Novel control mechanisms of lymphocyte trafficking

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Blood-circulating lymphocytes enter secondary lymphoid organs, including the spleen and lymph nodes (LNs), and migrate to distinct compartments where they survey for antigens. After spending several hours to a day in a secondary lymphoid organ, lymphocytes exit and traffic to other lymphoid organs where they continue antigen surveillance. Once lymphocytes encounter cognate antigens, they rapidly change their migration programs to stay in the lymphoid organ and induce adaptive immune responses. These lymphocyte trafficking events are governed by G protein-coupled receptors (GPCRs) that respond to chemotactic molecules represented by chemokines. Our study showed that inputs from adrenergic neurons inhibit lymphocyte exit from LNs through the β2-adrenergic receptor (β2AR) expressed on lymphocytes. This is mediated by the crosstalk between β2AR and chemokine receptors, CCR7 and CXCR4, where β2AR activation enhances LN retention signals mediated by the chemokine receptors. We found that the adrenergic neuron-mediated control of lymphocyte exit from LNs contributes to diurnal variations of adaptive immune responses. In investigating molecular mechanisms of the crosstalk between β2AR and chemokine receptors, we identified a protein complex consisting of copper metabolism MURR1 domain-containing (COMMD) 3 and COMMD8 (COMMD3/8 complex) as an adaptor that recruits GPCR kinase 6 to chemoattractant receptors and promotes lymphocyte chemotaxis. The COMMD3/8 complex plays essential roles in the control of B cell migration in vivo and induction of humoral immune responses. Disabling the COMMD3/8 complex alleviates the pathology in mouse models of autoimmune disease. Based on these findings, we discuss novel mechanisms for the control of lymphocyte trafficking and immunotherapeutic approaches targeting them.

References

(Related articles)

Nakai, A., Fujimoto, J., Miyata, H., Stumm, R., Narazaki, M., Schulz, S., Baba, Y., Kumanogoh, A. and Suzuki, K. The COMMD3/8 complex determines GRK6 specificity for chemoattractant receptors. *J. Exp. Med.* 216 (7): 1630-1647, 2019.

Suzuki, K., Hayano, Y., Nakai, A., Furuta, F. and Noda, M. Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes. *J. Exp. Med.* 213 (12): 2567-2574, 2016.

Nakai, A., Hayano, Y., Furuta, F., Noda, M. and Suzuki, K. Control of lymphocyte egress from lymph nodes through β_2-adrenergic receptors. *J. Exp. Med.* 211 (13): 2583-2598, 2014.

CV

04/1994 – 03/1998	School of Science, The University of Tokyo, Tokyo, Japan (BS in Chemistry)
04/1998 – 03/1999	Graduate School of Science, The University of Tokyo, Tokyo, Japan
04/1999 – 03/2003	Medical School, Osaka University, Osaka, Japan (MD)
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10/2007 – 03/2011	Postdoctoral fellow, University of California, San Francisco, USA
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S - 0 6

Roles of the GDF3-ALK7 axis in adiposity

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ALK7, a type I receptor in the transforming growth factor-β superfamily, is predominantly expressed in adipocytes and regulates lipid metabolism by modulating the master adipose transcription factors, C/EBPα and PPARγ. GDF3, a principal candidate of ALK7 ligand, is produced from adipose tissue macrophages in response to NLRP3 inflammasome activation and/or insulin secreted under nutrient-excess condition. This signaling axis within white adipose tissue physiologically plays a major role in storing excess intake energy as fat. However, the continuous activation enlarges adipocytes and induces a proinflammatory and insulin-resistant state. Inactivation of ALK7 or GDF3 protects mice from diet-induced and genetically prone obesity and insulin resistance. Furthermore, human subjects with ALK7 variants have an association with reduced waist-to-hip ratio and resistance to development of type 2 diabetes. The blockade of the GDF3-ALK7 signaling pathway, which targets the final outcome of obesity, fat accumulation, may be a safe and robust treatment for obesity and diabetes.

References

Hirayama I, Yi Z, Izumi S, Arai I, Suzuki W, Nagamachi Y, Kuwano H, Takeuchi T, and Izumi T (1999). Genetic analysis of obese diabetes in the TSOD mouse. Diabetes, 48, 1183-1191.

Mizutani S, Gomi H, Hirayama I, and Izumi T (2006). Chromosome 2 locus *Nidd5* has a potent effect on adiposity in the TSOD mouse. Mamm. Genome, 17, 375-384.

Yogosawa S, Mizutani S, Ogawa Y, and Izumi T (2013). Activin receptor-like kinase 7 suppresses lipolysis to accumulate fat in obesity through downregulation of peroxisome proliferator-activated receptor γ and C/EBPα. Diabetes, 62, 115-123.

Yogosawa S, and Izumi T (2013). Roles of activin receptor-like kinase 7 signaling and its target, peroxisome proliferator-activated receptor γ, in lean and obese adipocytes. Adipocyte, 2, 246-250.

Bu Y, Okunishi K, Yogosawa S, Mizuno K, Irudayam MJ, Brown CW, and Izumi T (2018). Insulin regulates lipolysis and fat mass in adipocytes by upregulating growth/differentiation factor 3 in adipose macrophages. Diabetes, 67, 1761-1772.

CV

EDUCATION/DEGREES

1982.3: Faculty of Medicine, University of Tokyo, M.D.
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POSITIONS/EMPLOYMENT

1982 – 1984: Resident (Internal Medicine), University of Tokyo Hospital, Mitsui Memorial Hospital
1984 – 1989: Physician and Research Fellow, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Institute of Diabetes Care and Research, Asahi Life Foundation
1989 – 1992: Research Associate, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo
1990 – 1994: Research Associate, Howard Hughes Medical Institute, Department of Pharmacology, University of Colorado School of Medicine, USA
1994 – 2000: Associate Professor, Department of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University
2000 – present: Professor, Institute for Molecular and Cellular Regulation, Gunma University
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Y - 0 7

Premature ovarian insufficiency in mice lacking phosphoinositide-metabolizing enzymes.

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Premature ovarian insufficiency (POI) is characterized by the cessation of menstruation before age 40 in humans. It is estimated that about 1% of women are affected by POI. In POI, ovarian function declines and ovulation stops, making pregnancy very difficult. Candidate genes causing follicular dysgenesis have been discovered mainly by phenotypes obtained from murine knockout models (1). FSH (follicle-stimulating hormone) receptors and BMP15 (Bone Morphogenetic Protein 15) and GDF9 (Growth Differentiation Factor 9), which are oocyte-derived growth factors belonging to the TGF-β (transforming growth factor-β) superfamily, are found to be genetically abnormal in sporadically occurring human POI. However, more than 50% of POIs are unexplained.

We have analyzed the physiological functions of phosphoinositides (PIPs), which are intracellular signaling phospholipids (2-5). In the process, we generated conditional PI(3,4,5)P3 (Phosphatidylinositol 3,4,5-trisphosphate) phosphatase-deficient mice (cKO mice) using transgenic mice expressing Cre recombinase under the control of the CD11b promoter and found that males were normal and fertile, while females were infertile. When 1-month-old cKO female mice were subjected to superovulation treatment with PMSG (pregnant mare serum gonadotropin) and hCG (Human chorionic gonadotropin), the cKO female mice did not ovulate at all, suggesting that they were developing POI. Histological analysis has found that cKO ovaries contain a large number of follicles with abnormal morphology, which are not found in wild-type ovaries. In this meeting, I will discuss the pathological analysis of cKO ovaries and report on the molecular mechanism of POI development due to abnormal PI(3,4,5)P3 metabolism.

References
(Related articles)

(1) Jiao X, Ke H, Qin Y, Chen ZJ. Molecular Genetics of Premature Ovarian Insufficiency. Trends Endocrinol Metab, 29, 795-807, 2018

(2) Morioka S, Nakanishi H, Yamamoto T, Hasegawa J, Tokuda E, Hikita T, Sakihara T, Kugii Y, Oneyama C, Yamazaki M, Suzuki A, Sasaki J, Sasaki T. A mass spectrometric method for in-depth profiling of phosphoinositide regioisomers and their disease-associated regulation. Nat Commun, 13, 83, 2022

(3) Koizumi A, Narita S, Nakanishi H, Ishikawa M, Eguchi S, Kimura H, Takasuga S, Huang M, Inoue T, Sasaki J, Yoshioka T, Habuchi T, Sasaki T. Increased fatty acyl saturation of phosphatidylinositol phosphates in prostate cancer progression. Sci Rep, 9, 13257, 2019

(4) Kimura H, Eguchi S, Sasaki J, Kuba K, Nakanishi H, Takasuga S, Yamazaki M, Goto A, Watanabe H, Itoh H, Imai Y, Suzuki A, Mizushima N, Sasaki T. Vps34 regulates myofibril proteostasis to prevent hypertrophic cardiomyopathy. JCI Insight, 2, e89462, 2017

(5) Kofuji S, Kimura H, Nakanishi H, Nanjo H, Takasuga S, Liu H, Eguchi S, Nakamura R, Itoh R, Ueno N, Asanuma K, Huang M, Koizumi A, Habuchi T, Yamazaki M, Suzuki A, Sasaki J, Sasaki T. INPP4B is a PtdIns(3,4,5)P3 phosphatase that can act as a tumor suppressor. Cancer Discov, 5, 730-739, 2015

CV

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1993 B.S., Faculty of Home Economics, Ochanomizu University

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2018-	Associate Professor:	Medical Research Institute Tokyo Medical and Dental University
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2008	Lecturer:	Akita University Graduate School of Medicine
2003	Assistant Professor:	Akita University Graduate School of Medicine
2000	Postdoctoral Fellow:	Tokyo Metropolitan Institute of Medical Science
1999	Technical Staff:	Ontario Cancer Institute, Canada
1995	Researcher:	Pharmaceutical Research Institute, Kyowa Hakko Kogyo Company

Y - 0 8

ACBD3 forms specialized ER-Golgi contact sites to drive the ER exit of STING

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[2]Graduate School of Pharmaceutical Science, Tokushima University.
[3]Kuramoto Division, Technical Support Department, Tokushima University.

Stimulator of interferon genes (STING) is an endoplasmic reticulum (ER)-resident receptor for cyclic di-nucleotides (CDNs) that triggers potent innate immune responses in both microbial infections and sterile inflammations. Upon CDN binding, STING moves from the ER to the Golgi apparatus, where it activates the downstream TBK1-IRF3 axis, leading to type-I interferon (IFN) induction. Although ER exit is a critical step in STING signaling, its mechanism is poorly understood. Here, we visualized the ER exit sites of STING by blocking its transport at low temperature. After ligand binding, STING formed dot-like structures (hereafter referred to as “STING ER exit foci”) at non-canonical ER exit sites. Using APEX2-based proximity proteomics and cross-linking immunoprecipitation coupled to mass spectrometry, we identified the Golgi-resident protein ACBD3/GCP60 as an essential and specific component of the STING ER exit foci. Subsequent imaging analysis and in vitro reconstitution using semi-intact cells revealed that ACBD3 recognized ligand-bound STING and concentrated it at specialized ER-Golgi contact sites. Moreover, depletion of ACBD3 diminished STING foci formation and inhibited STING exit from the ER to the Golgi, resulting in impaired type-I IFN responses. Our results identified the ACBD3-positive specialized ER-Golgi contact sites that function as platforms to trigger the ER exit of STING.

References
(Related articles)

CV

2/2019-Present	Senior Lecturer Tokushima University
10/2014-2/2019	Assistant Professor Tokushima University
2/2014-9/2014	Project Assistant Professor Tokushima University
4/2013-2/2014	Research Fellowship for Young Scientists Japan Society for the Promotion of Science
4/2011-3/2013	Program Specific Researcher Kyoto University
3/2011	Ph.D. in Medical Science Kanazawa University

Y - 0 9

Cell type-specific control of reward learning in the brain

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The nucleus accumbens (NAc) of the ventral striatum is thought to play a critical role in controlling reward signaling in the brain, and its dysfunction is associated with several psychiatric conditions associated with impaired reward learning, including drug addiction, depression, and schizophrenia. Within the NAc, neurons can be subdivided into two broadly equal populations, medium spiny neurons expressing either dopamine D1 or D2 receptors (D1-/D2-MSNs); however, the contribution of these two neuron types to reward learning has been unclear. Here in a series of experiments, we used a combination of transgenic and viral technologies to block neurotransmission from NAc D1- or D2-MSNs in mice performing behavioral tasks that required various types of reward learning, including Pavlovian and goal-directed learning. Our findings reveal differential functional roles for NAc cell types, with D1-MSNs necessary for Pavlovian reward learning, and D2-MSNs necessary for goal-directed reward learning as well as adaptive learning when task parameters are changed. These findings potentially identify new neurocircuit therapeutic targets for reward learning impairments in psychiatric conditions.

References
(Related articles)

Macpherson T, Kim J, Hikida T. (2022) *Nucleus* accumbens core dopamine D2 receptor-expressing neurons control reversal learning but not set-shifting in behavioral flexibility in male mice. *Frontiers in Neuroscience.* In Press.

