Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell and Molecular Medicine
幹細胞分子医学分野

Professor	Atsushi Iwama, M.D., Ph.D.	教　授　博士(医学)	岩　間　厚　志
Assistant Professor	Motohiko Oshima, Ph.D.	助　教　博士(医学)	大　島　基　彦
Assistant Professor	Yaeko Nakajima, Ph.D.	助　教　博士(医学)	中　島　やえ子
Assistant Professor	Masayuki Yamashita, M.D., Ph.D.	助　教　博士(医学)	山　下　真　幸

Stem cells have the remarkable capacity to both self-renew and give rise to many types of more specialized cells in the body, which explains their great therapeutic potential in regenerative medicine. But that's not the only reason stem cells have become such a hotbed of scientific inquiry. These cellular transformers also offer an invaluable research tool for probing the disease mechanisms that underpin cancer, aging and a host of other health problems. Our major interest is to elucidate the mechanisms of self-renewal and multi-lineage differentiation of hematopoietic stem cells (HSCs). We are also interested in how the deregulated HSC functions are associated with aging of our body and the development of age-related hematological malignancies. We approach these issues mainly from the view point of epigenetics.

1. The chromatin binding protein Phf6 restricts the self-renewal of hematopoietic stem cells

Satoru Miyagi[1,7,8], Patrycja Sroczynska[4,5], Yuko Kato[1], Yaeko Takagi-Nakajima[1], Motohiko Oshima[1], Ola Rizq[1], Naoya Takayama[3], Atsunori Saraya[1], Seiya Mizuno[6], Fumihiro Sugiyama[6], Satoru Takahashi[6], Yumi Matsuzaki[7], Jesper Christensen[4,5], Kristian Helin[4,5,8], and Atsushi Iwama[1,2] : [1]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Chiba, [2]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo, Tokyo, [3]Department of Regenerative Medicine, Graduate School of Medicine, Chiba University, Chiba, [4]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Denmark, [5]The Novo Nordisk Center for Stem Cell Biology (Danstem), University of Copenhagen, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark, [6]Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, [7]Department of Life Science, Faculty of Medicine, Shimane University, Izumo.

Recurrent inactivating mutations have been identified in the X-linked *PHF6* gene, encoding a chromatin-binding transcriptional regulator protein, in various hematological malignancies. However, the role of PHF6 in normal hematopoiesis and its tumor suppressor function remain largely unknown. We herein generated mice carrying a floxed *Phf6* allele and inactivated *Phf6* in hematopoietic cells at various developmental stages. The *Phf6* deletion in embryos augmented the capacity of hematopoietic stem cells (HSCs) to proliferate in cultures and reconstitute hematopoiesis in recipient mice. The *Phf6* deletion in neonates and adults revealed that cycling HSCs readily acquired an advantage in competitive repopulation upon the *Phf6* deletion, while dormant HSCs only did so after serial transplantations. *Phf6*-deficient HSCs maintained an enhanced repopulating capacity during serial transplantations; however, they did not induce any hema-

tological malignancies. Mechanistically, Phf6 directly and indirectly activated downstream effectors in TNFα signaling. The *Phf6* deletion repressed the expression of a set of genes associated with TNFα signaling, thereby conferring resistance against the TNFα-mediated growth inhibition on HSCs. Collectively, these results define Phf6 as a novel negative regulator of HSC self-renewal, implicating inactivating *PHF6* mutations in the pathogenesis of hematological malignancies, but also indicate that a *Phf6* deficiency alone is not sufficient to induce hematopoietic transformation.

