Bloodlines of haematopoietic stem cell research in Japan

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Haematopoietic stem cells (HSCs) can supply all blood cells throughout the adult life of individuals. Based on this property, HSCs have been used for bone marrow and cord blood transplantation. Among various stem cells, HSCs were recognized earliest and were studied most extensively, providing a model for other stem cells. Knowledge of HSC regulation has rapidly accumulated of late. Contributions of scientists in Japan to progress HSC biology are here briefly overviewed. Focusing on the original work accomplished in Japan in the last two decades, people who have led such activities are introduced and their relationships with one another are sketched.

Keywords: haematopoietic stem cells; self-renewal; differentiation; cytokines; niche

1. INTRODUCTION

Haematopoietic stem cells (HSCs) are defined as cells with self-renewal and multilineage differentiation potentials. This definition was given by J. E. Till & E. A. McCulloch, based on the studies of spleen colony-forming cells, a half-century ago (Till & McCulloch 1961; Till *et al.* 1964). Since then much effort has been devoted to the identification and isolation of such cells. Experimental bone marrow transplantation has served as the only reliable and sensitive method to detect HSCs. When bone marrow cells are transplanted into lethally irradiated myeloablated animals, the entire haematopoietic system is durably reconstituted, providing evidence that transplanted bone marrow cells contain cells capable of homing for bone marrow and reconstituting all blood lineages. With the persistence of multilineage reconstitution, such cells can be inferred to self-renew because short-living mature blood cells are otherwise not replenished. The study of HSCs has mostly relied on a mouse model due to the use of experimental transplantation.

In the competitive repopulation assay, originally introduced by Micklem *et al.* (1972) and one of the most frequently used transplantation systems, syngeneic test donor cells and competitor cells are transplanted together. Competitor cells not only ensure the survival of recipients ('radioprotection effect'), but also provide a means to evaluate the quantity and quality of HSCs. This assay was later established in a quantitative manner by C. J. Eaves' and D. E. Harrison's groups (Szilvassy *et al.* 1990; Harrison *et al.* 1993).

Yukihiko Kitamura had earlier noted independently that mutant mice are extremely useful for HSC studies. He was the first to report the HSC origin of mast cells as shown by transplantation with bone marrow cells from beige mice (Kitamura *et al.* 1977). Long before

one knew that c-Kit and its ligand, respectively, are expressed in HSCs and their microenvironment, he had made the important observation that W/W^v mice as well as Sl/Sl^d mice are deficient in mast cells, assuming that the W gene product is expressed in mast cells and the Sl gene product is expressed in fibroblasts (Kitamura 1989). His foresight has been appreciated by a number of HSC researchers.

In vitro colony assays, originally invented by two groups in the mid-1960s (Pluznik & Sachs 1965; Bradley & Metcalf 1966), led to the discovery of a number of colony-stimulating factors or cytokines in the following years. Granulocyte colony-stimulating factor (Nagata *et al.* 1986; Nomura *et al.* 1986) and thrombopoietin (Kato *et al.* 1996) were molecularly cloned in Japan independently. *In vitro* colony assays remain extremely useful not only for the analysis of a variety of haematopoietic progenitors in normal and mutant mice (Metcalf 2005), but also for the characterization of HSCs because HSCs can also give rise to colonies *in vitro* in the presence of an appropriate combination of cytokines (Takano *et al.* 2004). Early establishment of functional assays underlies the advancement in the studies of HSCs beyond those of other stem cells.

In the field of immunology, interleukin (IL)-2 (Taniguchi *et al.* 1983), IL-4 (Noma *et al.* 1986), IL-5 (Takatsu *et al.* 1988) and IL-6 (Hirano *et al.* 1986) were also found in Japan. The serial discoveries from IL-6 to the suppressor of cytokine signalling 1 have been recently reviewed by Kishimoto (2005). Identification of various cytokines led to a rash of cytokine receptor cloning (Nakayama *et al.* 1992), followed by extensive signal transduction analyses (Miyajima *et al.* 1992; Ihle 2001). Among these, of special note is the discovery of signal transducers: gp130 (Hibi *et al.* 1990) common beta chain (β c; Kitamura *et al.* 1991) and common gamma chain (γ c; Takeshita *et al.* 1992). The gp130 is shared by the receptors of IL-6, IL-11, IL-27, leukaemia inhibitory factor, oncostatin M and other factors; the β c is shared