Macpherson T, Matsumoto M, Gomi, H, Morimoto J, Uchibe E, Hikida T. (2021) Parallel and hierarchical neural mechanisms for adaptive and predictive behavioral control. *Neural Networks*. 144:507-521.

Macpherson T, Hikida T. (2019) Role of basal ganglia neurocircuitry in the pathology of psychiatric disorders. *Psychiatry and Clinical Neuroscience.*73(6): 289-301.

Macpherson T, Hikida T. (2018) Nucleus accumbens dopamine D1-receptor-expressing neurons control the acquisition of sign-tracking to conditioned cues in mice. *Frontiers in Neuroscience.* 12: 418.

Macpherson T, Morita M, Wang Y, Sasaoka T, Sawa A, Hikida T. (2016) Nucleus accumbens dopamine D2-receptor expressing neurons control behavioral flexibility in a place discrimination task in the IntelliCage. *Learning and Memory.* 23 (7): 359-364.

CV

Tom Macpherson completed his PhD in Behavioral & Clinical Neuroscience at the University of Sussex, UK (2009-2014), studying the role of $GABA^A$ receptors in controlling addiction to cocaine in mice. This work led to new insights into the mechanisms by which GABA receptors in the nucleus accumbens (NAc) can modulate the influence of local dopamine release following cocaine administration (Maguire et al., 2014).

Upon graduation from his PhD, he was granted a JSPS Postdoctoral Fellowship and moved to Kyoto University to take up a postodoctoral research position (2014-2016). During this time, his research led to several major breakthroughs in our understanding of striatal neurocircuit control of Pavlovian conditioning and behavioral flexibility and resulted in the award of a second JSPS Postdoctoral Fellowship grant to continue his research for a further two years (2016-2018).

Since 2018, Tom Macpherson has been working as an assistant professor at Osaka University's Institute of Protein Research. His research incorporates cutting-edge neural imaging, artificial intelligence, genetic, molecular, and behavioral analyses to elucidate the neural mechanisms underlying motor, cognitive, and limbic control within the brain.

Session4

S - 0 7

Molecular mechanisms underlying autophagosome biogenesis

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Autophagy is an intracellular degradation system conserved among eukaryotes and contributes to cellular homeostasis through degradation of harmful or superfluous materials. When autophagy is induced, isolation membrane suddenly appears in the cytosol, which expands and seals into a double-membrane structure termed an autophagosome, during which cytoplasmic materials are sequestered within it. Autophagosome then fuses with the lysosome and the sequestered materials are degraded by lysosomal hydrolases. Autophagosome biogenesis is mediated by dozens of core autophagy-related (Atg) proteins, which are known to gather at the pre-autophagosomal structure (PAS) upon autophagy induction. However, it remained elusive how Atg proteins gather and cooperate to execute autophagosome biogenesis.

In order to reveal the molecular mechanisms of autophagosome biogenesis, we performed structural analyses on all core Atg proteins and established the structural basis of these proteins1,2). During these studies, we noticed that many of Atg proteins are abundant with intrinsically disordered regions (IDRs), which we could visualize using high-speed atomic force microscopy (HS-AFM)3,4). Since IDRs are known to be related to liquid-liquid phase separation (LLPS), a common mechanism for constructing membraneless organelles or condensates, we studied the role of LLPS in autophagy and found that the PAS is a liquid-like condensate formed by LLPS of the Atg1 complex5). Moreover, we also found that autophagy selectively degrades liquid-like protein condensates formed by LLPS but not solid-like aggregates6,7).

After the PAS is organized, initial isolation membrane is generated from the PAS, which then expands using phospholipids mainly derived from the endoplasmic reticulum (ER). The mechanism of how bulk phospholipids are transferred from the ER to the isolation membrane remained elusive. Using in vitro systems, we found that Atg2 and Atg9, which localize at the ER-isolation membrane contact site, possess intermembrane and interleaflet lipid transfer activities, respectively. Based on in vitro and in vivo data, we proposed a novel mechanism that Atg2 and Atg9 collaborate to transfer phospholipids from the ER to the isolation membrane for membrane expansion8,9). We also found that Atg8, a ubiquitin-like protein tightly bound to isolation membranes, possesses membrane perturbation activity in vitro and that Atg8 uses this activity to promote autophagosome biogenesis10)

References
(Related articles)

1) Suzuki et al., Structural biology of the core autophagy machinery. *Curr. Opin. Struct. Biol.* 43, 10-17 (2017).
2) Matoba & Noda, Structural catalog of core Atg proteins opens new era of autophagy research. *J. Biochem.* 169, 517-525 (2021).
3) Yamamoto et al., The intrinsically disordered protein Atg13 mediates supramolecular assembly of autophagy initiation complexes. *Dev. Cell* 38, 86-99 (2016).
4) Kodera et al., Structural and dynamics analysis of intrinsically disordered proteins by high speed atomic force microscopy. *Nat. Nanotech.* 16, 181-189 (2021).
5) Fujioka et al., Phase separation organizes the site of autophagosome formation. *Nature* 578, 301-305 (2020).
6) Yamasaki et al., Liquidity is a critical determinant for selective autophagy of protein condensates. *Mol. Cell* 77, 1163-1175 (2020).
7) Fujioka & Noda, Biomolecular condensates in autophagy regulation. *Curr. Opin. Cell Biol.* 69, 23-29 (2021).
8) Osawa et al., Atg2 mediates direct lipid transfer between membranes for autophagosome formation. *Nat. Struct. Mol. Biol.* 26, 281-288 (2019).
9) Matoba et al., Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion. *Nat. Struct. Mol. Biol.* 27, 1185-1193 (2020).
10) Maruyama et al., Membrane perturbation by lipidated Atg8 underlies autophagosome biogenesis. *Nat. Struct. Mol. Biol.* 28, 583-593 (2021).

CV

EDUCATION

2001	Ph.D. from University of Tokyo, Graduate School of Pharmaceutical Sciences Supervisor: Professor Yoshinori Satow
1998	M.S. in the pharmaceutical sciences from University of Tokyo
1996	B.S. in the pharmaceutical sciences from University of Tokyo

WORK EXPERIENCE

2022 to present	Professor at Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan.
2017 to 2021	Laboratory Head at Institute of Microbial Chemistry, Tokyo, Japan.
2011 to 2017	Chief Researcher at Institute of Microbial Chemistry, Tokyo, Japan.
2008-2011	Lecturer with tenure at Hokkaido University, Sapporo, Japan.
2005-2008	Research Associate at Hokkaido University, Sapporo, Japan.
2001-2005	Postdoctoral fellow at Hokkaido University, Sapporo, Japan.

RESEARCH

My research interest is to understand the molecular mechanisms of autophagy and the roles of liquid-liquid phase separation in various life phenomena.

Y - 1 0

Gut microbiota modulates inflammation in the central nervous system

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While genetic factors are primarily responsible for autoimmune diseases, environmental factors including diet and infections also contribute to their risk. Accumulating evidence has indicated that one of the major environmental factors, gut microbiota, plays a significant role in the pathogenesis of autoimmune diseases including multiple sclerosis (MS). MS is an autoimmune disease characterized by chronic inflammation and demyelination in the central nervous system (CNS), and pro-inflammatory autoreactive T cells, especially T helper 17 (TH17) cells, are key players in the CNS inflammation. Studies of experimental autoimmune encephalomyelitis (EAE; an animal model of MS), as well as human studies, have implicated gut microbiota dysbiosis in the development or severity of MS. However, it remains unclear how gut microbes act on the inflammation of extra-intestinal tissues such as the CNS.

We found that two distinct gut microbes coordinately activate autoreactive TH17 cells that respond specifically to myelin oligodendrocyte glycoprotein (MOG) and worsen EAE. After induction of EAE in mice, MOG-specific CD4+ T cells are observed in the small intestine, where a newly isolated strain in the family Erysipelotrichaceae acts as an adjuvant to enhance the TH17 responses. Shotgun sequencing of the small intestinal microbiota also revealed a strain of Lactobacillus reuteri that possesses peptides that potentially mimic MOG. Mice that are co-colonized with these two strains showed EAE symptoms that were more severe than those of germ-free or monocolonized mice. We also performed EAE experiments using gnotobiotic mice colonized with a synthetic human gut microbiota. Dietary fiber deprivation in these mice leads to the increase in Akkermansia muciniphila and decrease in short chain fatty acids (SCFAs) in the intestine. Although these features have also been observed in MS patients and are thought to contribute to the disease pathogenesis, removal of A. municiphila and addition of SCFAs did not affect EAE symptoms. Comprehensive metabolomic analysis of the intestine revealed that metabolite X is increased upon the fiber deprivation and that the metabolite X promotes the antigen-specific T cell activation.

Our study highlights some potential targets for microbiome-focused therapeutic approaches in MS, although further studies are needed to validate the their roles in humans.

References

(Related articles)

1. Miyauchi E, Shimokawa C, Steimle A, Desai MS, Ohno H. The impact of the gut microbiome on extra-intestinal autoimmune diseases. Nat Rev Immunol. 2022;10.1038/s41577-022-00727-y.

2. Miyauchi E, Kim SW, Suda W, et al. Gut microorganisms act together to exacerbate inflammation in spinal cords. Nature. 2020;585(7823):102-106.

3. Takewaki D, Suda W, Sato W, et al. Alterations of the gut ecological and functional microenvironment in different stages of multiple sclerosis. Proc Natl Acad Sci U S A. 2020;117(36):22402-22412.

4. Duscha A, Gisevius B, Hirschberg S, et al. Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism. Cell. 2020;180(6):1067-1080.e16.

5. Desai MS, Seekatz AM, Koropatkin NM, et al. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. Cell. 2016;167(5):1339-1353.e21.

6. Berer K, Gerdes LA, Cekanaviciute E, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc Natl Acad Sci U S A. 2017;114(40):10719-10724.

CV

2022-current	**Associate Professors**, Laboratory of Mucosal Ecosystem Design, Institute for Molecular and Cellular Regulation, Gunma University
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2016-2019	**Special Postdoctoral Researcher (SPDR) in RIKEN**, Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences (IMS)
2013-2015	**Research Fellow of Japan Society for the Promotion of Science (PD)**
2012-2015	**Postdoctoral fellow**, Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences (IMS), RIKEN
2009-2012	**Research Fellow of Japan Society for the Promotion of Science (DC1)**
2009-2012	**Ph.D. (Dr. of Agriculture)**, Graduate School of Biosphere Science, Hiroshima University
2010-2011	**Visiting PhD Student (JSPS Excellent Young Researcher Overseas Visit Program),** Alimentary Pharmabiotic Centre, University College Cork, Ireland

Y - 1 1

Immune cell dynamics and role in central nervous system diseases

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In recent years, the linkage between the nervous system and the immune system has been the focus of much attention. In addition to neurodegenerative diseases such as Alzheimer's disease, the involvement of the immune system has begun to be strongly implicated in the pathogenesis of psychiatric disorders such as autism spectrum disorder (ASD). It is also becoming clear that immune cells are involved in brain development and aging. The interaction between various immune cells and nervous system cells is thought to be important in various events such as pathological conditions, development, and aging.

Using a mouse model of cerebral infarction, we have reported on the regulatory mechanisms of inflammation and neurological symptoms by immune responses after cerebral infarction. In this symposium, we will introduce immune responses in the brain during various CNS diseases and brain development.

References
(Related articles)

Ito M*, Komai K, Mise-Omata S, Iizuka-Koga M, Noguchi Y, Kondo T, Sakai R, Matsuo K, Nakayama T, Yoshie O, Nakatsukasa H, Chikuma S, Shichita T, Yoshimura A*. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. Nature. 2019; 565(7738): 246-250

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Department of Microbiology and Immunology, Keio University School of Medicine.

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Department of Pathological Science, Graduate School of Medical Science, Kyushu University.

2011 B.S. (Biomedical Science)
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Y - 1 2

Strategies for the expansion of cerebral cortex in primates

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Expansion of the cerebral cortex is one of the important factors that archives higher cognitive abilities and sensorimotor functions. Notably, primates including human have markedly expanded cerebral cortex. However, it is still unclear how species-specific expansion period of neural progenitors is determined and mechanisms underlying massive enlargement of human cerebral cortex. Recent progresses in the field of neural development have led to the discovery of intrinsic differences of neural progenitors among species. The differences in proliferative and neurogenic properties of the canonical neural progenitors have been considered to be responsible for the cortical expansion. On the other hand, the cerebrospinal fluid (CSF), which is produced from the choroid plexus (ChP), also influences neural progenitors, CSF contains various secreting molecules including growth factors and supports neural progenitor proliferation. Interestingly, human embryo has markedly developed ChP, raising a possibility that the differences in CSF contributes to the expansion of cerebral cortex.

We have identified several secreting factors from the embryonic ChP which are preferentially expressed in macaque monkey (Macaca fascicularis). We would found that these factors promoted proliferation of primate neural progenitors, as well as murine ones. We would like to propose that these primate specific CSF factors play important roles in the expansion of cerebral cortex.

References
(Related articles)

1) Human-specific genomics signatures of neocortical expansion.
Florio M. et al. 2017 42: 33-44, Current Opinion in Neurobiology

2) Cerebral cortex expansion and folding: what have we learned?
Fernandez V. et al. 2016 35(10): 1021-1044, The EMBO journal

CV

Education
2005 Ph.D., Graduate School of Medicine, Kyoto University.

