

Japan Academy Prize to:

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for “Elucidation of Microenvironments Essential for the Maintenance of
Hematopoietic Stem Cells, Hematopoiesis, and Bone”

Outline of the work:

All blood cell types, including red blood cells, immune cells and platelets, are generated from hematopoietic stem cells (HSCs) in bone marrow, which is the tissue filling the space between bone surfaces, throughout adult life. HSCs are rare cells and cannot survive and expand on their own, and thus the special microenvironment, termed the HSC niche, in which HSCs reside, is required for their maintenance. However, the identity of the HSC niche has been a subject of long standing debate due to the lack of specific markers for mesenchymal lineages within bone marrow. Since 2003 it has been generally assumed that a population of bone-lining osteoblasts creates the HSC niche; however, the *in vivo* role of osteoblasts in hematopoiesis remains unclear and there is no genetic and histological evidence to support this hypothesis.

To understand the properties of bone marrow microenvironments, Prof. Takashi Nagasawa first focused his studies on cytokines that support lympho-hemopoiesis and isolated the chemokine, CXCL12 (also known as SDF-1 or PBSF) as a molecule that stimulates the growth of B cell precursors (ref. 1). Prof. Nagasawa generated mice deficient in CXCL12 or its receptor CXCR4 and demonstrated that CXCL12-CXCR4 signaling is essential for colonization of bone marrow by HSCs during ontogeny (refs. 2 and 8), maintenance of a pool of HSCs in adult bone marrow (refs. 12 and 17), and development of immune cells, including B cells, plasmacytoid dendritic cells (pDCs) and NK cells (refs. 7, 10, 11, 13 and 15). Further, CXCL12-CXCR4 signaling was also shown to be necessary for cardiogenesis and formation of arteries in the gastrointestinal tract during ontogeny (refs. 2 and 5). These findings identified CXCL12 as the long-sought environmental signal essential for homing of HSCs to the bone marrow.

Subsequently, Prof. Nagasawa generated mice, in which the GFP (green fluorescent protein) reporter gene was knocked into the CXCL12 locus, and identified a population of reticular cells expressing high levels of CXCL12, termed CXCL12-abundant reticular (CAR) cells in bone marrow. Further studies revealed that most HSCs are in contact with CAR cells, that *in vivo* CAR cell ablation leads to severely impaired maintenance of hematopoietic stem and progenitor cells (HSPCs), and that CAR cells are the major producer of CXCL12 and SCF, another factor essential for HSC maintenance (refs. 12, 14 and 19). These findings demonstrate that CAR cells create niches for HSCs and hematopoiesis. Collectively, these studies have contributed to the emergence of new concept that cellular niches for tissue stem cells are more abundant than tissue stem cells.

Prof. Nagasawa also demonstrated that the transcription factors Foxc1 and Ebf3 are preferentially expressed in CAR cells, that deletion of Foxc1 in CAR cells leads to severely reduced HSPC numbers and

markedly increased adipocyte numbers in the bone marrow, and that deletion of *Ebfl* and *Ebf3* in CAR cells severely reduces HSPC numbers and markedly increases bone in bone marrow (refs. 18 and 20). These findings indicate that the CAR cell-specific transcription factors *Foxc1* and *Ebf1/3* are essential for inhibiting differentiation of CAR cells into adipocytes and osteoblasts, respectively as well as for HSPC niche formation, providing the first example of a niche cell type required for tissue stem cell maintenance. Finally, Prof. Nagasawa's genetic fate tracing studies revealed that CAR cells are self-renewing mesenchymal stem cells (MSCs), from which almost all osteoblasts and adipocytes are generated in adult bone marrow (ref. 20).

In summary, Prof. Nagasawa has identified the major cellular component of the bone marrow microenvironment for HSC maintenance and hematopoiesis, a key cytokine essential for HSPC niche function, the molecular basis of HSPC niche formation, and essential CAR cell properties. Additionally, he has identified marrow-specific MSCs. Clinically, an inhibitor of CXCL12-CXCR4 signaling has been approved for use to mobilize HSCs from their niches to the peripheral blood for collection and subsequent hematopoietic transplantation. Prof. Nagasawa's work has substantially advanced our understanding of hematopoiesis, bone remodeling and tissue stem cell behavior, thereby profoundly impacting hematology, immunology, stem cell biology, skeletal biology, and medicine.