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by the receptors of IL-3, IL-5 and GM-CSF; the γc is shared by the receptors of IL-2, IL-4, IL-7, IL-9 and IL-15. Since gp130 and βc receptors are expressed in HSCs, the signal transduction initiated by these signal transducers should play roles in HSC regulation. IL-3, IL-6, IL-11 and IL-27 can act on HSCs in combination with c-Kit ligand due to the co-expression of their respective α chain receptors among them. The gp130 signalling and βc signalling seem to affect the HSC fate somewhat differently. Whether the effects of IL-6, IL-11 and IL-27 differ from one another is not clear. Further work will be required to understand how cytokine signalling modulates the fate of HSCs.

Many Japanese researchers are involved in the field of HSC research, although much early work was done in the USA and other foreign countries. Among them Makio Ogawa is worth a special mention. He graduated with Tadamitsu Kishimoto from Osaka University School of Medicine in 1964. After a residency in the USA, he decided to study HSC biology. As a graduate student at the University of Toronto, Ogawa had the opportunity to learn from McCulloch himself the logical designing of experiments. Ogawa then began to work in his own laboratory at the Medical University of South Carolina, where he has made tremendous contributions to HSC biology. Ogawa studied the effects of various cytokines on HSCs and their progeny by using semi-solid culture systems. He clarified many aspects of regulatory mechanisms in haematopoiesis, with the assistance of many Japanese post-doctoral researchers who undertook fellowships in his laboratory from time to time. Among them were Tatsutoshi Nakahata, Toshio Suda and Hiroaki Kodama, who later became leaders in the field of haematopoiesis research in Japan. It is now clear that cytokines play key roles in the regulation of HSCs. Overviews and paradigms of cytokine and HSC interactions are due principally to Ogawa's initiatives (Ogawa 1993). Ogawa thus remains one of the most influential scientists in the history of HSC study in Japan.

In this review HSC studies performed in Japan in the last 20 years are the focus. Papers published by Japanese researchers are preferentially cited. Since many studies have certainly contributed to the progress of HSC biology, to review them all here is impossible. We thus have selected studies that we consider both highly original and memorable. As usual, personal biases are unavoidable in this sort of review; since it reflects our experiences, and is our account of events, rather than being a definitive history.

2. THE ORIGIN OF MODERN HAEMATOPOIETIC RESEARCH IN JAPAN

Fumimaro Takaku has been a strong driving force in the progress of haematology in Japan. When Jichi Medical School in Tochigi prefecture was founded in 1972, having Kiku Nakao as president, Takaku brought all his fellows from the University of Tokyo to this new institute and started the haematology department. Among them was Yasusada Miura who was interested in yolk-sac haematopoiesis at that time (Miura & Wilt 1970). Soon after Miura started his experiments in his own laboratory while Takaku continuously contributed to the establishment of clinical haematology in a new

hospital. Hideaki Mizoguchi, whose lifelong interest has been megakaryopoiesis, Shigetaka Asano, who would later contribute to the discovery of granulocyte colony-stimulating factor and its clinical applications (Asano 1991), Shigekazu Nagata, who would make tremendous contribution to the understanding of apoptosis (Nagata 1997), and Kei-ya Osawa, who would later become one of the leaders in the field of gene therapy in Japan (Ozawa *et al.* 2000; Kume *et al.* 2002), all at one time or another worked together with Miura and Takaku there. After Takaku returned to the University of Tokyo in 1982, Miura took over his position and directed the haematology department. From the mid-1980s to the early 1990s, experiments in Miura's laboratory were largely carried out or directed by Toshio Suda. Kazuo Motoyoshi, who succeeded in the purification of macrophage colony-stimulating factor from human urine (Motoyoshi *et al.* 1986), and Norio Komatsu, who established the UT7 megakaryocytic cell line (Komatsu *et al.* 1991), also were there at the same time. With respect to the haematopoiesis studies in Japan, Miura's institute was, in effect, their Mecca and Miura their pioneer.