Employment
2021- Visiting Assistant Professor, Shiga University of Medical Science
2021- JST FOREST scientist
2009- Assistant professor, Institute of Molecular Embryology and Genetics, Kumamoto University
2006-2009 JSPS research fellow (PD), Institute of Molecular Embryology and Genetics, Kumamoto University
2006 Post-doctoral fellow, Institute of Molecular Embryology and Genetics, Kumamoto University
2002-2005 JSPS research fellow (DC1), Institute for Virus Research, Kyoto University

Session5

S - 0 8

Generation of RNA aptamers and their applications in molecular biology

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RNA has a great potential to specifically bind to various kinds of targets by forming unique three-dimensional structures. RNA molecules that bind to targets of interest, which widely range from chemicals, recombinant proteins to viruses and whole living cells, are called as aptamers. Currently, aptamers serve as a new modality for medical agents and as an experimental tool in molecular biology. Nucleic acid aptamers are isolated from large combinatorial libraries using a unique iterative selection-amplification process known as systematic evolution of ligands by exponential enrichment (SELEX). By modifying SELEX methods to suit proteins of interest, we developed aptamers against P2RY2, a GPCR, and TGF-β1 as a unique GPCR modulator and medical agent, respectively. As for generating aptamers targeting P2RY2, we established a new selection strategy named “VLP-SELEX” using virus-like particles (VLPs) for stabilizing GPCRs with their native structures, and showed unique functions of aptamers as a positive allosteric modulator (PAM) agonist for P2RY2. Regarding anti-TGF-β1 aptamers, we developed the aptamers as medical agents possessing chemical modifications and showed that the aptamers enhance anti-cancer drug efficacy in xenograft model. Herein, I would like to introduce and discuss these aptamers and their application in molecular biology.

References

(Related articles)

01. Takahashi M*, Hashimoto Y, Nakamura Y. “Anti-TGF-β1 aptamer enhances therapeutic effect of tyrosine kinase inhibitor, gefitinib, on non-small cell lung cancer in xenograft model.” *Mol Ther Nucleic Acids.* 2022 June 29 online.

02. Takahashi M*, Amano R, Ozawa M, Martinez A, Akita K, Nakamura Y*. “Nucleic acid ligands act as a PAM and agonist depending on the intrinsic ligand binding state of P2RY2.” *Proc Natl Acad Sci U S A.*. 2021 May 4 118 (18) e2019497118

03. Takahashi M. “Nucleic acid aptamers emerging as modulators of G-protein coupled receptors: Challenge to difficult cell-surface proteins" *Cells.* 2022 June 11(11), 1825 (Review)

04. Takahashi M. “Aptamers targeting cell-surface proteins" *Biochimie.* 2016 Dec 131, 77-84 (Review)

CV

[Education]
2008 Ph.D.in Science, Aoyama Gakuin University
2003 B.S. in Science, Aoyama Gakuin University

[Professional Career]
2015-present: Project Associate Professor, Project Division of RNA Medical Science, IMSUT
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2009-2014: Post-Doctoral Fellow, Department of Molecular Pharmacology, National Institute of Neuroscience, NCNP
2007-2009: Research Fellow of Japan Society for the Promotion of Science (JSPS) (DC2, PD)
2005-2007: Junior Research Associate (JRA), RIKEN

S - 0 9

Identification of chromatin instability at the single-cell level from transcriptome data

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To understand complex human organs at a single-cell resolution, we need to analyze the function and organization of each cell type and state and study their interactions. Single-cell transcriptome analysis is a data-driven approach to identifying cell types and functions by acquiring gene expression data on a whole-gene scale without prior identification of cell types and states in organs and tissues.

Single-cell RNA-seq methods to obtain reads from the full-length RNA and its data analysis methods are needed to understand diseases that involve changes in RNA structure, such as cancer. Single-cell RNA sequencing methods can simultaneously measure thousands to tens of thousands of single-cell transcriptomes (Sasagawa Y. et al. Genome Biol. 2018, Mereu E. et al. Nature Biotech. 2020). However, these high-throughput single-cell RNA sequencing methods determine only 3'-end truncated RNA sequences and cannot capture changes in RNA structural variation. In addition, in principle, non-poly A RNA, estimated to account for 50% of all RNA in typical mammalian cells, cannot be detected. The detection of non-polyA RNAs will lead to the discovery of new disease markers.

Therefore, we developed the world's first Single-cell Full-length Total RNA-seq method RamDA-seq (Hayashi T. et al. Nature Comm. 2018; Nature Digest 2018, Morita R. et al. Nature 2021). We have also developed a method to identify cell-specific genomic regions expressed from data obtained with this method (Matsumoto H. NARGAB 2020) and its visualization method Millefy (Ozaki H. BMC Genomics, 2020). We are currently developing robotic experiment automation and artificial intelligence techniques to identify the genomic instability that leads to abnormal cellular functions. In this talk, I will introduce the progress of the development of these technologies.

References
(Related articles)

1. Ritsuko Morita, et al. Tracing the origin of hair follicle stem cells. Nature. 09 June 2021
2. Hiroshi Ochiai, Tetsutaro Hayashi, Mana Umeda, Mika Yoshimura, Akihito Harada, Yukiko Shimizu, Kenta Nakano, Noriko Saitoh, Hiroshi Kimura, Zhe Liu, Takashi Yamamoto, Tadashi Okamura, Yasuyuki Ohkawa, Itoshi Nikaido. Genome-wide analysis of transcriptional bursting-induced noise in mammalian cells. Science Advances 17 Jun 2020: Vol. 6, no. 25, eaaz6699.
3. Elisabetta Mereu et al. Benchmarking Single-Cell RNA Sequencing Protocols for Cell Atlas Projects. Nature Biotechnology. 06 April 2020.
4. Haruka Ozaki, Tetsutaro Hayashi, Umeda Mana, Itoshi Nikaido. Millefy: visualizing cell-to-cell heterogeneity in read coverage of single-cell RNA sequencing datasets. BMC Genomics volume 21, Article number: 177, 2020.
5. Hirotaka Matsumoto, Tetsutaro Hayashi, Haruka Ozaki, Koki Tsuyuzaki, Mana Umeda, Tsuyoshi Iida, Masaya Nakamura, Hideyuki Okano, Itoshi Nikaido. A NMF-based approach to discover overlooked differentially expressed gene regions from single-cell RNA-seq data. NAR Genomics and Bioinformatics, Volume 2, Issue 1, March 2020.
6. Tetsutaro Hayashi*, Haruka Ozaki*, Yohei Sasagawa, Mana Umeda, Hiroki Danno, Itoshi Nikaido. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. Nature Communications. 9, Article number: 619, 2018.

CV

Employment:

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- Apr. 2013 - Mar. 2018: Unit Leader, Bioinformatics Research Unit, Advanced Center for Computing and Communication, RIKEN, Japan.
- Apr. 2014 - Mar. 2016: Senior Research Scientist, Preventive medicine and applied genomics unit, Advanced Center for Computing and Communication, RIKEN, Japan. (concurrent)
- Jun. 2006 – Mar. 2013: Researcher, Functional genomics unit, Center for Developmental Biology, RIKEN Kobe Institute
- Apr. 2004 – May. 2006: Research fellow, Division of Functional Genomics & Systems Medicine, Research Center for Genomic Medicine, Saitama Medical School

Education:

- Apr. 2001 – Mar. 2004 Doctor’s Degree Course Division of Genome Information Resources, Science of Biological Supramolecular Systems, Graduate School of Integrated Science, Yokohama City University

S - 1 0

Chromatin regulation during skeletal muscle regeneration

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The selective incorporation of appropriate histone variants into chromatin is critical for the regulation of genome function. Although many histone variants have been identified, a complete list has not been compiled. We previously screened the mouse genome in silico, and we identified 14 uncharacterized H3 genes, among which13 are similar to H3.3 and one is similar to the human testis-specific H3 variant, H3T/H3.4. Although some of these genes were previously annotated as pseudogenes, their tissue-specific expression was confirmed by RNA sequencing. However, many of their functions are still unknown. Therefore, we have been analyzing the function of these unknown histone H3 genes. In particular, we have been analyzing H3mm7, H3mm13, and H3mm18,which are expressed in skeletal muscle. In this talk, I would like to report on the progress of the analysis and the latest findings, as well as introduce the original technologies that have supported these analyses.

References
(Related articles)

1. +Honda M, Oki S, Kimura R, Harada A, Maehara K, Tanaka K, Meno C, *Ohkawa Y.
   High-depth spatial transcriptome analysis by photo-isolation chemistry.
   Nat Commun. 2021 Jul 20;12(1):4416. doi: 10.1038/s41467-021-24691-8.
2. +Handa T, Harada A, Maehara K, Sato S, Nakao M, Goto N, Kurumizaka H, *Ohkawa Y, *Kimura H.
   Chromatin integration labeling for mapping DNA-binding proteins and modifications with low input.
   Nat Protoc. 15(10):3334-3360. 2020.
3. *Oka M, Mura S, Otani M, Miyamoto Y, Nogami J, Maehara K, Harada A, Tachibana T, Yoneda Y, Ohkawa Y.
   Chromatin-bound CRM1 recruits SET-Nup214 and NPM1c onto HOX clusters causing aberrant HOX expression in leukemia cells.
   Elife. 8 e46667. 2019.
4. +Harada A, Maehara K, Handa T, Arimura Y, Nogami J, Hayashi-Takanaka Y, Shirahige K, Kurumizaka H, *Kimura H, *Ohkawa Y.
   A chromatin integration labelling method enables epigenomic profiling with lower input.
   Nat Cell Biol. Feb;21(2):287-296. 2019.
5. +Harada A, Maehara K, Ono Y, Taguchi H, Yoshioka K, Kitajima Y, Xie Y, Sato Y, Iwasaki T, Nogami J, Okada S, Komatsu T, Semba Y, Takemoto T, Kimura H, Kurumizaka H, *Ohkawa Y.
   Histone H3.3 sub-variant H3mm7 is required for normal skeletal muscle regeneration.
   Nat Commun. 9(1):1400. 2018.

CV

2003 Ph.D. Graduate School of Medicine, Osaka University
2003-2006 Postdoctoral Fellow, Department of Cell Biology, University of Massachusetts Medical School
2006-2011 Project Associate Professor, Graduate School of Medical Sciences, Kyushu University
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2016- Professor, Department of Transcriptomics, Medical Institute of Bioregulation, Kyushu University

Session6

S - 1 1

Regulation on stem cells in myeloid leukemia: a metabolic perspective

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Cancer cells reprogram and rewire cellular metabolic networks for survival, proliferation, and adaptation to microenvironment. Such altered metabolism often leads to dependency on specific nutrients, and therefore, targeting cancer-specific metabolism would be an effective therapeutic strategy. Towards this goal it is essential to identify metabolic vulnerabilities in human malignancy. In this talk I would like to share our recent findings on the roles of essential role of the branched-chain amino acid (BCAA) metabolism in the maintenance of leukemia stem cells and its functional contribution to disease progression in human cancer.

References

(Related articles)

- Hattori A, Tsunoda M, Konuma T, Kobayashi M, Nagy T, Glushka J, Tayyari F, McSkimming D, Kannan N, Tojo A, Edison A, Ito T.
  Cancer progression by reprogrammed BCAA metabolism in myeloid leukemia. **Nature** 545, 500-504, (2017).
- Fox RG, Lytle NK, Jaquish DV, Park FD, Ito T, et al.
  Image-based detection and targeting of therapy resistance in pancreatic adenocarcinoma. **Nature**, 534, 407-11, (2016).
- Zimdahl B*, Ito T*, Blevins A, Bajaj J, Konuma T, Weeks J, et al.
  Lis1 regulates asymmetric division in hematopoietic stem cells and in leukemia. **Nat. Genet.**, 46, 245–52, (2014).
- Ito T, Kwon HY, Zimdahl B, Congdon KL, Blum J, Lento WE, et al.
  Regulation of myeloid leukaemia by the cell-fate determinant Musashi. **Nature**, 466, 765–8, (2010).

CV

Bio:

Dr. Ito received his Ph.D. in Pharmaceutical Sciences from the University of Tokyo in 2006 for his work on transcriptional regulation by eukaryotic RNA polymerase II. He then shifted his academic focus to stem cell and cancer biology and joined the group of Tannishtha Reya at Duke University Medical Center and later UC San Diego. After the postdoctoral training, Dr. Ito was appointed as an assistant professor in 2013 in the Department of Biochemistry and Molecular Biology at the University of Georgia and then promoted to an Associate Professor. In 2019, Dr. Ito joined the Institute of Life and Medical Sciences at Kyoto University as a professor. His research group studies the molecular mechanisms and regulation of cell fates in stem cells and cancer with a particular focus on the role of RNA binding proteins and metabolic reprogramming in cancer progression and drug resistance.