2. KDM2B in polycomb repressive complex 1.1 functions as a tumor suppressor in the initiation of T-cell leukemogenesis

Yusuke Isshiki[1,2,3], Yaeko Nakajima-Takagi[1,4], Motohiko Oshima[1,4], Kazumasa Aoyama[1], Mohamed Rizk[1,4], Shuhei Kurosawa[1,4], Atsunori Saraya[1], Takashi Kondo[5], Emiko Sakaida[2,3], Chiaki Nakaseko[6], Koutaro Yokote[3], Haruhiko Koseki[5], and Atsushi Iwama[1,4] : [1.] Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Chiba, [2.] Department of Hematology, Chiba University Hospital, Chiba, [3.] Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, [4.] Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo, Tokyo, [5.] Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, [6.] Department of Hematology, International University of Health and Welfare, Narita.

KDM2B together with RING1B, PCGF1, and BCOR or BCORL1 comprise polycomb repressive complex 1.1 (PRC1.1), a non-canonical PRC1 that catalyzes H2AK119ub1. It binds to non-methylated CpG islands through its zinc finger-CxxC (ZF-CxxC) DNA binding domain and recruits the complex to target gene loci. Recent studies identified the loss of function mutations in the PRC1.1 gene, *BCOR* and *BCORL1* in human T-cell acute lymphoblastic leukemia (T-ALL). We previously reported that *Bcor* insufficiency induces T-ALL in mice, supporting a tumor suppressor role for BCOR. However, the function of BCOR responsible for tumor suppression, either its co-repressor function for BCL6 or that as a component of PRC1.1, remains unclear. We herein examined mice specifically lacking the ZF-CxxC domain of KDM2B in hematopoietic cells. Similar to *Bcor*-deficient mice, *Kdm2b*-deficient mice developed lethal T-ALL mostly in a NOTCH1-dependent manner. A ChIP sequence analysis of thymocytes revealed the binding of KDM2B at promoter regions, at which BCOR and EZH2 co-localized. KDM2B target genes markedly overlapped with those of NOTCH1 in human T-ALL cells, suggesting that non-canonical PRC1.1 antagonizes NOTCH1-mediated gene activation. KDM2B target genes were expressed at higher levels than the others and were marked with high levels of H2AK119ub1 and H3K4me3, but low levels of H3K27me3, suggesting that KDM2B target genes are transcriptionally active or primed for activation. These results indicate that PRC1.1 plays a key role in restricting excessive transcriptional activation by active NOTCH1, thereby acting as a tumor suppressor in the initiation of T-cell leukemogenesis.

Publications

1. Isshiki Y, Nakajima-Takagi Y, Oshima M, Aoyama K, Rizk M, Kurosawa S, Saraya A, Kondo T, Sakaida E, Nakaseko C, Yokote K, Koseki H, and Iwama A. KDM2B in polycomb repressive complex 1.1 functions as a tumor suppressor in the initiation of T-cell leukemogenesis. Blood Adv 3(17):2537-2549, 2019.
2. Nitta E, Itokawa N, Yabata S, Koide S, Hou LB, Oshima M, Aoyama K, Saraya A, and Iwama A. Bmi1 counteracts hematopoietic stem cell aging by repressing target genes and enforcing the stem cell gene signature. Biochem Biophys Res Commun 2019 Oct 31. pii: S0006-291X(19)32054-6.
3. Mohamed R, Ola R, Oshima M, Nakajima-Takagi Y, Koide S, Saraya A, Isshiki Y, Chiba T, Yamazaki S, Ma A, Jin J, Iwama A, and *Mimura N. Akt Inhibition Synergizes with PRC2 Inhibition in the Treatment of Multiple Myeloma. Cancer Sci. 110(12):3695-3707, 2019.
4. Takaishi K, Takeuchi M, Tsukamoto S, Takayama N, Oshima M, Kimura K, Isshiki Y, Kayamori K, Hino Y, Oshima-Hasegawa N, Mitsukawa S, Takeda Y, Mimura N, Ohwada C, Iseki T, Nakamura S, Eto K, Iwama A, Yokote K, Nakaseko C, Sakaida E. Suppressive effects of anagrelide on cell cycle progression and the maturation of megakaryocyte progenitor cell lines in human induced pluripotent stem cells. Haematologica 2019 Sep 5. pii: haematol.2018.214841.
5. Kato Y, Hou LB, Miyagi S, Nitta E, Aoyama K, Shinoda D, Yamazaki S, Kuribayashi W, Isshiki Y, Koide S, Si S, Saraya A, Matsuzaki Y, van Lohuizen M, and Iwama A. Bmi1 restricts the adipogenic differentiation of bone marrow stromal cells to maintain the integrity of the hematopoietic stem cell niche. Exp Hematol 76:24-37, 2019.
6. Kanatsu-Shinohara M, Yamamoto T, Toh H, Kazuki Y, Imoto J, Ikeo K, Oshima M, Shirahige K, Iwama A, Nabeshima Y, Sasaki H and Shinohara T. Aging of mouse spermatogonial stem cells by 2

