Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell and Molecular Medicine
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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. Epigenetic traits inscribed in chromatin accessibility in aged hematopoietic stem cells

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Hematopoietic stem cells (HSCs) exhibit considerable cell-intrinsic changes with age. Here, we present an integrated analysis of transcriptome and chromatin accessibility of aged HSCs and downstream progenitors. Alterations in chromatin accessibility preferentially take place in HSCs with aging, which gradually resolve with differentiation. Differentially open accessible regions (open DARs) in aged HSCs are enriched for enhancers and show enrichment of binding motifs of the STAT, ATF, and CNC family transcription factors that are activated in response to

external stresses. Genes linked to open DARs show significantly higher levels of basal expression and their expression reaches significantly higher peaks after cytokine stimulation in aged HSCs than in young HSCs, suggesting that open DARs contribute to augmented transcriptional responses under stress conditions. However, a short-term stress challenge that mimics infection is not sufficient to induce persistent chromatin accessibility changes in young HSCs. These results indicate that the ongoing and/or history of exposure to external stresses may be epigenetically inscribed in HSCs to augment their responses to external stimuli.

2. Unraveling unique features of plasma cell clones in POEMS syndrome with single cell analysis

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POEMS syndrome is a rare monoclonal plasma cell disorder, with unique symptoms distinct from those of other plasma cell neoplasms, including high serum VEGF levels. Because the prospective isolation of POEMS clones has not yet been successful, their real nature remains unclear. Herein, we performed single-cell RNA-Seq of BM plasma cells from patients with POEMS syndrome and identified POEMS clones that had Ig λ light chain (IGL) sequences (IGLV1-36, -40, -44, and -47) with amino acid changes specific to POEMS syndrome. The proportions of POEMS clones in plasma cells were markedly smaller than in patients with multiple myeloma (MM) and patients with monoclonal gammopathy of undetermined significance (MGUS). Single-cell transcriptomes revealed that POEMS clones were CD19$^+$, CD138$^+$, and MHC class II^{lo}, which allowed for their prospective isolation. POEMS clones expressed significantly lower levels of c-MYC and CCND1 than MM clones, accounting for their small size. VEGF mRNA was not upregulated in POEMS clones, directly indicating that VEGF is not produced by POEMS clones. These results reveal unique features of POEMS clones and enhance our understanding of the pathogenesis of POEMS syndrome.

3. Insufficiency of non-canonical PRC1 synergizes with JAK2V617F in the development of myelofibrosis

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Insufficiency of polycomb repressive complex 2 (PRC2), which trimethylates histone H3 at lysine 27, is frequently found in primary myelofibrosis and promotes the development of JAK2V617F-induced myelofibrosis in mice by enhancing the production of dys-

plastic megakaryocytes. Polycomb group ring finger protein 1 (Pcgf1) is a component of PRC1.1, a non-canonical PRC1 that monoubiquitylates H2A at lysine 119 (H2AK119ub1). We herein investigated the impact of PRC1.1 insufficiency on myelofibrosis. The deletion of Pcgf1 in JAK2V617F mice strongly promoted the development of lethal myelofibrosis accompanied by a block in erythroid differentiation. Transcriptome and chromatin immunoprecipitation sequence analyses showed the de-repression of PRC1.1 target genes in Pcgf1-deficient JAK2V617F hematopoietic progenitors and revealed Hoxa cluster genes as direct targets. The deletion of Pcgf1 in JAK2V617F hematopoietic stem and progenitor cells (HSPCs), as well as the overexpression of Hoxa9, restored the attenuated proliferation of JAK2V617F progenitors. The overexpression of Hoxa9 also enhanced JAK2V617F-mediated myelofibrosis. The expression of PRC2 target genes identified in PRC2-insufficient JAK2V617F HSPCs was not largely altered in Pcgf1-deleted JAK2V617F HSPCs. The present results revealed a tumor suppressor function for PRC1.1 in myelofibrosis and suggest that PRC1.1 insufficiency has a different impact from that of PRC2 insufficiency on the pathogenesis of myelofibrosis.