S - 1 2

Modeling hematopoietic stem cell development in a dish

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Inducing transplantable hematopoietic stem cells (HSCs) from pluripotent stem cells (PSCs) in culture has been a long outstanding challenge in developmental biology and regenerative medicine. During the ontogeny of vertebrate species, HSCs derive from a specialized population of endothelial cells known as hemogenic endothelial cells (HECs). HECs are not merely the developmental origin of HSCs: they also generate various types of blood cells independently of the HSC development. In the case of mouse embryos, HECs in the extraembryonic yolk sac give rise to erythro-myeloid progenitors and lymphoid-myeloid progenitors. However, the yolk sac HECs do not generate HSCs. In the intraembryonic region, HECs of the dorsal aorta generate diverse blood precursors, including multipotent hematopoietic progenitors, before the emergence of HSCs. HSCs are eventually formed from the late HECs in the dorsal aorta as the last wave of the definitive hematopoiesis. The HSC-independent hematopoietic lineages with different developmental origins and pathways have been an obstacle to the induction of HSCs from PSCs in culture. The successful derivation of HSCs from PSCs will require the culture method to correctly follow the developmental pathway toward the definitive HSC without deviating to the easy-to-make HSC-independent lineages. To this end, it is necessary to precisely identify intermediate precursors in various developmental stages between the pluripotent epiblasts and the definitive HSCs. The signaling molecules driving the proliferation and differentiation of each intermediate precursor should also be identified. Single-cell RNA-sequencing of mouse embryos provides valuable insights into the process of HSC development. This technology helps identify candidate cell populations of intermediate precursors and deduce the signaling pathways activated in the precursors. For verification, the candidate precursors are isolated from mouse embryos and cultured in the presence of deduced signaling molecules to induce their differentiation into transplantable HSCs. Once the culture conditions are determined by tracing the developmental stages step-by-step, these conditions can be directly applied to mouse embryonic stem cells to induce HSCs in culture. An example of our ongoing approach in line with this strategy will be discussed.

References
(Related articles)

Tsuruda, M., S. Morino-Koga, and M. Ogawa. Hematopoietic stem cell-independent differentiation of mast cells from mouse intraembryonic VE-cadherin^{+} cells. Stem Cells 40: 332-345, 2022. DOI: 10.1093/stmcls/sxac001

Tsuruda, M., S. Morino-Koga, and M. Ogawa. Bone morphogenetic protein 4 differently promotes distinct VE-cadherin^{+} precursor stages during the definitive hematopoietic development from embryonic stem cell-derived mesodermal cells. Exp. Hematol. 103: 40-51.e7, 2021. DOI: 10.1016/j.exphem.2021.08.008

Tsuji-Tamura, K., S. Morino-Koga, S. Suzuki, and M. Ogawa. The canonical smooth muscle cell marker TAGLN is present in endothelial cells and is involved in angiogenesis. J. Cell Sci. 134 (15): jcs254920, 2021. DOI: 10.1242/jcs.254920

Ahmed, T., K. Tsuji-Tamura, and M. Ogawa. CXCR4 signaling negatively modulates the bipotential state of hemogenic endothelial cells derived from embryonic stem cells by attenuating the endothelial potential. Stem Cells 34:2814-2824, 2016. DOI: 10.1002/stem.2441

CV

Name (Surname, First name): Ogawa, Minetaro

Education:

1980–1984	Faculty of Pharmaceutical Science, Kanazawa University, Kanazawa, Japan Bachelor of Pharmacy
1984–1986	The Master's Course in Pharmaceutical Science, Kanazawa University Master of Pharmacy
1986–1987	The Doctoral Course in Pharmaceutical Science, Kanazawa University
1992	Ph.D. (Doctor of Pharmacy, Kanazawa University)

Work Experience:

1987–1992	Assistant Professor, Institute for Medical Immunology, Kumamoto University School of Medicine, Kumamoto, Japan
1992–1993	Assistant Professor, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine
1993–1995	Assistant Professor, Department of Molecular Genetics, Faculty of Medicine, Kyoto University, Kyoto, Japan
1995–1997	Member Scientist, Basel Institute for Immunology, Basel, Switzerland
1997–2000	Assistant Professor, Department of Molecular Genetics, Graduate School of Medicine, Kyoto University
2000–2002	Associate Professor, Department of Molecular Genetics, Graduate School of Medicine, Kyoto University
2002 to present	Professor, Institute of Molecular Embryology and Genetics, Kumamoto University

Poster Session

P - 0 1

A cross-talk between microtubules and focal adhesions regulates dynamics of actin cytoskeleton

Nishimura, Yukako[1,2]; Nisha Bte Mohd Rafiq[2] ;Sergey V. Plotnikov[3]; Visalatchi Thiagarajan[2]; Zhen Zhang[2]; Meenubharathi Natarajan[2]; Shidong Shi[2]; Virgile Viasnoff[2,4,5]; Gareth E. Jones[6]; Pakorn Kanchanawong[2,7] and Alexander D. Bershadsky[2,8]

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The interrelationship between microtubules and the actin cytoskeleton in mechanoregulation of integrin-mediated adhesions is poorly understood. Here, we show that the effects of microtubules on a major type of cell-matrix adhesion, focal adhesions, are mediated by KANK family proteins connecting the adhesion protein talin with microtubule tips. Both total microtubule disruption and microtubule uncoupling from adhesions by manipulations with KANKs trigger a massive assembly of myosin-IIA filaments. Myosin-IIA filaments, augmenting the focal adhesions, are indispensable effectors in the microtubule-dependent regulation of integrin-mediated adhesions. Myosin-IIA filament assembly depends on Rho activation by the RhoGEF, GEF-H1, which is trapped by microtubules when they are connected with integrin-mediated adhesions via KANK proteins but released after their disconnection. Thus, microtubule capturing by integrin-mediated adhesions modulates the GEF-H1-dependent effect of microtubules on the myosin-IIA filaments. Subsequent actomyosin reorganization then remodels the focal adhesions, closing the regulatory loop.

P - 0 2

Extracellular Domains I and II of CD44 mediate its trans-homophilic dimerization and tumor cluster aggregation

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CD44 molecule (CD44) is a surface glycoprotein on cancer stem cells. However, its utility as a therapeutic target for managing metastases remains to be fully evaluated. We examined the biochemical properties of CD44 in homotypic tumor cell aggregation. The standard CD44 form (CD44s) mainly assembled as intercellular homodimers (*trans*-dimers) in tumor clusters rather than intracellular dimers (*cis*-dimers) present in single cells. Based on machine learning-based computational modeling combined with experimental mutagenesis tests, substituted 10 these residues in Domain I or 5 residues in Domain II abolished CD44 dimerization and reduced tumor cell aggregation *in vitro*. Importantly, the substitutions in Domain II dramatically inhibited lung colonization in mice. The CD44 dimer-disrupting substitutions decreased downstream P21-activated kinase 2 (PAK2) activation, suggesting that PAK2 activation in tumor cell clusters is CD44 *trans*-dimer-dependent. These results shed light on the biochemical mechanisms of CD44-mediated tumor cell cluster formation and may help inform the development of therapeutic strategies to prevent tumor cluster formation and block cluster-mediated metastases.

P - 0 3

Epigenetic dysregulations in the lysine methyltransferase (KMT2) deficient endometrial cancer cells

Ryosuke Kobayashi[1], Reika-Kawabata-Iwakawa[2], Makoto Sugiyama[3], Takehiro Yokobori[2], Sumiyo Morita[1], Takuro Horii[1], Masahiko Nisiyama[2] and Izuho Hatada[1]

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Endometrial cancer (EMC) is the most common gynecological malignancy in Japan. The lysine methyltransferase 2 (KMT2) family genes are frequently mutated in patients of EMC. Specially, KMT2C/D, which are involved in H3K4me1 deposition in enhancer regions, were mutated in 28.7% and 21.0%, respectively in EMC. However, the biological consequences of KMT2 mutations for the endometrial cancer development has not been shown. To challenge these issues, we established KMT2-deficient EMC cell lines using CRISPR/Cas9 genome editing. Ablation of KMT2 showed repression of cell growth and migration in vitro. On the other hand, three-dimensional culture system revealed that spheroids formed from KMT2-deficient cells had structural abnormality with spike-like protrusion compared to those from intact cells. Morphological analysis by transmission electron microscope revealed that KMT2-deficient cells showed disruption of cellular polarity. RNA-seq and ChIP-seq analysis demonstrated that genes involved in epithelial functions such as adhesion or extracellular matrix organization were largely repressed in KMT2-deficient cells, and active enhancer marks accompanied with these genes were also disappeared. These data suggest that KMT2 ablation disrupt epithelial functions through epigenetic dysregulations in EMC cells.

P - 0 4

A novel E3 ligase is involved in Unfolded Protein Response through Ubiquitination of eS7A and up-regulation of Hac1 production in yeast

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The endoplasmic reticulum (ER) is the major intracellular calcium reservoir in the cell and is arranged in a dynamic tubular network involved in gluconeogenesis and lipid synthesis. Accumulation of unfolded or misfolded proteins in ER due to various stresses induces ER stress. Unfolded stress response (UPR) is a conserved cellular pathway involved in protein quality control to maintain homeostasis under ER stress. In the yeast UPR signaling pathway, the ER-located transmembrane protein Ire1 is crucial for splicing of the HAC1 pre mRNA, HAC1(u) to produce HAC1i mRNA that encodes the Hac1, a transcriptional factor essential for UPR.
We recently reported that E3 ligase Not4 was involved in UPR in yeast, Saccharomyces cerevisiae. Not4-mediated mono-ubiquitination of eS7A is crucial for increasing the translation efficiency of HAC1i mRNA. More recently, we identified GRR1 as an essential E3 ligase for resistance to Tunicamycin (Tm), an ER stress-inducing drug. Grr1p is involved in eS7A ubiquitination and translation of HAC1i mRNA, however, the role of eS7 ubiquitination by Grr1p in the translation of HAC1i mRNA remains unknown.
In this study, we have investigated the relationship between Grr1p-mediated ubiquitination of eS7A and up-regulation of Hac1 expression to reveal the function of GRR1 under ER stress conditions. We found that Grr1p forms a polyubiquitin chain on the K83 residue of eS7A under ER stress conditions. In addition, Hel2, another E3 ligase that polyubiquitinated eS7A via K63 linkage, was not involved in the translation of HAC1i mRNA. These results suggest that non-K63-linked polyubiquitination by Grr1p may be required for the translation of HAC1i mRNA. In summary, we propose that polyubiquitination of eS7A by Grr1p is crucial for the translation of HAC1i and UPR.

P - 0 5

Molecular mechanism of YAP-dependent hepatocyte elimination

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Background and Purpose: The oncogene product Yes-associated protein (YAP) is a transcriptional cofactor that serves in the nucleus to enhance cell proliferation. It has been reported that YAP is highly expressed in the nucleus in cancer tissues of various organs including liver, and that the nuclear level of YAP correlates with poor prognosis of patients. Activation of YAP in mouse liver also induces hepatocarcinogenesis. On the other hand, we found that when YAP activated forms were induced in a mosaic fashion with liver injury, YAP-expressing hepatocytes were eliminated within one week. However, the molecular mechanism of how activated YAP-expressing precancerous hepatocytes are eliminated is unknown. The aim of this study was to elucidate this hepatocyte elimination mechanism.
Methods: Using the hydrodynamic tail vein injection (HTVi) method, we introduced a plasmid expressing YAP (2SA), an activated form of YAP, into mouse liver. Hepatocytes expressing activated YAP were isolated by FACS and subjected to comprehensive gene expression analysis using RNA-seq.
Results: Several genes were altered in an activated YAP-dependent manner. Ontology analysis of the identified genes showed that transcription of stress-responsive genes was upregulated by YAP. These genes included Rho-guanine nucleotide exchange factors, which functions in actin cytoskeletal remodeling, and several chemokines involved in macrophage activation. On the other hand, transcription of metabolism-related genes was suppressed by YAP.
Discussion: These results suggest that cell morphology changes mediated by actin remodeling, immune cell recruitment mediated by chemokines, and changes in intracellular metabolism are involved in the elimination of YAP-activated cells. In this way, the liver maintains homeostasis by eliminating YAP-activated precancerous hepatocytes, and disruption of this elimination mechanism is thought to increase the risk of hepatocarcinogenesis. The results of this study are also expected to lead to the development of therapeutic and preventive methods for hepatic cancer targeting the mechanism of abnormal cell elimination.

P - 0 6

Application of CDK4/6 inhibition therapies to PDACs

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The CDK4/6-dependent RB1 mono-phosphorylation triggers cells to pass through the G1-S checkpoint in cell cycle following RB1 hyper-phosphorylation. Thus, CDK4/6 inhibitors (CDK4/6i) are emerging cancer therapeutic tools. However, many intrinsic or extrinsic factors and mutational landscapes often halt the effectiveness of CDK4/6i therapies. Pancreatic ductal adenocarcinomas (PDAC) are depicted by very frequent incidence of Kras mutation and rare RB1 mutation. Here we investigated the effects of a CDK4/6i, Palbociclib, on PDAC lines harboring KrasG12D or KrasG12C mutation, and assessed the mechanisms leading to therapy resistance. RB7LP is a constitutively active form mutant of RB1, lacking N-terminal end with all serines substituted to unphosphorylatable forms. RB7LP overexpression in cells phenocopies the CDK4/6i effects including senescence-like phenotypes and partial apoptosis. Therefore, RB7LP-overexpressing cells may serve as a good model to parallelly examine the mechanism of intrinsic resistance to CDK4/6i. We screened chemical libraries to seek molecules that enhance the cytotoxic action of RB7LP in PDACs, which identified several kinase inhibitors. One of them appeared to target a signaling molecule X. We further observed that CDK4/6 inhibition in PDACs increased the activity of X and moreover that of a receptor kinase Y upstream of X. Importantly, inhibition of either X or Y dramatically synergized with Palbociclib treatment in PDAC lines. We are currently investigating the mechanism whereby CDK4/6i treatment increases activity of X and Y, and gathering proof of concepts that may support future application of CDK4/6i to PDAC patients in combination with a rationally selected kinase inhibitor.