3. THE POWER OF CELL SORTERS

Although the concept of self-renewing HSCs with multi-lineage differentiation potential had been around for many years, the prospective isolation of such cells was never possible due to the paucity of HSCs in the bone marrow. HSCs, as detected by competitive repopulation assays, represent of the order of 1 in 10^4 – 10^5 bone marrow cells in the adult mouse. HSCs had to be identified and prospectively isolated before their detailed characterization at cellular and molecular levels could occur. J. W. Visser *et al.* were the first to apply a fluorescence-activated cell sorter (FACS) to HSC purification from mouse bone marrow (Baines & Visser 1983). G. J. Spangrude and I. L. Weissman had attempted to fractionate mouse bone marrow cells by using FACS and a combination of antibodies to various cell surface antigens. They reported in 1988 that Thy-1-low, Sca-1-positive lineage markers negative cells within adult mouse bone marrow are highly enriched in HSCs (Spangrude *et al.* 1988). Since then Sca-1 has served as the best positive marker for HSC enrichment in the mouse. During a post-doctoral fellowship, Hiromitsu Nakauchi worked on the molecular cloning of genes that encode CD8 and other lymphocyte cell surface markers, using FACS and transfectants, with L. A. Herzenberg at Stanford University. In the mid-1980s, Nakauchi set up a FACS facility similar to that of Herzenberg in his own laboratory at the RIKEN Tsukuba Life Science Centre (TLSC), while his scientific interests slowly shifted from immunology to studies of cell differentiation.

In the late 1980s, Miura and Suda realized that flow cytometry was necessary for their experiments. Suda decided to collaborate with Nakauchi to keep up with Weissman in HSC purification. The RIKEN TLSC, where Nakauchi was, lies approximately 50 km from Jichi Medical School. Seiji Okada and Hideo Ema were among the several young people chosen to make frequent trips from Jichi Medical School to Nakauchi's laboratory and back.

This collaboration soon worked out well. Purification of mouse HSCs was improved by using an anti-c-Kit antibody developed by Shin-Ichi Nishikawa (Okada *et al.* 1992). The FACStar was equipped with a clone-sorting system that was originally designed for cell cloning such as hybridoma cloning. With a special emphasis on the importance of clonal analysis, a prototype of single-cell culture was established (Ema *et al.* 1990). It was Nakauchi's dream to reconstitute the bone marrow of a lethally irradiated mouse by transplanting a single purified HSC. Surprisingly, this dream came true in the following 5 years, a period during which the focus of Nakauchi's research shifted completely from immunology to stem cell biology.

HSCs clonally give rise to both progenitor cells and themselves. Evidence for this was given by transplantation studies using abnormal chromosomal markers (Wu *et al.* 1968; Abramson *et al.* 1977) and retroviral integration markers (Dick *et al.* 1985; Lemischka *et al.* 1986; Keller & Snodgrass 1990). Evidence that was more direct and less inferential, however, was lacking. While HSC transplantation at the single-cell level had been attempted, a relatively low degree of purification prevented the analysis of single cells in substantial numbers (Smith *et al.* 1991; Spangrude *et al.* 1995). Masatake Osawa and Nakauchi were seeking another marker for HSCs to upgrade the degree of purification attainable. At that time, an anti-mouse CD34 monoclonal antibody (clone 49E8) made by Makoto Otsu in Hirofumi Hamada's laboratory in the Cancer Institute of JFCR became available. Nakauchi's group immediately tested this antibody for HSC purification. Surprisingly, they found that the CD34-negative or low fraction among c-Kit⁺, Sca-1⁺ Lin⁻ cells (CD34⁻KSL cells) is highly enriched in HSCs, although CD34 had been believed to be a candidate human HSC marker (Osawa *et al.* 1996). The significance of this work is that long-term reconstituting cells can be clearly distinguished from short-term reconstituting cells and CFU-S by CD34 expression levels and that only one cell is sufficient to reconstitute the entire haematopoietic system of recipient mice for a long period. The presence of both self-renewal and multilineage differentiation potentials in single cells and the existence of heterogeneity among HSCs were formally reported by Ema *et al.* (2005). Successful single-cell reconstitution led to more precise and quantitative studies of *in vitro* self-renewal of HSCs (Ema *et al.* 2000; Seita *et al.* 2007). The recent development of in-droplet single-cell immunostaining by this group has provided a way to look into intracellular molecular events in small numbers of HSCs (Ema *et al.* 2006). Using this method, we have uncovered striking similarities between the dormant state of HSCs and that of *Caenorhabditis elegans* dauer formation (Yamazaki *et al.* 2006).