4. YAP1/TAZ activity maintains vascular integrity and organismal survival

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Radiation therapy is one of the major treatment modalities for patients with cancers. However, ionizing radiation (IR) damages not only cancer cells but also the surrounding vascular endothelial cells (ECs). Hippo pathway effector genes Yap1 and Taz are the two transcriptional coactivators that have crucial roles in tissue homeostasis and vascular integrity in various organs. However, their function in adult ECs at the steady state and after IR is poorly understood. Here, we report sex- and context-dependent roles of endothelial YAP1/TAZ in maintaining vascular integrity and organismal survival. EC-specific Yap1/Taz deletion compromised systemic vascular integrity, resulting in lethal circulation failure preferentially in male mice. Furthermore, EC-specific Yap1/Taz deletion induced acute lethality upon sublethal IR that was closely associated with exacerbated systemic vascular dysfunction and circulation failure. Consistent with these findings, RNA-seq analysis revealed downregulation of tight junction genes in Yap1/Taz deleted ECs. Collectively, our findings highlight the importance of endothelial YAP1/TAZ for maintaining adult vascular function, which may provide clinical implications for preventing organ injury after radiation therapy.

5. A high prevalence of myeloid malignancies in progeria with Werner syndrome is associated with p53 insufficiency

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Werner syndrome (WS) is a progeroid syndrome caused by mutations in the WRN gene, which encodes the RecQ type DNA helicase for the unwinding of unusual DNA structures and is implicated in DNA replication, DNA repair, and telomere maintenance. patients with WS are prone to develop malignant neoplasms, including hematological malignancies. However, the pathogenesis of WS-associated hematological malignancies remains uncharacterized. Here we investigated the somatic gene mutations in WS-associated myelodysplastic syndrome/acute myeloid leukemia (MDS/AML). Whole-exome sequencing (WES) of 4 patients with WS with MDS/AML revealed that all patients had somatic mutations in TP53 but no other recurrent mutations in MDS/AML. TP53 mutations were identified at low allele frequencies at more than one year before the MDS/AML stage. All 4 patients had complex chromosomal abnormalities including those that involved TP53. Targeted sequencing of nine patients with WS without apparent blood abnormalities did not detect recurrent mutations in MDS/AML except for a PPM1D mutation. These results suggest that patients with WS are apt to acquire TP53 mutations and/or chromosomal abnormalities involving TP53, rather than other MDS/AML-related mutations. TP53 mutations are frequently associated with prior exposure to chemotherapy; however, all four patients with WS with TP53 mutations/deletions had not received any prior chemotherapy, suggesting a pathogenic link between WRN mutations and p53 insufficiency. These results indicate that WS hematopoietic stem cells with WRN insufficiency acquire competitive fitness by inactivating p53, which may cause complex chromosomal abnormalities and the subsequent development of myeloid malignancies. These findings promote our understanding of the pathogenesis of myeloid malignancies associated with progeria.