References

1. Nagasawa, T., Kikutani, H., and Kishimoto, T. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. **Proc. Natl. Acad. Sci. USA**, 91; 2305–2309, 1994.
2. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. **Nature**, 382; 635–638, 1996.
3. Nagasawa, T., Nakajima, T., Tachibana, K., Iizasa, H., Bleul, C. C., Yoshie, O., Matsushima, K., Yoshida, N., Springer, T. A., and Kishimoto, T. Molecular cloning and characterization of a murine pre-B-cell growth-stimulating factor/stromal cell-derived factor 1 receptor, a murine homolog of the human immunodeficiency virus 1 entry coreceptor fusin. **Proc. Natl. Acad. Sci. USA**, 93; 14726–14729, 1996.
4. Murakami, T., Nakajima, T., Koyanagi, Y., Tachibana, K., Fujii, N., Tamamura, H., Yoshida, N., Waki, M., Matsumoto, A., Yoshie, O., Kishimoto, T., Yamamoto, N., and Nagasawa, T. A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection. **J. Exp. Med.**, 186; 1389–1393, 1997.
5. Tachibana, K., Hirota, S., Iizasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., Kitamura, Y., Matsushima, K., Yoshida, N., Nishikawa, S., Kishimoto, T., and Nagasawa, T. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. **Nature**, 393; 591–594, 1998.
6. Kawabata, K., Ujikawa, M., Egawa, T., Kawamoto, H., Tachibana, K., Iizasa, H., Katsura, Y., Kishimoto, T., and Nagasawa, T. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. **Proc. Natl. Acad. Sci. USA**, 96; 5663–5667, 1999.
7. Egawa, T., Kawabata, K., Kawamoto, H., Amada, K., Okamoto, R., Fujii, N., Kishimoto, T., Katsura, Y., and Nagasawa, T. The earliest stages of B cell development require a chemokine stromal cell-derived factor/pre-B cell growth-stimulating factor. **Immunity**, 15; 323–334, 2001.
8. Ara, T., Tokoyoda, K., Sugiyama, T., Egawa, T., Kawabata, K., and Nagasawa, T. Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny. **Immunity**, 19; 257–267, 2003.
9. Ara, T., Nakamura, Y., Egawa, T., Sugiyama, T., Abe, K., Kishimoto, T., Matsui, Y., and Nagasawa, T.

- Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). **Proc. Natl. Acad. Sci. USA**, 100; 5319–5323, 2003.
10. Tokoyoda, K., Egawa, T., Sugiyama, T., Choi, B.-I., and Nagasawa, T. Cellular niches controlling B lymphocyte behavior within bone marrow during development. **Immunity**, 20; 707–718, 2004.
 11. Nagasawa, T. Microenvironmental niches in the bone marrow required for B-cell development. **Nat. Rev. Immunol.**, 6; 107–116, 2006.
 12. Sugiyama, T., Kohara, H., Noda, M., and Nagasawa, T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. **Immunity**, 25; 977–988, 2006.
 13. Kohara, H., Omatsu, Y., Sugiyama, T., Noda, M., Fujii, N., and Nagasawa, T. Development of plasmacytoid dendritic cells in bone marrow stromal cell niches requires CXCL12-CXCR4 chemokine signaling. **Blood**, 110; 4153–4160, 2007.
 14. Omatsu, Y., Sugiyama, T., Kohara, H., Kondoh, G., Fujii, N., Kohno, K., and Nagasawa, T. The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. **Immunity**, 33; 387–399, 2010.
 15. Noda, M., Omatsu, Y., Sugiyama, T., Oishi, S., Fujii, N., and Nagasawa, T. CXCL12-CXCR4 chemokine signaling is essential for NK-cell development in adult mice. **Blood**, 117; 451–458, 2011.
 16. Nagasawa, T., Omatsu, Y., and Sugiyama, T. Control of hematopoietic stem cells by the bone marrow stromal niche: the role of reticular cells. **Trends Immunol.**, 32; 315–320, 2011.
 17. Greenbaum, A., Hsu, Y.-M. S., Day, R. B., Schuettpelz, L. G., Christopher, M. J., Borgerding, J. N., Nagasawa, T., and Link, D. C. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. **Nature**, 495; 227–230, 2013.
 18. Omatsu, Y., Seike, M., Sugiyama, T., Kume, T., and Nagasawa, T. Foxc1 is a critical regulator of haematopoietic stem/progenitor cell niche formation. **Nature**, 508; 536–540, 2014.
 19. Shimoto, M., Sugiyama, T., and Nagasawa, T. Numerous niches for hematopoietic stem cells remain empty during homeostasis. **Blood**, 129; 2124–2131, 2017.
 20. Seike, M., Omatsu, Y., Watanabe, H., Kondoh, G., and Nagasawa, T. Stem cell niche-specific Ebf3 maintains the bone marrow cavity. **Genes Dev.**, 32; 359–372, 2018.