P - 0 7

Structural basis of Marburg virus helical nucleoprotein-RNA complex formation

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Marburg virus (MARV), which belongs to the family Filoviridae in the order Mononegavirales, causes lethal hemorrhagic fever in humans and non-human primates. MARV poses a threat to public health; however, no vaccine or antiviral drugs have yet been licensed.
The nucleoprotein (NP) binds the non-segmented, single-stranded, negative-sense viral genomic RNA (vRNA) to form the helical NP－RNA complex. The helical NP-RNA complex acts as a scaffold for nucleocapsid formation and as a template for viral genome transcription and replication. Proper interactions between NPs and vRNA are required for the helical assembly and their function. However, the structural basis for the helical assembly remains largely elusive.
Here, to elucidate the structural basis for the nucleocapsid formation, we determined the structure of the MARV NP-RNA complex using cryo-electron microscopy. Structure-based mutational analysis of MARV and Ebola virus (EBOV) NPs identified residues critical for helix assembly and subsequent viral RNA synthesis. Most of the residues were conserved in filoviruses, suggesting that filoviruses share common molecular mechanisms for nucleocapsid formation. The findings advance our understanding of filovirus morphogenesis and contribute to the development of novel antivirals against MARV and EBOV.

P - 0 8

Abnormal pH environment interrupts cell competition-mediated developmental robustness

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Our bodies are formed through repeated cell division from a single fertilized egg. During this process of embryonic development, defective cells are suddenly generated due to genomic errors. We found that these defective cells are eliminated by cell competition in zebrafish embryos. The defective cells are recognized by the neighboring normal cells and are eliminated via cell death. This cell competition supports precise embryogenesis and confers developmental robustness. Interestingly, recent NGS analysis has revealed that cell population originating from mutant cell at early embryo are involved in various diseases such as Alzheimer's disease and diabetes. These mutant cells occurring during embryogenesis may have evaded elimination due to disruption of cell competition. However, the factors that disrupt the elimination by cell competition are not well understood. In this study, we focused on the environmental perturbations. As a result, we found that acidic pH environment disrupts cell competition. As does alkaline pH environment. The mechanism of suppression of cell competition by the abnormal pH environment is becoming clear that the abnormal pH environment acts on the sensing mechanism of abnormal cells by neighboring normal cells. Thus, a new factor for disrupting cell competition has been uncovered. Interestingly, it has been reported that the extracellular pH environment changes to acidic due to various situations such as cancer microenvironment, hypoxia, and inflammation, suggesting that cell competition may also be disrupted in these situations.

P - 0 9

Altered behavior and increased neuroinflammation in Importin α4/KPNA4 KO mice

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Importins are a family of proteins that assist newly synthesized polypeptide molecules to be transported from the cytoplasm into the nucleus. Importin αs (Imp αs/KPNAs), are a type of Importin that bind to classical nuclear localization signals contained in the sequences of cargo proteins and form a cargo-Impα-Importin β trimer, which allow the cargo to move through nuclear pore complexes into the nucleus. The characteristics of Imp αs related to nucleocytoplasmic transport have been extensively studied, but the physiological implications of such cellular functions are still under investigation. 3 Impα subtypes are expressed highly in the mammalian brain and have been associated to psychiatric disease and/or alterations in mouse behavior, bringing up the importance of Impαs in brain function and behavioral regulation.
In particular, the Importin α4 protein (KPNA4) has been known to transport NF-κB transcription factors to the nucleus, and decreased expression has been associated to psychiatric symptoms of schizophrenia and decreases in NF-κB signaling. To examine the behavioral phenotypes of Kpna4 KO mice, we conducted a behavioral task battery. A KPNA4 KO mouse line showed behavioral abnormalities including increased anxiety and reduced sensorimotor gating, providing evidence that KPNA4 is associated with brain function and behavior. To further characterize the alterations in Kpna4 KO mice, we measured inflammatory cytokines from several different brain regions and primary cultured cells and found increases in levels of inflammatory cytokines in KO. We aim to elucidate the molecular background underlying the behavioral abnormalities through characterization of cellular gene expression patterns.

P - 1 0

RHBDL2 has essential roles for glutaminolysis and chemoresistance in triple negative breast cancer

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The current therapeutic modalities for triple-negative breast cancer (TNBC) are limited, and the development of new molecular targeting therapeutic approaches is required. We here report the pivotal role of cancer-specific intramembrane serine protease RHBDL2 (Rhomboid-like 2) in regulating glutaminolysis in chemoresistance of TNBC. RHBDL2 expression is significantly associated with poor prognosis in TNBC. Furthermore, RNA interference (RNAi)-mediated attenuation of RHBDL2 in TNBC cells significantly suppressed cell proliferation and progression, whereas ectopic RHBDL2 overexpression in HCC1806 cells drove their proliferation. Notably, RHBDL2 interacts with ASCT2 (SLC1A5: solute carrier family one member 5), a significant glutamine transporter, thereby inducing glutamine uptake, promoting glutaminolysis and oxidative phosphorylation, resulting in chemoresistance to docetaxel and taxanes in TNBC cells. These findings suggest that the RHBDL2-ASCT2 axis plays a critical role in the regulation of glutaminolysis and may be a promising therapeutic target for TNBC treatment.

P - 1 1

Physiological functions of novel proteins encoded by hidden ORFs

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Although significant progress has been made in genome, epigenome, and transcriptome analyses in recent years, but the progress of translatome analysis remains behind compared to them. In translatome analysis, we are particularly focusing on "extension of known open reading frames (ORFs)" and "identification of novel ORFs".
Translatome analysis relies on ribosome profiling (Ribo-seq), but Ribo-seq data contain a lot of experimental noise. We have therefore established a novel method termed TISCA to precisely identify translation initiation sites, and have identified many proteins that are translated from Non-AUG initiation codons (Ichihara K., Matsumoto A*. et al., Nucleic Acids Res., 2021). Such Non-AUG initiation codons are often present upstream of the canonical AUG initiation codon, producing N-extended proteins (extension of known ORFs). Some of the extended N-terminal regions contain localization signals, which result in different subcellular localizations. We are currently analyzing mice that specifically lack N-extended proteins.
Furthermore, although long non-coding RNAs (lncRNAs) are defined as RNAs that do not translate proteins, we found that some lncRNAs translate polypeptides of less than 100 amino acids (identification of novel ORFs) (Matsumoto A. et al., Nature, 2017). These novel polypeptides regulate the functions of VDAC and HDAC3, and knockout mice showed diverse phenotypes such as abnormal social behavior, delayed skin regeneration and infertility (Nita A., Matsumoto A*. et al., PLoS Genet., 2021, Mise S., Matsumoto A*. et al., Nat. Commun., 2022).
Thus, we have succeeded in "extension of known ORFs" and "identification of novel ORFs" and revealed that these hidden proteins contribute to various physiological functions.

P - 1 2

The female-specific regulation of meiotic cell cycle in murine germ cells

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The meiosis is specialized cell cycle to produce haploid cells that is triggered by the activation of meiotic genes by STRA8-MEIOSIN. As mitosis-meiosis transition occurred in S phase, meiotic gene activation should coincide with S phase. In the mammalian gametogenesis, meiotic initiation is occurred in sex-specific timing. In testis, meiosis continuously initiated throughout their life after puberty. In contrast, in the ovary, all germ cells enter meiosis synchronously in embryonic stage and keep cells in meiotic prophase for long term. Although such sexual difference is well known, the underlying mechanism to ensure sexually different mode of meiosis remain elusive. Here, we identified retinoblastoma family protein, RB1 and p107 (RB-like 1) as STRA8 interacting protein. RB is well known as a repressor of S phase entry during cell cycle. To elucidate the physiological relevance of STRA8-RB interaction, we generated mutant mice that express STRA8 lacking LXCXE motif (Stra8ΔRB), in which STRA8 cannot bind to RB but preserves intact interaction with MEIOSIN. In Stra8ΔRB mutant mice, whereas male mice produce functional sperm, female prematurely lose oocytes before puberty. To address the role of RB-STRA8 interaction in meiotic initiation, we conducted single cell-RNA-sequencing analyses using STRA8-expressing cells from, control, Stra8ΔRB and STRA8-null ovaries. Transcriptome analysis revealed that STRA8-null cells highly expressed pluripotent genes, suggesting STRA8 functions to induce differentiation prior to meiosis. We also revealed that without RB-STRA8 interaction, S phase entry and expression of the Meiosin were compromised. As a result, meiosis progression was delayed in Stra8ΔRB germ cells. Our present data suggests that STRA8 have central role to induce cell differentiation, S phase entry and meiotic initiation to ensure production of functional oocytes.

P - 1 3

Formation of pericentromeric heterochromatin via ZNF518s that link satellite DNA to heterochromatin

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Aneuploidy is caused by chromosomal missegregation and frequently observed in cancers and hematological diseases, making it important to understand the molecular mechanisms underlying chromosomal segregation. The intricate structure formed at the centromere/kinetochore plays an integral role in the faithful segregation of chromosomes. For instance, heterochromatin present at pericentromeric alpha-satellites is essential for proper chromosomal segregation, although how heterochromatin is targeted to pericentromeres is not fully understood. Here, we demonstrate a novel mechanism by which two homologous zinc-finger proteins of ZNF518s (ZNF518A and ZNF518B) play roles in heterochromatin formation at pericentromeres. We investigate this mechanism by determining the cellular localization of ZNF518s, centromeric proteins, such as CENP-A and CENP-B, and heterochromatin factors, including HP1 and histone H3K9 tri-methylation. The results indicate that the multiple segments within ZNF518s contribute to their interactions with CENP-B, HP1, and G9a histone H3K9 methyltransferase. This study identifies the novel mechanism underlying pericentromeric heterochromatin formation, which is mediated by the central components, ZNF518s.

P - 1 4

Aurora A-dependent polyubiquitination of OLA1 regulates centrosome number through controlling the recruitment of PCM proteins

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BRCA1/BARD1 functions in a variety of cellular processes, including DNA repair and centrosome regulation. We have identified Obg-like ATPase 1 (OLA1) as a BARD1-interacting protein. OLA1 binds to BRCA1 and BARD1 and localizes to centrosome throughout the cell cycle, whereas its localization decreases in G2 phase. Knockdown or overexpression of OLA1 causes centrosome amplification, suggesting that adequate level of OLA1 expression is important for the regulation of centrosome number. We have found that OLA1 is polyubiquitinated by Aurora A, which is known as a mitotic kinase, following degradation via the proteasome in G2 phase.
In this study, we found that the expression level of OLA1 negatively regulates the recruitment of g-tubulin and pericentrin, a conserved pericentriolar materials (PCM) scaffold protein, to centrosome in G2 phase. Furthermore, the E3 ubiquitin ligase activity of Aurora A was involved in the recruitment of these PCM proteins to centrosome in G2 phase and in the regulation of centrosome number. Interestingly, we also found that the phosphomimetic variant, OLA1-T124E, enhanced the polyubiquitination of OLA1, but not the non-phosphorylated variant, OLA1-T124A. NIMA-related kinase 2 (NEK2), which is a potential kinase for T124 residue of OLA1, promoted OLA1 polyubiquitination by Aurora A.
These suggest that Aurora A-dependent ubiquitination of OLA1 regulates centrosome number through controlling the recruitment of PCM proteins to G2 phase.

P - 1 5

The role of small GTPase protein Rab35 in brain development

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The largest family of small GTPases, Rab proteins function as molecular switches that regulate intracellular membrane trafficking. Rab35, a highly conserved Rab in metazoans, is a multifunctional small GTPase that regulates endocytic recycling of protein cargos to plasma membrane, cytoskeletal rearrangement, cytokinesis. However, its physiological importance in mammalian development remains unclear. Here, we generated systemic Rab35-knockout mice and found that Rab35 is essential for early embryogenesis. Interestingly, brain-specific Rab35-knockout mice exhibited defects in brain functions such as anxiety-related behaviours. Moreover, they showed hippocampal malformation due to the impaired migration of pyramidal neurons, although evident cerebral cortex formation defects were not observed. Quantitative proteomics indicated that the loss of Rab35 significantly affected the levels of several proteins associated with endocytic trafficking, as well as some neural cell adhesion molecules. Altogether, our findings reveal that Rab35 plays a crucial role in the developing hippocampus by regulating the expression of cell adhesion molecules.