Publications

1. Shiroshita K, Kobayashi H, Watanuki S, Karigane D, Tamaki S, Haraguchi M, Itokawa N, Koide S, Masamoto Y, Nakamura-Ishizu A, Kurokawa M, Iwama A, Okamoto S, Kataoka K, Takubo K. Reversion to quiescence preserves hematopoietic stem cells following genome editing. Cell Reports Method in press.
2. Takano J, Ito S, Dong Y, Sharif J, Nakajima-Takagi Y, Umeyama T, Han YW, Isono K, Kondo T, Iizuka Y, Miyai T, Mizutani-Koseki Y, Ikegaya M, Sakihara M, Nakayama M, Ohara O, Hasegawa Y, Hashimoto K, Arner E, Klose RJ, Iwama A, Koseki H, and Ikawa T. PCGF1-PRC1 links chromatin repression with DNA replication during hematopoietic cell lineage commitment. Nat Commun 13(1): 7159, 2022.
3. Jimbo K, Hattori A, Koide S, Ito T, Sasaki K, Iwai K, Nannya Y, Iwama A, Tojo A, and Konuma T. Genetic deletion and pharmacologic inhibition of E3 ubiquitin ligase HOIP impairs the propagation of myeloid leukemia. Leukemia 2022 Nov 9. doi: 10.1038/s41375-022-01750-7.
4. Isshiki Y, Oshima M, Mimura N, Kayamori K, Miyamoto-Nagai Y, Seki M, Nakajima-Takagi Y, Kanamori T, Iwamoto E, Muto T, Tsukamoto S, Takeda Y, Ohwada C, Misawa S, Ikeda JI, Sanada M, Kuwabara S, Suzuki Y, Sakaida E, Nakaseko C, Iwama A. Unraveling unique features of plasma cell clones in POEMS syndrome by single-cell analysis. JCI insight 7(20):e151482, 2022.
5. Poplineau M, Platet N, Mazuel A, Hérault L, N'guyen L, Koide S, Nakajima-Takagi Y, Kuribayashi W, Carbuccia N, Haboub L, Vernerey J, Oshima M, Birnbaum D, Iwama A, Duprez E. Non-canonical EZH2 drives retinoic acid resistance of variant acute promyelocytic leukemias. Blood 140:2358-2370, 2022.
6. Aoyama K, Itokawa N, Oshima M, and Iwama A. Epigenetic memories in hematopoietic stem and progenitor cells (Review). Cells 11:2187, 2022.
7. Uemura S, Yamashita M, Aoyama K, Yokomizo T, Oshima M, Nishio M, Masuko M, Takizawa J, Sone H, Yamada Y, Suzuki A, and Iwama A. YAP1/TAZ activity maintains vascular integrity and organismal survival. Biochem Biophys Res Commun 619:117-123. 2022.
8. Itokawa N, Oshima M, Koide S, Takayama N, Kuribayashi W, Nakajima-Takagi Y, Aoyama K, Yamazaki S, Yamaguchi K, Furukawa Y, Eto K, Iwama A. Epigenetic memories inscribed in chromatin accessibility in aged hematopoietic stem cells. Nat Commun 13(1): 2691, 2022.
9. Yamashita M and Iwama A. Aging and clonal behavior of hematopoietic stem cells (Review). Int J Mol Sci 23(4):1948, 2022.
10. Jimbo K, Nakajima-Takagi Y, Ito T, Koide S, Nannya, Y, Iwama A, Tojo A, Konuma T. Immunoglobulin superfamily member 8 maintains cancer stem cells through inhibition of β-catenin degradation in myeloid leukemia. Leukemia 36:1550-1562,

2022.
11. Kato H, Maezawa Y, Nishijima D, Iwamoto E, Takeda J, Kanamori T, Yamaga M, Mishina T, Takeda Y, Izumi S, Hino Y, Nishi H, Ishiko J, Takeuchi M, Kaneko H, Koshizaka M, Mimura N, Kuzuya M, Sakaida E, Takemoto M, Shiraishi Y, Miyano S, Ogawa S, Iwama A, Sanada M, Yokote K. High prevalence of myeloid malignancies in progeria with Werner syndrome is associated with p53 insufficiency. Exp Hematol 109:11-17, 2022.
12. Shinoda D, Nakajima-Takagi Y, Oshima M, Koide S, Aoyama K, Saraya A, Harada H, Rahmutulla B, Kaneda A, Yamaguchi K, Furukawa Y, Koseki H, Shimoda K, Tanaka T, Sashida G, and Iwama A. Insufficiency of non-canonical PRC1 synergizes with JAK2V617F in the development of myelofibrosis. Leukemia 36:452-463, 2022.
13. Nakata M, Honda H, Iwama A, Terui K, Komatsu S, Shibata R, Hishiki T. Wnt5a plays a critical role in anal opening in mice at an early stage of embryonic development. Pediatr Surg Int 38(5):743-747, 2022.