4. THE CHARLESTON LINEAGE

At Ogawa's laboratory in Charleston, South Carolina, Tatsutoshi Nakahata observed the appearance of a particular class of colonies consisting of primitive cells with blast-like morphology in methylcellulose culture

when mouse bone marrow or spleen cells were cultured with conditioned medium from pokeweed mitogen-stimulated spleen cells (PWMCM) for 16 days. These blast colonies contained colony-forming cells with the potential to give rise to neutrophils, macrophages, erythroblasts and megakaryocytes (Nakahata & Ogawa 1982; Nakahata *et al.* 1982). This so-called 'blast colony assay' was further established by Toshio Suda and his wife Junko by using 5-fluorouracil-treated spleen cells (Suda *et al.* 1983) and IL-3 instead of PWMCM (Suda *et al.* 1985). Using micromanipulation techniques, Toshio and Junko Suda provided experimental evidence for the stochastic nature of lineage commitment in their seminal work, comparing the differentiation potentials in paired daughter cells produced by individual blast cells (Suda *et al.* 1984a,b).

After returning to Japan from Ogawa's laboratory, Nakahata and Suda performed experiments along the lines established by Ogawa. Soon after, however, they found their own ways to study HSCs. Both had initially trained in paediatrics; Nakahata chose to continue as a paediatrician, while Suda chose to leave clinical paediatrics entirely. Nakahata's researches are directed towards human HSCs and applications to clinical haematology, in particular *ex vivo* expansion of HSCs using cytokines such as soluble IL-6 or aorta-gonad-mesonephros region-derived stromal cells (Nakahata 2001). His group has recently reported a protocol for efficient induction of blood cells from monkey embryonic stem (ES) cells (Umeda *et al.* 2004). This is expected to be applied to human ES cells soon. Nakahata has also contributed to the generation of NOD/SCID/common gamma-chain-deficient mice as a tool to study human HSCs *in vivo* (Hiramatsu *et al.* 2003).

Suda's researches are directed towards more fundamental biology. In concept, niches are the microenvironments where stem cells reside in tissue (Moore & Lemischka 2006). Adult HSCs supposedly exist in bone marrow niches (Schofield 1978). However, unlike skin epidermal stem cells in the bulge (Alonso & Fuchs 2003) and gut epithelial stem cells in the crypt (Radtko & Clevers 2005), HSCs are not localized to particular anatomical sites in the bone marrow. This makes identification of HSC niches difficult, although osteoblasts (Calvi *et al.* 2003; Zhang *et al.* 2003) and vascular endothelial cells (Heissig *et al.* 2002; Kiel *et al.* 2005) have been suggested as HSC niche components. To identify HSC niches, we need to mark HSCs specifically. Otherwise, to find niche signals that control HSCs will hardly be possible. Suda, nonetheless, has been addressing this issue with his unique but reasonable hypothesis that the importance for HSCs of niches lies in their enabling HSCs to stay in the G₀ phase of cell cycle and in the protection from proliferative and oxidative stresses provided by the niche (Suda *et al.* 2005). Fumio Arai and Suda have found that angiopoietin-1 (Ang-1)/Tie2 signal transduction plays a role in protecting HSCs from cytokine stimulation (Arai *et al.* 2004). This study further suggested that osteoblasts secrete Ang-1 to enhance N-cadherin-dependent adhesion of HSCs to osteoblasts. Nobuyuki Takakura and Suda reported that Ang-1 promotes HSC adhesion to fibronectin (Takakura *et al.* 1998). They also reported that HSCs secrete Ang-1 that attracts vascular endothelial cells to migrate towards

HSCs (Takakura *et al.* 2000). In addition to its role in angiogenesis, Ang-1 seems to play complex roles in the interactions between HSCs and their niches.