P - 1 6

Identification and validation of multi-specific interactions of a tumor suppressor protein PRELP

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The small leucine-rich repeat proteoglycans (SLRPs) are a family of secreted proteins, many of that regulate various cellular functions through the interactions with different proteins in the extracellular environment. Proline and arginine-rich end leucine-rich repeat protein (PRELP) is a SLRP, known to function as a tumor suppressor and that mRNA of PRELP is down-regulated in many types of cancer, although the details of the molecular mechanism have yet to be fully clarified. Since we hypothesized that PRELP regulates tumor cell proliferation through some interactions with different proteins as other SLRPs, we investigated the new interactions of PRELP to characterize the molecular mechanism of PRELP-mediated tumor suppression.
We first assessed the interaction of PRELP with transforming growth factor-β1 (TGFβ1), a growth factor known to interact with several SLRPs. A pull-down assay showed that PRELP interacted with TGFβ1 as other SLRPs. Then, we also carried out co-immunoprecipitation coupled with mass spectrometry (CoIP-MS) to comprehensively identify novel interactions between PRELP and membrane proteins. The proteomic analysis identified various membrane proteins that would interact with PRELP, including two growth factor receptors, insulin-like growth factor I receptor (IGFI-R) and low-affinity nerve growth factor receptor (p75NTR). Surface plasmon resonance (SPR) analysis using recombinant proteins validated the direct binding of PRELP to TGFβ1, IGFI-R, and p75NTR with around micromolar affinities. Furthermore, cell-based analysis using recombinant PRELP protein showed that PRELP suppressed cell growth and affected cell morphology of A549 lung carcinoma cells, also at micromolar concentration, which is consistent with the micromolar affinities measured by SPR analysis. Our results indicate that PRELP regulates the tumor cell proliferation triggered by the weak-affinity multi-specific interactions (H. Kosuge *et al., J. Biol. Chem*. 2021; 296: 100278).

P - 1 7

Establishment of a high-content polymer array screening system to explore niche mimicries for cancerous cells

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Chemical-based approaches are one of the gold standard therapies for cancers. However, fundamentally new modalities still need to be developed due to the resistant properties of refractory cancers against conventional small molecular compounds that act inside cells. Thus, macromolecules controlling cells from outside (i.e., microenvironment) are considered possible key options to tackle this issue. Although some synthetic polymers with bio-functionalities have been reported, the presence of polymer scaffolds that affect cancer nature and their detailed mechanisms of action remains to be demonstrated. Here, the groundworks for a high-content polymer screening system have been set up to explore functional polymers that specifically regulate cancer cells. To fabricate the microwell plates for such screening, sixty-nine acrylate heteropolymers were designed with two from 14 kinds of biocompatible monomers, photo-polymerized at the defined condition of 254 nm UV illumination and immobilized on the acrylate-functionalized glass bottom surface. Residual monomers were washed out by ethanol and water, and the immobilization of polymers was confirmed by using fluorescein-O-acrylate. Finally, adult p53-/- mouse-derived astrocytes (AS) and their transformed ones by RasV12 oncogenic mutant (AS-RasV12) were mixed and seeded onto polymer arrays. The numbers of cells were evaluated at 24 and 96 hours after seeding, and two hit polymers with different growth-supporting preferences for AS and AS-RasV12 cells were identified. One cancer-friendly polymer grew AS-RasV12 by 22.7-fold and AS by 14.2-fold, and another polymer grew AS-RasV12 3.2-fold and AS 5.8-fold. Further investigation to reveal the mechanisms of action how these hit polymers specifically support cancerous cells will help us uncover unknown cancer characteristics, leading to the development of new modalities and the identification of new therapeutic targets.

P - 1 8

Lysosomes regulate oncogenic signals in acute myelogenous leukemia

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Even though a majority of leukemia cells are killed by chemotherapy, persistent leukemic stem cells (LSCs) are still not eradicated with current treatments, due to various drug-resistance mechanisms. Recent studies have revealed that nutrients, such as amino acid, sugar, and lipid, are critical determinants of cell proliferation and differentiation of LSCs, indicating metabolic pathways have critical roles in regulation of "stemness" in LSCs. In this study, to understand hallmarks of metabolic regulation of stemness in acute myelogenous leukemia (AML), we screened metabolic pathways that critical for maintenance of undifferentiated status of AML cells. For this purpose, we establisted sgRNA libraries comprehensively targeting molecules in human metabolisms. Cas9-expressing HL-60 cells were transduced with these sgRNA libraries, and then forced-differentiated cells by CRISPR/Cas9-mediated gene-deletion were sorted. We found that sgRNA in the differentiated cells enriched targeted-genes that contribute particular metabolic pathways: lysosomal acidification, iron-sulfur clusters, and electron transport in mitochondrial. We confirmed that pharmacological inhibition of lysosome acidification efficiently induced cell differentation and apoptosis in AML cell lines. RNA-seq indicated that inhibition of lysosome acidification suppress transcription of oncogenes. We also found that H3K27ac occupancy around an oncogene was significantly reduced by inhibition of lysosome acidification. Furthermore, combination therapy with another anti-cancer drug and inhibitor of lysosome acidification, synergistically suppressed tumor formation *in vivo*. These data indicated that lysosome regulates expression of oncogenes, and lysosome acidification is a promissing therapetic target for AML.

P - 1 9

Identification of factors essential for CXCR4 signaling in leukemic cells using CRISPR dropout screens

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Blood cells, including immune cells, are generated from hematopoietic stem and progenitor cells (HSPCs) in the bone marrow. HSPCs are in contact with and maintained by special microenvironments, known as niches, in the bone marrow. CXCL12 as well as stem cell factor, which are produced by HSPC niches, are essential for HSPC maintenance.

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignant neoplasm of the bone marrow, and the outcome of treatment-refractory and/or relapsed T-ALL is poor. CXCL12 is essential for the migration of leukemic cells to the bone marrow and the retention and maintenance of leukemic cells in the bone marrow; however, factors essential for CXCL12-induced chemotaxis of leukemic cells are not fully described.

To identify genes essential for the regulation of CXCL12-induced chemotaxis of leukemic cells, we carried out a genome-wide CRISPR/Cas9 screen in a chemotaxis assay with a human T-ALL cell line. Cas9-expressing leukemic cells were transduced with the human CRISPR library, cultured for 10 days, and used for a chemotaxis assay toward CXCL12. Input cells and migrated cells were harvested to determine their gRNA content. The genome-wide screen identified 1,072 genes which were significantly depleted from the migrated cells, suggesting that these genes are essential for the regulation of CXCL12-indeced chemotaxis of the leukemic cells. *CXCR4* (encoding CXCR4, the main physiological receptor for CXCL12) and *RHOA* (encoding RHOA, a guanosine triphosphatase essential for CXCL12-induced chemotaxis) were highly depleted from the migrated cells, confirming the robustness of our screen. Some genes which had not been reported to regulate CXCL12-induced chemotaxis were highly depleted from the migrated cells.

Thus, we develop a new screening system which effectively identify genes essential for a CXCL12-induced chemotaxis by combining the genome-wide CRISPR-Cas9 dropout screening system and the chemotaxis assay system. Our screening strategy can be used to explore factors essential for another chemokine-induced chemotaxis of other types of cells.

P - 2 0

An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies

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Antibodies against the receptor-binding-domain of the SARS-CoV-2 spike protein prevent SARS-CoV-2 infection. However, the effects of antibodies against other spike protein domains are largely unknown. Here, we screened a series of anti-spike monoclonal antibodies from COVID-19 patients, and found that some of antibodies against the N-terminal-domain (NTD) induced the open conformation of receptor binding domain (RBD) and thus enhanced the binding capacity of the spike protein to ACE2 and infectivity of SARS-CoV-2. Mutational analysis revealed that all the infectivity-enhancing antibodies recognized a specific site on the NTD. Structural analysis demonstrated that all the infectivity-enhancing antibodies bound to NTD in a similar manner. The antibodies against this infectivity-enhancing site were detected at high levels in severe patients. Moreover, we identified antibodies against the infectivity-enhancing site in uninfected donors, albeit at a lower frequency. These findings demonstrate that not only neutralizing antibodies but also enhancing antibodies are produced during SARS-CoV-2 infection.

P - 2 1

Induction of cellular senescence by sustained NFκB activation

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Nuclear Factor-kappa B (NFκB) is an important transcription factor implicated in various biological phenomena such as cancer, inflammation, aging, and immune response. The canonical pathway of NFκB has multiple feedbacks, and activation of NFκB is known to exhibit oscillation behavior. NFκB inhibitor alpha (IκBα) is one of target genes of NFκB, but also mediates a powerful negative feedback loop that may remove NFκB from DNA and result in oscillatory NFκB activity. Knockout of the IκBα gene induces sustained NFκB activation in cells stimulated with tumor necrosis factor α (TNFα), leading to gene expression distinct from oscillatory NFκB activation (Cheng et al., Science, 2021). However, whether and how this change in the dynamics of NFκB activity link to cell properties or fate is not fully understood.
In breast cancer MCF7 cells, knockdown of IκBα and stimulation of TNFα induced sustained activation of NFκB, which promoted cellular senescence phenotypes, including increased cell size, cell cycle arrest, elevated SA-βgal activity, and upregulated senescence-associated secretory phenotype (SASP) gene expression. Furthermore, metabolomic and transcriptomic analyses revealed that the sustained activation of NFκB induced some characteristic metabolic alterations associated with cellular senescence. In this presentation, we would like to discuss the molecular mechanisms of cellular senescence caused by the altered dynamics of NFκB activity due to IκBα knockdown.

P - 2 2

Endoplasmic Reticulum Stress Sensor IRE1 Directly Detects Misfolded Insulin by Oligomerization

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The mammalian endoplasmic reticulum (ER) is an organelle that produces secretory proteins such as insulin, collagen, and IgG. Sometimes, the accumulation of misfolded proteins (ER stress) is caused by external environmental changes or the production of secretory proteins in excess of acceptable levels. Since ER stress can cause various diseases, cells have an ER stress response mechanism (UPR) that regulates the amount of molecular chaperones. The ER stress sensor, IRE1, initiates the UPR by oligomer formation at a microscopic scale under stress conditions. Interestingly, cells regulate UPR depending on the quantity and quality of stress, but the molecular mechanism of regulation is unknown. In particular, it is completely unclear how IRE1 recognizes the "stress level". In this study, we investigated the hypothesis that the more misfolded proteins there are, the larger IRE1 oligomers are formed. In the presence of misfolded insulin, the IRE1 oligomeric state was investigated by electrophoresis, mass spectrometry, and so on. The results showed that IRE1 changes its oligomeric state depending on the amount of misfolded insulin. This change in the oligomeric state suggests that IRE1 senses the accumulation level of misfolded proteins as the level of ER stress.

P - 2 3

Essential role of ER membrane complex subunit 1 (EMC1) in B cell development

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Introduction
Endoplasmic reticulum (ER) is a multifunctional organelle which contributes to quality control of proteins and cellular calcium homeostasis. It has been suggested that the ER homeostasis is closely involved in B cell differentiation and antibody secretion, but its molecular basis remains unclear. Store-operated Ca^{2+} entry (SOCE) mediated by STIM1, which can induce a sustained influx of extracellular Ca^{2+}, is chief source of Ca^{2+} signals and extremely important for maintaining ER homeostasis. Recently, we have identified the ER membrane complex subunit 1 (EMC1) as a novel STIM1-binding partner to regulate SOCE. In addition, EMC1 has been reported to be a member of EMC family which consist of ten subunits and function as "insertase" which promotes protein insertion into plasma membrane, suggesting the important role of EMC1 on ER homeostasis by regulating Ca^{2+} and protein quality control. In this study, we aimed to understand the function of EMC1 in B cell development.

Results and Discussion
We generated mice with B cell-specific deletion of EMC1 (EMC1 BKO mice) and found that SOCE and B cell development was impaired in the absence of EMC1. Given that B cell differentiation in STIM BKO mice is normal, it is considered that the abnormal B cell differentiation in EMC1 BKO mice is due to reasons other than SOCE disorders. To address the cause of defect in B cell development, we performed proteome analysis. The results showed that chemokine receptors (CXCR4, CCR6, EBI2) are decreased in EMC1-deficient B cells. CXCR4 is particularly associated with maintaining peripheral B cell compartment. Therefore, our findings suggest that EMC1 support insertion of CXCR4 into plasma membrane and that regulates B cell development.

P - 2 4

RNA phase transition disrupts α-Synuclein proteostasis

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The mechanism underling dysfunction of cellular proteostasis on prion-like proteins (such as α-synuclein (α-Syn), tau and FUS) leading to pathogenesis of neurodegeneration remains unclear. Recently, we reported that the binding of an RNA secondary structure G-quadruplex (G4RNA) to a prion-like protein FMRpolyG causes its liquid-to-solid phase transition, leading to neurodegeneration in a hereditary neurodegenerative disease, Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) (*Sci Adv.* 2021). Here, we introduce the possibility that G4RNA is a key pathogen on the phase transition of α-Syn that cause sporadic synucleinopathy. Purified α-Syn protein preferentially bound to guanine-rich RNA sequences by RNA Bind-n-Seq using a pool of random RNA oligonucleotides *in vitro*. We confirmed that purified α-Syn protein binds to G4 structure formed RNA specifically, but not other structures in EMSA. In addition, purified α-Syn protein underwent liquid-liquid phase separation, and the addition of G4RNA promoted the liquid-to-solid phase transition under molecular crowding. In mouse primary neurons, G4RNA aggregation was immediately observed under cellular stress conditions, thereafter co-aggregation of α-Syn with G4RNA was occurred. We confirmed that artificial assembly of G4RNA using an optogenetic approach initiated α-Syn aggregation, thereby elicits neuronal dysfunction in mouse primary neurons. These results suggest that G4RNA assembly evoked by various cellular stress trigger to develop aggregation of α-Syn, which may be a cellular mechanism underlying onset of sporadic synucleinopathy.