Wnt7b-Jnk pathway activation. Proc Natl Acad Sci USA 116(33):16404-16409, 2019.
7. Miyagi S, Sroczynska P, Kato Y, Nakajima-Takagi Y, Oshima M, Rizq O, Takayama N, Saraya A, Mizuno S, Sugiyama F, Takahashi S, Matsuzaki Y, Christensen J, Helin K, and Iwama A. The chromatin binding protein Phf6 restricts the self-renewal of hematopoietic stem cells. Blood 133(23):2495-2506, 2019.
8. Kanayama K, Chiba T, Oshima M, Kanzaki H, Koide S, Saraya A, Miyagi S, Mimura N, Kusakabe Y, Saito T, Ogasawara S, Suzuki E, Ooka Y, Maruyama H, Iwama A, Kato N. Genome-wide mapping of bivalent histone modifications in hepatic stem/progenitor cells. Stem Cells Int 2019 Apr 1;2019:9789240.
9. Hayashi Y, Harada Y, Kagiyama Y, Nishikawa S, Ding Y, Imagawa J, Shingai N, Kato N, Kitaura J, Hokaiwado S, Maemoto Y, Ito A, Matsui H, Kitabayashi I, Iwama A, Komatsu N, Kitamura T, and Harada H. NUP98-HBO1–fusion generates phenotypically and genetically relevant chronic myelomonocytic leukemia pathogenesis. Blood Adv 3(7):1047-1060, 2019
10. Poplineau M, Vernerey J, Platet N, N'guyen L, Hérault L, Esposito M, Saurin AJ, Guilouf C, Iwama A, Duprez E. PLZF limits enhancer activity during hematopoietic progenitor aging. Nucleic Acids Res 47(9):4509-4520, 2019.
11. Kubota S, Tokunaga K, Umezu T, Yokomizo-Nakano T, Sun Y, Oshima M, Tan KT, Yang H, Kanai A, Iwanaga E, Asou N, Maeda T, Nakagata N, Iwama A, Ohyashiki K, Osato M, and Sashida G. Lineage-specific RUNX2 super-enhancer activates MYC and promotes the development of blastic plasmacytoid dendritic cell neoplasm. Nat Commun 10(1):1653, 2019.
12. Sashida G, Oshima M, Iwama A. Deregulated Polycomb functions in myeloproliferative neoplasms. Int J Hematol 110(2):170-178, 2019.
13. Nagao Y, Mimura N, Takeda J, Yoshida K, Shiozawa Y, Oshima M, Aoyama K, Saraya A, Koide S, Rizq O, Hasegawa Y, Shiraishi Y, Chiba K, Tanaka H, Nishijima D, Isshiki Y, Kayamori K, Kawajiri-Manako C, Oshima-Hasegawa N, Tsukamoto S, Mitsukawa S, Takeda Y, Ohwada C, Takeuchi M, Iseki T, Misawa S, Miyano S, Ohara O, Yokote K, Sakaida E, Kuwabara S, Sanada M, Iwama A, Ogawa S, and Nakaseko C. Genetic and transcriptional landscape of plasma cells in POEMS syndrome. Leukemia 33:1723-1735, 2019.