Through the analysis of mice deficient in the ataxia-telangiectasia-mutated (*Atm*) gene, Keisuke Ito, Atsushi Hirao and Suda made the surprising observation that administration of an anti-oxidative agent (*N*-acetyl-L-cysteine) or of a p38 mitogen-activated protein kinase (MAPK) inhibitor to this mutant mouse can reverse the age-associated marked reduction of repopulating activity that constitutes their defect in HSC phenotype (Ito *et al.* 2004, 2006). Bmi1-deficient HSCs exhibit severely reduced repopulating activity from embryonic stages onward (Lessard & Sauvageau 2003; Park *et al.* 2003; Iwama *et al.* 2004). The expression of p16^{Ink4a} and p19^{Arf} is derepressed by the lack of Bmi1 or *Atm* (Ito *et al.* 2004; Iwama *et al.* 2004; Oguro *et al.* 2006). Interestingly, *Atm*-deficient HSCs can be rescued by overexpression of Bmi1 as well (Ito *et al.* 2004). Phosphorylation of p38 MAPK, somehow, resulted in the derepression of p16^{Ink4a} and p19^{Arf} expression. These findings led to the discovery that p38 MAPK is constitutively phosphorylated in HSCs after serial transplantation (Ito *et al.* 2006). An additional surprising finding was that continuous administration of p38 MAPK inhibitor to mice that have received bone marrow cells can prevent 'decline' in the repopulating activity in HSCs. This effect is more apparent during serial transplantation (Ito *et al.* 2006). Suda is well-versed in the logical deconstruction of various complicated data; his in-depth knowledge allows him to produce inferences and to deduce interrelationships, making him the best storyteller in this field.

5. A SCIENTIST WITH PHILOSOPHY

Satimaru Seno was the one who first organized annual workshops to promote haematopoietic studies in Japan. Yukihiko Kitamura took over his role in the late 1980s. At one of these workshops, Shin-Ichi Nishikawa reported that mutation in macrophage colony-stimulating factor is responsible for osteopetrosis in (op/op) mouse (Yoshida *et al.* 1990). This was perhaps the first of his many unexpected contributions and insights. Nishikawa has performed a variety of work in haematopoiesis, lymphopoiesis, vasculogenesis, melanocytogenesis, and so on; in retrospect, all of it has been on stem cell biology. His research seems very simple, testing unique ideas with solid techniques—of course, both the inspirations and the techniques are not simple! Nishikawa made several valuable tools with great help from his wife Satomi Nishikawa. Monoclonal antibodies against receptor tyrosine kinases (c-Kit, Flk-1 and PDGFR) are good examples. c-Kit is perhaps the most important functional marker for HSCs. Anti-c-Kit antibody with neutralizing activity was used to show that c-Kit signalling is important for the development of melanocytes (Nishikawa *et al.* 1991) and of haematopoietic progenitors (Ogawa *et al.* 1991). *Flk-1* was cloned from foetal liver HSCs by W. Matthews and I. R. Lemischka (Matthews *et al.* 1991) and proved important in early vasculogenesis and haematopoiesis (Shalaby *et al.* 1995). Using anti-Flk-1 antibody, Nishikawa found that Flk-1 is one of the earliest markers of mesoderm (Nishikawa *et al.* 1998). Using

anti-PDGF receptor antibody, his group also suggested that PDGFR is a mesoendoderm marker (Tada *et al.* 2005). Nishikawa has also contributed to the development of S-clone, a serum-free medium in which all components are defined, while some commercially available serum-free media still contain undefined proteins. S-clone medium has become essential to our HSC culture system. He distributed his materials without restriction all over the world as soon as the papers reporting them were published, as all scientists should, though few actually do. Nishikawa is full of unique ideas that sometimes look unrealistic. Before becoming involved in *in vitro* ES cell differentiation projects some years ago, Nishikawa proposed a project to his laboratory members. According to his wife, however, nobody wanted to perform the experiments proposed by him because they sounded so unrealistic and too risky. Nishikawa thus—after a long absence from bench work—decided to do the experiments himself. He set up a FACS facility and started sorting ES cells as he had proposed. He took particular pleasure in being the first author of the paper reporting this work (Nishikawa *et al.* 1998), which was followed by numerous papers from his own laboratory and elsewhere. His philosophy of work has been highly inspirational for us.