International Symposium

I - 0 1

The potential role of multi-drug resistance protein ABCB1 in tumor suppression in bats

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Most cancer research utilizes cancer-prone, short-lived models such as mice and rats. However, emerging evidence suggests that long-lived mammals such as elephants, whales, and naked mole rats have unique anticancer strategies that could potentially inform new treatment strategies in humans. Although longevity is positively correlated with body size in general, bats are unique since bats live much longer (up to 40 years) than other mammals of equivalent size. Bats are also known to have extremely low cancer incidence. However, the underlying unique anti-cancer mechanisms of bats remain elusive. We discovered that bat-derived cells exhibit enhanced DNA damage resistance to toxic chemical exposure compared to human and mouse cells. We found that bat cells accumulate less chemicals than human and mouse cells and that efficient drug efflux mediated by the ABC transporter, ABCB1, underlies this improved response to genotoxic reagents. Increased drug efflux and high expression of ABCB1 are conserved across multiple bat species, while ABCB1 is expressed in only limited tissues in humans and mice. As the accumulation of DNA damage is one of the primary causes of cancer and senescence, we propose that genotoxic efflux by ABCB1 is a potential mechanism that contributes to the low cancer incidence and longevity in bats. In this talk, I would like to introduce the novel role of ABCB1 in tumor suppression in bats and our current research on developing potential novel treatment strategies in humans using bat ABCB1 as a tool.

References

(Related articles)

Koh J, Itahana Y, Mendenhall I, Low D, Soh EXY, Guo AK, Chionh YT, Wang LF, Itahana K (2019) ABCB1 protects bat cells from DNA damage induced by genotoxic compounds. Nat Commun. 0:1:2820.

(Others)

1. Lee Y, Itahana Y, Ong CC, Itahana K (2022) Redox-dependent AMPK inactivation disrupts metabolic adaptation to glucose starvation in xCT-overexpressing cancer cells J Cell Sci. jcs.259090.
2. Guo AK, Itahana Y, Seshachalam VP, Chow HY, Ghosh S, Itahana K (2021) Mutant TP53 interacts with BCAR1 to contribute to cancer cell invasion Br J Cancer. 124:1:299-312.
3. Alagu J, Itahana Y, Sim F, Chao SH, Bi X, Itahana K (2018) Tumor suppressor p14ARF enhances IFNγ-activated immune response by inhibiting PIAS1 via SUMOylation. J Immunol. 201:2:451-464.
4. Lee HY, Itahana Y, Schuechner S, Ogris E, Virshup DM, Itahana K (2018) Ca^{2+}-dependent demethylation of phosphatase PP2Ac promotes glucose deprivation-induced cell death independently of inhibiting glycolysis. Sci Signal. 11:512: pii: eaam7893.
5. Itahana Y, Zhang J, Göke J, Vardy LA, Han R, Iwamoto K, Engin C, Robson P, Pouladi MA, Colman A, Itahana K (2016) Histone modifications and p53 binding poise the p21 promoter for activation in human embryonic stem cells. Sci Rep. 6: 28112.
6. Yeo SY, Itahana Y, Guo AK, Han R, Iwamoto K, Nguyen TH, Bao Y, Kleiber K, Wu YJ, Bay BH, Voorhoeve PM, Itahana K (2016) Transglutaminase 2 contributes to a TP53-induced autophagy program to prevent oncogenic transformation. eLife 5:e07101.
7. Itahana Y, Han R, Barbier S, Lei Z, Rozen S, Itahana K (2015) The uric acid transporter SLC2A9 is a direct target gene of the tumor suppressor p53 contributing to antioxidant defense. Oncogene 34:14:1799-810.
8. Neo SH, Itahana Y, Alagu J, Kitagawa M, Guo A, Lee SH, Tang K, Itahana K (2015) TRIM28 is an E3 ligase for ARF-mediated NPM1/B23 SUMOylation that represses centrosome amplification. Mol Cell Biol. 35:16:2851-63.
9. Itahana K and Zhang Y (2008) Mitochondrial p32 is a critical mediator of ARF-induced apoptosis. Cancer Cell 13:6:542-553 (featured article).
10. Itahana K, Mao H, Jin A, Itahana Y, Clegg HV, Lindström MS, Bhat K, Godfrey VL, Evan GI, Zhang Y (2007) Targeted inactivation of Mdm2 RING finger E3 ubiquitin ligase activity in the mouse reveals mechanistic insights into p53 regulation. Cancer Cell 12:4:355-366 (cover article).
11. Itahana K, Bhat KP, Jin A, Itahana Y, Hawke D, Kobayashi R, Zhang Y (2003) Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. Mol Cell. 12:5:1151-1164.

CV

Dr. Itahana obtained B.S. and Ph.D. degrees at the Faculty of Science, Kyoto University in Japan and received postdoctoral training at the laboratory of Dr. Judith Campisi, Lawrence Berkeley National Laboratory to study the role of p53 in cellular senescence (Itahana et al., JBC 2002 and MCB 2003). Subsequently, he joined the laboratory of Dr. Yanping Zhang, University of North Carolina at Chapel Hill to study the regulation of MDM2 degradation in vivo (Itahana et al., Cancer Cell 2007) and the novel functions of ARF (Itahana et al., Mol Cell 2003 and Cancer Cell 2008). After he started his own laboratory at Cancer & Stem Cell Biology Program, Duke-NUS Medical School Singapore in 2009, he continued to focus on tumor suppression and the ARF-MDM2-p53 pathway. Recent mouse models challenged the long-held view of canonical functions of the ARF-MDM2-p53 pathway such as cell cycle arrest, apoptosis, and senescence and highlighted the importance of non-canonical, diverse roles of this pathway. Therefore, his laboratory has focused on uncovering novel non-canonical functions of this pathway by searching for new downstream targets of p53 and new binding proteins of ARF and p53. He demonstrated novel roles of ARF in preventing genomic instability caused by centrosome amplification (MCB 2015) and activating immune response by inhibiting PIAS1 via SUMOylation (J Immunol 2018). He also demonstrated the first beneficial link between p53 and uric acid for an antioxidant function to prevent cancer (Oncogene 2015) and identified that the p53-TGM2 pathway is a critical barrier to prevent oncogenic transformation by promoting autophagy (eLife 2016). He revealed the unique epigenetic strategies in human embryonic stem cells (hESCs) that control p53 target gene expression for maintaining homeostasis and genomic stability in ESCs (Sci Rep 2016). He also revealed new roles for p53 in response to histone methylation inhibitor (Clin Cancer Res 2012) and to irradiation in quiescent cells (BBRC 2015) and the role of BCAR1 in mutant p53-mediated invasion (Br J Cancer 2020). His other ongoing research projects include the role of PP2A (Sci Signal 2018) and AMPK (J Cell Sci 2022) in cancer metabolism and the role of ABCB1 in tumor suppression and longevity in bats (Nat Commun 2019). He is currently an Associate Professor at Duke-NUS Medical School, and he continues to work on the non-canonical functions of ARF and p53, tumor suppression in bats, cancer metabolism, and aging.

Y - 0 4

The membrane-linked adaptor FRS2b fashions a cytokine-rich microenvironment that promotes breast cancer carcinogenesis

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Although it is held that pro-inflammatory changes precede the onset of breast cancer, the underlying mechanisms remain obscure. Here, we demonstrate that FRS2β, an adaptor protein expressed in a small subset of mammary epithelial cells, triggers the pro-inflammatory changes that induce stroma in precancerous mammary tissues and is responsible for the disease onset. FRS2β-deficiency in a mouse mammary tumor virus (MMTV)-ErbB2 genetic background markedly attenuated mammary tumorigenesis. FRS2β (+/+) tumors contained ample stroma, on the other hand, very little stroma was observed in FRS2β (-/-) tumors, suggesting that FRS2β is required for the formation of tumor stroma. Importantly, tumor cells derived from MMTV-ErbB2 mice failed to form tumors when they were grafted in the FRS2β-deficient precancerous mammary tissues, indicating that FRS2β plays essential role in mammary tumorigenesis.

We also demonstrated that co-localization of FRS2β and the NEMO subunit of the IkB kinase (IKK) complex in early endosomes led to activation of nuclear factor-kB (NF-kB), a master regulator of inflammation. Moreover, inhibition of the activities of the NF-kB-induced cytokines, CXC chemokine ligand (CXCL)12 and insulin-like growth factor (IGF)1, abrogated tumorigenesis, indicating that the production of IGF1 and CXCL12 in premalignant mammary tissues creates a cytokine-enriched microenvironment that is necessary for mammary tumorigenesis.

Human breast cancer tissues that express higher levels of FRS2β contain more stroma. Furthermore, patients with higher expression levels of FRS2β in breast cancer tissues had poorer prognosis. In conclusion, the elucidation of the FRS2β-NF-kB axis uncovers the unknown molecular link between the pro-inflammatory changes and the onset of breast cancer.

References
(Related articles)

Y. Takeuchi[†], N. Kimura[†], T. Murayama[†], Y. Machida, D. Iejima, T. Nishimura, M. Terashima, Y. Wang, M. Li, R. Sakamoto, M. Yamamoto, N. Itano, Y. Inoue, M. Ito, N. Yoshida, J. Inoue, K. Akashi, H. Saya, K. Fujita, M. Kuroda, I. Kitabayashi, D. Voon, T. Suzuki, A. Tojo and N. Gotoh. The membrane-linked adaptor FRS2b fashions a cytokine-rich inflammatory microenvironment that promotes breast cancer carcinogenesis. [†]Equally contribution. *Proc Natl Acad Sci USA.*, 2021 Oct 26;118(43):e2103658118.

CV

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I - 0 2

Predicting primary resistance to cancer targeted therapies at diagnosis: lessons from chronic myeloid leukemia

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Primary resistance to targeted therapies is a significant barrier to achieving optimal responses in a broad range of human cancers with druggable oncogenes. This includes chronic myeloid leukemia, (CML) where pharmacologic targeting of the BCR-ABL1 kinase with tyrosine kinase inhibitors (TKI) has proved immensely successful. However, even in CML, the promise of targeted therapies has not been fully realized since clinical responses to first-line TKIs remain highly heterogeneous with 10−15% of patients at either end of the response spectrum enjoying either major molecular responses (MMR) or progressing to blast crisis (BC).1, 2 To date, the factors contributing to CML response heterogeneity and primary TKI resistance remain largely unknown, and cannot be fully accounted for by somatic mutations in the BCR-ABL1 kinase domain that are a common cause of secondary TKI resistance, i.e. following an initial clinical response.3 In order to identify bone marrow (BM) cell types contributing to response heterogeneity, we performed single cell RNA-sequencing of pretreatment chronic phase (CP) BM mononuclear cells (MNC) samples from patients with: optimal responses (MMR) to first-line imatinib, sub-optimal responses to imatinib but favorable responses to second- or third-line TKIs; and pan-TKI resistance with eventual BC progression. We generated a single cell atlas from 163,146 BM MNCs, and comparing across prognostic groups, obtained a transcriptional and cellular landscape of progressive TKI resistance. Using our atlas, and employing machine-learning, we identified leukemia stem cell (LSC) and NK cell gene expression profiles predicting imatinib response with >80% accuracy, as well as eight statistically significant features in pretreatment BM MNCs which correlated with either sensitivity (MMR) or extreme resistance to imatinib (eventual BC transformation). We will discuss: 1. Ongoing work to understand how differential transcriptional programs within CML LSCs and NK cells functionally contribute to primary TKI response heterogeneity; 2. Development of antibody-based panels to predict TKI responses at the time of diagnosis; and 3. Novel hypotheses arising from our observations.