6. CONTRIBUTION OF STROMAL CELLS IN THE STUDY OF HSCs

T. M. Dexter was the first to show that long-term bone marrow cultures can recapitulate *in vitro* haematopoiesis, particularly myelopoiesis (Dexter *et al.* 1977). A heterogeneous population of adherent bone marrow cells is established prior to the inoculation of haematopoietic cells. Cultures are incubated with 5% CO₂ in air at 33°C. At weekly intervals, half the supernatant medium is replaced with freshly prepared medium. When mouse bone marrow cells are cultured under 'Dexter conditions', the production of granulocyte/macrophage progenitors is reportedly sustained for up to 20 weeks (Moore *et al.* 1979). This culture system consists of haematopoietic and stromal cell elements, and offers opportunities for study of the interaction between HSCs and niches. The long-term culture-initiating cell assay (Sutherland *et al.* 1990) and cobblestone area-forming cell assay (Ploemacher *et al.* 1989) were developed based on Dexter conditions.

Replacement of heterogeneous stromal cells by a defined stromal cell line improves the reproducibility of experiments and facilitates molecular approaches to discover molecules that play a role in the interactions between HSCs and their niches. In addition, it is possible that stromal cells capable of supporting HSCs are as rare in bone marrow as are HSCs (Moore *et al.* 1997). In this regard, functional stromal cells should be separated from non-functional ones.

Hiroaki Kodama has been excellent at the establishment of stromal cell lines from mouse bone marrow. To date his three cell lines, MC3T3-E1, MC3T3-G2/PA6 (PA6) and OP9, have been widely used internationally. Proceeding from a classic protocol used to make the immortalized mouse fibroblast 3T3 cell line, Kodama initially established the MC3T3-E1 cell line from a calvaria of newborn mice (Kodama *et al.* 1981).

MC3T3-E1 cells can be differentiated into osteoblasts that then form calcified bone *in vitro* (Sudo *et al.* 1983). Kodama similarly established the PA6 cell line from a calvaria of newborn mice. This cell line strongly supports the maintenance of HSCs in culture (Kodama *et al.* 1982a,b). However, when bone marrow cells or purified HSCs are co-cultured with PA6 cells, macrophages predominantly proliferate. Kodama simply did not like macrophages covering the monolayer of his stromal cell line. To exclude macrophages from culture, Kodama next established the OP9 cell line from the calvaria of op/op mice. This cell line, as expected, maintains HSCs well in culture (Kodama *et al.* 1994). Toru Nakano unexpectedly found that mouse ES cells efficiently differentiate into blood cells when co-cultured with OP9 cells (Nakano *et al.* 1994, 1996). Of interest is that vasculogenesis also can be supported by OP9 cells (Takakura *et al.* 1998). Delta-1-expressing OP9 cells have been used to generate CD4/CD8 double-positive T cells in culture without thymus microenvironment (Schmitt & Zuniga-Pflucker 2002; Schmitt *et al.* 2004). Galectin-1 isolated from OP9 cells has been recently shown to support the proliferation of neutral stem cells (Sakaguchi *et al.* 2006). Kodama once said that in creating his cell lines he had played his part; they would find people who appreciated their value. Indeed, they have been appreciated—Kodama is recognized in Japan as a legendary creator of stromal cell lines.

7. HSC ATTRACTING FACTOR

Stroma cell-derived factor-1 (SDF1, also designated as CXCL12) was identified in PA6 cells by expression cloning (Nagasawa *et al.* 1994). SDF1, belonging to the chemokine family, supports B cell proliferation in culture. CXC-chemokine receptor 4 (CXCR4) is the receptor for SDF1 and also an entry receptor for some strains of HIV-1 virus (Bleul *et al.* 1996; Nagasawa *et al.* 1996b; Oberlin *et al.* 1996). Studies of mutant mice deficient in SDF1 or CXCR4 have shown that SDF1/CXCR4 signalling is essential for B cell development (Nagasawa *et al.* 1996a; Ma *et al.* 1998). Moreover, it has been suggested that, during embryonic development, foetal liver HSCs migrate into the spleen and the bone marrow in an SDF1-dependent manner (Ara *et al.* 2003b). Conditional knockout mice for CXCR4 showed that the number of HSCs in adult bone marrow is significantly reduced after CXCR4 is deleted (Sugiyama *et al.* 2006). A certain portion of HSCs migrate out of and return to their niches under the physiological steady state of haematopoiesis (Wright *et al.* 2001). SDF1 seemingly acts as an attractant factor for HSCs to recruit them back to their niches. SDF1/CXCR4 signalling is reportedly also required for angiogenesis, neurogenesis, and migration of primordial germ cells (Nagasawa *et al.* 1996a; Tachibana *et al.* 1998; Ara *et al.* 2003a).

8. TRANSCRIPTIONAL REGULATION IN HSC DEVELOPMENT

Transcription factors such as Runx1 (formerly designated AML1), Gata2 (Tsai *et al.* 1994), Scl (Porcher *et al.* 1996) and Moz (Katsumoto *et al.* 2006; Thomas *et al.* 2006) play roles in the development of HSCs.

RUNX1 was identified in acute myeloid leukaemia with the chromosomal translocation t(8;21) by Miyoshi *et al.* (1991). The translocation breakpoints form a fusion gene consisting of RUNX1 and ETO (Miyoshi *et al.* 1993). Runx1 knockout mice showed normal yolk sac haematopoiesis but lacked foetal liver haematopoiesis, suggesting that Runx1 is essential for definitive haematopoiesis; perhaps also for the generation of HSCs (Okuda *et al.* 1996). Conditional knockout mice for Runx1, however, showed that Runx1 is mainly required for megakaryocyte and lymphocyte differentiation (Ichikawa *et al.* 2004). Further work is needed to know whether Runx1 is also required for the maintenance of the HSC pool in adult mice. In many cases, roles of transcription factors in developmental haematopoiesis seem to differ from those in adult haematopoiesis.

9. HAEMATOPOIETIC PROGENITORS AND DIFFERENTIATION PATHWAYS

Koichi Akashi, who used to work with Weissman, returned to Japan after doing explosive work at Harvard University. He prospectively identified various haematopoietic progenitor populations lying between HSCs and mature cells, such as common lymphoid progenitors (Kondo *et al.* 1997) and myeloid progenitors (Akashi *et al.* 2000). Although relationships among these populations in terms of differentiation pathways are not completely understood, these populations have proved useful for the analysis of a variety of mutant mice to examine differentiation problems in blood lineage (Kozar *et al.* 2004; Zhang *et al.* 2004; Iwasaki *et al.* 2005). These progenitor populations, together with HSC populations, have been used for the studies of cancer stem cells (Passegue *et al.* 2004; Yilmaz *et al.* 2006). One hopes that Akashi will maintain his research activity and establish a new bloodline of research investigators in Japan.

10. MAINTENANCE OF THE BLOODLINES

What are the prospects for these bloodlines of HSC research? Nishiwaka, Nakahata, Suda and Nakauchi remain active in research and have become influential in biomedical politics in Japan. Some of the scientists trained under these people have already become independent and started their own investigations. However, activity in HSC research in Japan may not at present be so high as it used to be, since many people have already left this field for other stem cell fields. Perhaps this is because the HSC field has a long history and there are no more simple experiments to do. In order to maintain the bloodlines of HSC research, younger scientists must eventually take over and further extend the work originally initiated in Japan. The HSC has been a flagship for other stem cells, leading stem cell biology research. We are just beginning to understand how HSCs are regulated in molecular terms. The authors hope that the bloodlines of HSC study in Japan will continue to grow and contribute to the further development of stem cell science worldwide.

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