References

1. Braun TP, Eide CA, Druker BJ. Response and Resistance to BCR-ABL1-Targeted Therapies. Cancer Cell 2020 Apr 13; 37(4): 530-542. PMC7722523.
2. Holyoake TL, Vetrie D. The chronic myeloid leukemia stem cell: stemming the tide of persistence. Blood 2017 Mar 23; 129(12): 1595-1606.
3. Krishnan V, Kim DDH, Hughes TP, Branford S, Ong ST. Integrating genetic and epigenetic factors in CML risk assessment: toward gene expression-based biomarkers. Haematologica 2021 Oct 7; 107(2): 358-370.
4. Takeuchi S, Hase T, Shimizu S, et al. Phase I study of vorinostat with gefitinib in BIM deletion polymorphism/epidermal growth factor receptor mutation double-positive lung cancer. Cancer Sci 2020 Feb; 111(2): 561-570. PMC7004511.
5. Tanimoto A, Takeuchi S, Arai S, Fukuda K, Yamada T, Roca X, Ong ST, Yano S. Histone Deacetylase 3 Inhibition Overcomes BIM Deletion Polymorphism-Mediated Osimertinib Resistance in EGFR-Mutant Lung Cancer. Clin Cancer Res 2017 Jun 15; 23(12): 3139-3149.
6. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 2012 Apr; 18(4): 521-528.
7. Sinnakannu JR, Lee KL, Cheng S, Li J, Yu M, Tan SP, Ong CCH, Li H, Than H, Anczukow-Camarda O, Krainer AR, Roca X, Rozen SG, Iqbal J, Yang H, Chuah C, Ong ST. SRSF1 mediates cytokine-induced impaired imatinib sensitivity in chronic myeloid leukemia. Leukemia 2020 Jul; 34(7): 1787-1798. PMC7682023.
8. Ng KP, Manjeri A, Lee KL, Huang W, Tan SY, Chuah CT, Poellinger L, Ong ST. Physiologic hypoxia promotes maintenance of CML stem cells despite effective BCR-ABL1 inhibition. Blood 2014 May 22; 123(21): 3316-3326.
9. Ko TK, Javed A, Lee KL, et al. An integrative model of pathway convergence in genetically heterogeneous blast crisis chronic myeloid leukemia. Blood 2020 Jun 25; 135(26): 2337-2353.
10. Lim S, Saw TY, Zhang M, Janes MR, Nacro K, Hill J, Lim AQ, Chang CT, Fruman DA, Rizzieri DA, Tan SY, Fan H, Chuah CT, Ong ST. Targeting of the MNK-eIF4E axis in blast crisis chronic myeloid leukemia inhibits leukemia stem cell function. Proc Natl Acad Sci U S A 2013 Jun 18; 110(25): E2298-2307. PMC3690864.

CV

I completed my undergraduate and medical training at Cambridge University in the UK, and subsequently trained as a hematologist/oncologist at the University of Chicago, where I also began my laboratory research with Michelle Le Beau. My independent research career started in 2001 when I began my studies in cancer drug resistance. While our studies focus on chronic myeloid leukemia (CML), the implications of our work have been extended to other human cancers, including EGFR-mutated non-small cell lung cancer,4-6 and more recently, glioblastoma. In 2007, I joined Duke-NUS, a collaboration between Duke University and the National University of Singapore to help build the first American-style post-baccalaureate medical school in Asia.

My research career began with foundational work on the role of dysregulated mRNA translation in resistance to imatinib (Gleevec). In recent years my team has made significant contributions to the understanding of BCR-ABL1-independent factors in CML patient response heterogeneity, including microenvironmental factors (extracellular cytokine signaling via SRSF1, physiologic hypoxia),[7, 8] germline polymorphisms (the *BIM* deletion polymorphism[6]), and more recently, BCR-ABL1-independent epigenetic reprogramming of leukemia stem cells.[9] Several of our discoveries have led to early phase clinical trials conducted by myself or international collaborators, including leading Japanese researchers,[4, 5] and drug development efforts uncovered by our discovery-driven projects.[10] A common thread that runs through our work is the use of cutting-edge technologies to interrogate primary patient samples, efforts undertaken in close collaboration with leading computational biologists, followed by bench-based experiments to dissect, confirm, and therapeutically overcome novel mechanisms of drug resistance. Our work has been published in leading scientific journals including Nature Medicine, Proceedings of the National Academy of Sciences (USA), Blood, Cancer Research, Leukemia, and Oncogene. We have also received current and past funding from the US NIH, National Medical Research Council Singapore, and the Leukemia & Lymphoma Society. Our past and current service activities include membership of the Singapore Medical Council, NIH and ASH Global Research Award review panels, and Directorship of the Duke-NUS MD-PhD Programme. Our research teams have also received international awards and recognition, including career development awards from the American Society of Clinical Oncology (STO), European Society of Hematology International John Goldman Award (Vaidehi Krishnan), and election to the American Society of Clinical Investigation (STO).

S - 0 3

Identification and functional analysis of senescent cells in tumor microenvironment

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Cellular senescence is triggered by diverse genotoxic stimuli. An important hallmark of senescent cells is its inability to proliferate in response to physiological mitotic stimuli, which can prevent tumorigenesis. Another is the appearance of Senescence-associated secretory phenotypes (SASP), such as robust secretion of numerous cytokines, which may cause deleterious effects on tissue microenvironment. Recent studies indicate that the elimination of senescent cells accumulated in the body during aging ameliorates age-related diseases including cancer, thus promoting the healthy lifespan (references 1 and 2). Therefore, cellular senescence likely plays both positive and negative roles in cancer. Recently, we generated a p16Ink4a-CreERT2-tdTomato mouse to analyze the in vivo characteristics of senescent cells (reference 3). Our new mouse model and single-cell analyses suggest that in vivo senescent cells exhibited heterogeneous senescence-associated phenotypes during aging. Currently, we are trying to apply this approach to some cancer models, and we found that cancer-related senescent cells also are composed of several cell types and have various characteristics. Further analysis will provide a molecular basis for context-dependent effects of cellular senescence in cancer.

References

1. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, Saltness RA, Jeganathan KB, Verzosa GC, Pezeshki A, Khazaie K, Miller JD, van Deursen JM. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan.Nature. 2016 11;530(7589):184-9. doi: 10.1038/nature16932

2. Johmura Y, Yamanaka T, Omori S, Wang TW, Sugiura Y, Matsumoto M, Suzuki N, Kumamoto S, Yamaguchi K, Hatakeyama S, Takami T, Yamaguchi R, Shimizu E, Ikeda K, Okahashi N, Mikawa R, Suematsu M, Arita M, Sugimoto M, Nakayama KI, Furukawa Y, Imoto S, Nakanishi M. Senolysis by glutaminolysis inhibition ameliorates various age-associated disorders. Science. 2021 15;371(6526):265-270. doi: 10.1126/science.abb5916.

3. Omori S, Wang TW, Johmura Y, Kanai T, Nakano Y, Kido T, Susaki EA, Nakajima T, Shichino S, Ueha S, Ozawa M, Yokote K, Kumamoto S, Nishiyama A, Sakamoto T, Yamaguchi K, Hatakeyama S, Shimizu E, Katayama K, Yamada Y, Yamazaki S, Iwasaki K, Miyoshi C, Funato H, Yanagisawa M, Ueno H, Imoto S, Furukawa Y, Yoshida N, Matsushima K, Ueda HR, Miyajima A, Nakanishi M. Generation of a p16 Reporter Mouse and Its Use to Characterize and Target p16 high Cells In Vivo. Cell Metab. 2020 3;32(5):814-828.e6. doi: 10.1016/j.cmet.2020.09.006.

CV

[Education]

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I - 0 3

Systems Genetics identifies WWP2 as a new target for fibrotic disease in fibroblasts and macrophages

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[4] Institute for Big Data and Artificial Intelligence in Medicine, School of Science, China Pharmaceutical University, Nanjing, China

Pathological cardiac fibrosis is a final common pathology in inherited and acquired heart diseases that causes cardiac electrical and pump failure. Here, we use systems genetics to identify a pro-fibrotic gene network in the diseased heart and show that this network is regulated by the E3 ubiquitin ligase WWP2, specifically by the WWP2-N terminal isoform. Importantly, the WWP2-regulated pro-fibrotic gene network is conserved across different cardiac diseases characterized by fibrosis: human and murine dilated cardiomyopathy and repaired tetralogy of Fallot. Transgenic mice lacking the N-terminal region of the WWP2 protein show improved cardiac function and reduced myocardial fibrosis in response to pressure overload or myocardial infarction. In primary cardiac fibroblasts, WWP2 positively regulates the expression of pro-fibrotic markers and extracellular matrix genes. TGFβ1 stimulation promotes nuclear translocation of the WWP2 isoforms containing the N-terminal region and their interaction with SMAD2. WWP2 mediates the TGFβ1-induced nucleocytoplasmic shuttling and transcriptional activity of SMAD2.

Moreover, we show that its myeloid-specific deletion reduces cardiac fibrosis. Using the same model, we establish the functional heterogeneity of macrophages and define an early pro-fibrogenic phase driven by Ccl5-expressing Ly6chigh monocytes. Among other cardiac macrophage subtypes, WWP2 dysfunction primarily affects the Ccl5-dependent infiltration and activation of Ly6chigh monocytes, which causes reduced myofibroblast trans-differentiation. WWP2 interacts with IRF7, promoting its non-degradative monoubiquitination, nuclear translocation and transcriptional activity, including upstream Ccl5. Thus, WWP2 plays also a role as a key regulator of IRF7-mediated Ccl5/Ly6chigh monocyte axis in heart fibrosis.

References
(Related articles)

Schafer, S. et al. IL-11 is a crucial determinant of cardiovascular fibrosis. Nature 552, 110–115 (2017)

Moreno-Moral, A., Pesce, F., Behmoaras, J. & Petretto, E. Systems genetics as a tool to identify master genetic regulators in complex disease. Methods Mol. Biol. 1488, 337–362 (2017)

Petretto, E. et al. Integrated genomic approaches implicate osteoglycin (Ogn) in the regulation of left ventricular mass. Nat. Genet. 40, 546–552 (2008)

Moreno-Moral, A., Mancini, M., D'Amati, G., Camici, P. & Petretto, E. Transcriptional network analysis for the regulation of left ventricular hypertrophy and microvascular remodeling. J. Cardiovasc. Transl. Res. 6, 931–944 (2013)

Rotival, M. & Petretto, E. Leveraging gene co-expression networks to pinpoint the regulation of complex traits and disease, with a focus on cardiovascular traits. Brief. Funct. Genomics 13, 66–78 (2014)

Heinig, M. et al. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature 467, 460–464 (2010)

Kang, H. et al. Kcnn4 is a regulator of macrophage multinucleation in bone homeostasis and inflammatory disease. Cell Rep. 8, 1210–1224 (2014)

CV

Associate Professor Enrico Petretto is Director of the Centre for Computational Biology (CCB) and leads the Systems Genetics Group at Duke-NUS Medical School in Singapore, which was stablished as a landmark collaboration between two world-ranking institutions of higher education: Duke University (USA) and the National University of Singapore (NUS). He is also Theme Leader for Bioinformatics and Systems Genetics at the Institute of Molecular and Translational Cardiology (IMTC) for the study of Brugada Syndrome and Sudden Death, Policlinico San Donato University and Research Hospital in Milan (Italy), Adjunct Professor at the Institute of Big Data and Artificial Intelligence, China Pharmaceutical University (CPU) in Nanjing (China) and Senior Lecturer at Imperial College London.

Enrico Petretto has developed a research programme in **Systems Genetics** to study the genetic regulation of cellular pathways and gene networks that drive human disease. His lab uses Systems Biology and integrative genomics approaches to identify new drug targets for various complex disease, including cardiometabolic (e.g., cardiomyopathies, Brugada Syndrome) and fibrotic disease, diabetic nephropathy, cancer and important brain disorders such as epilepsy and Alzheimer's disease. The identified drug targets are then followed up experimentally in Petretto's lab using several cellular and pre-clinical disease models. Enrico Petretto has published more than 130 papers in top tier journals such as *Nature*, *Nature Genetics*, *Nature Communications*, *Nature Neuroscience*, *Nature Immunology*, *Cell*, *Circulation*, *European Heart Journal*, *etc*., with more than 9,000 citations to date (see Google Scholar).

Selected papers include: Integrated genomic approaches implicate osteoglycin (Ogn) in the regulation of left ventricular mass (*Nature Genetics* 2008); A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk (*Nature* 2010); Systems-genetics identifies Sestrin 3 as a regulator of a proconvulsant gene network in human epileptic hippocampus (*Nature Communications* 2015); Systems genetics identifies a convergent gene network for cognition and neurodevelopmental disease (*Nature Neuroscience* 2016); A predictive computational framework for direct reprogramming between human cell types (*Nature Genetics* 2016); IL11 is a critical determinant of cardiovascular fibrosis (*Nature* 2017); A systems-level framework for drug discovery identifies Csf1R as an anti-epileptic drug target (*Nature Communications* 2018); Changes in macrophage transcriptome associate with systemic sclerosis and mediate GSDMA contribution to disease risk (*Annals of the Rheumatic Diseases* 2018); WWP2 regulates pathological cardiac fibrosis by modulating SMAD2 signaling (*Nature Communications* 2019); A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation (*Nature Neuroscience* 2019); Whole-genome sequencing of Finnish type 1 diabetic siblings discordant for kidney disease reveals DNA variants associated with diabetic nephropathy (*Journal of the American Society of Nephrology* 2020); Wnt-regulated lncRNA discovery enhanced by in vivo identification and CRISPRi functional validation (*Genome Medicine* 2020); Brugada Syndrome Genetics is Associated with Phenotype Severity (*European Heart Journal* 2020); EpiMogrify models H3K4me3 data to identify signaling molecules that improve cell fate control and maintenance (*Cell Systems* 2020); Transcriptional signature in microglia associated with Aβ plaque phagocytosis (*Nature Communications* 2021).

For more details on Enrico Petretto's group and research activities please visit enricopetretto.com.