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Stem Cell Exhaustion and Leukemogenesis

Bindya Jacob[1] and Motomi Osato[1,2*]

[1]Institute of Molecular and Cell Biology, Singapore 138673, Singapore
[2]Cancer Science Institute of Singapore, National University of Singapore, Singapore 117456, Singapore

ABSTRACT

Cancer is the result of a combination of genetic alterations, which aid transformation of cells. However, oncogenic alterations also simultaneously induce some detrimental effects on the cells such as apoptosis, senescence, and differentiation. Such negative effects caused by certain oncogenic events are overcome by other cooperating genetic hits. We propose stem cell exhaustion as a novel detrimental effect that is caused by a wide variety of oncogenic alterations. Interestingly, in most cases, the stem cell exhaustion due to oncogenic alterations is preceded by an abnormal expansion of stem/progenitor cells. This preceding stem/progenitor cell expansion may be a key feature that still promotes cancer development, along with cooperating hits that rescue stem cell exhaustion. This review summarizes current knowledge about hematopoietic stem cell exhaustion and the mechanisms to overcome stem cell exhaustion in cancer development. J. Cell. Biochem. 107: 393–399, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: Runx1; AML1; HEMATOPOIETIC STEM CELL; NICHE

A number of reports have recently emerged, focusing on the effect of various genetic alterations on hematopoietic stem cell (HSC) activity. It is becoming increasingly evident that a large number of genes are involved in maintaining the integrity of HSC in terms of both function and numbers. Many of these genes are involved in cancer-causing genetic alterations, resulting in enhanced stem cell activity, corroborating the current paradigm that the increase of stem cell number and/or function results in a cancer prone status. However, conversely, some cancer-causing genetic alterations also result in the decrease of HSC, namely HSC exhaustion. In this review, we discuss stem cell exhaustion, mainly in the context of the *RUNX1* gene, which is frequently mutated in human leukemias.

Runx1 AND HSC EXHAUSTION

The *RUNX1/AML1* gene is a key regulator of hematopoiesis and is one of the most frequently mutated genes in human leukemias. *Runx1* knockout mice have largely illustrated the role of Runx1 in hematopoiesis. In wild-type mice, *Runx1* expression is seen as early as E6.5 in the yolk sac and at E8.5 at all sites from which definitive hematopoietic cells emerge including the yolk sac, the umbilical and omphalomesenteric arteries, and the aorta gonad mesonephros (AGM) region [North et al., 1999; Samokhvalov et al., 2007]. In *Runx1* knockout embryos, there is a complete absence of definitive fetal liver hematopoiesis [Okuda et al., 1996] though primitive hematopoiesis is not drastically impaired [Yokomizo et al., 2008]. *Runx1* knockout embryos are unable to generate definitive hematopoietic cells from the hematogenic endothelial cells in the AGM region [Yokomizo et al., 2001] and die at E12.5 from hemorrhage [Okuda et al., 1996; Wang et al., 1996].

Conditional knockout mice have recently demonstrated the role of Runx1 in adult hematopoiesis [Taniuchi et al., 2002; Ichikawa et al., 2004; Growney et al., 2005; Putz et al., 2006; Motoda et al., 2007]. *Runx1*-excised adult mice have an expanded, immunophenotypically defined HSC compartment. In addition, *Runx1* knockout mice show accumulation of megakaryoblasts and immature lymphocytes due to abnormal megakaryocyte differentiation and defective T- and B-cell development, respectively. Excision of *Runx1* does not inhibit maturation of the myeloid lineage. These mice survive for more than a year, indicating that there is long-term HSC activity in these animals to some extent. This finding is surprising in light of the hematopoietic defect observed in *Runx1* knockout embryos. Ichikawa et al. [2004] did not report the development of leukemia or other disease phenotypes in their conditional *Runx1* knockout model. Another conditional knockout mouse model developed by Taniuchi et al. [2002] and Motoda et al. [2007] also did not show any spontaneous leukemia phenotypes. Growney et al. [2005] showed a modest myeloproliferative

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*Correspondence to: Prof. Motomi Osato, MD, Institute of Molecular and Cell Biology, 61 Biopolis Drive, Singapore 138673, Singapore. E-mail: motomi@imcb.a-star.edu.sg
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phenotype in Runx1-excised mice, with mild expansion of myeloid cells in the bone marrow (BM) and spleen. Nevertheless, there was no spontaneous leukemia development in these mice. Putz et al. [2006] also showed the moderate myeloproliferation with splenomegaly in their *Runx1* conditional knockout model, and three out of six mice studied in detail developed lymphoma. These differences in the development of lymphoma/myeloproliferative phenotypes could be due to differences in targeting strategies.

Genetic alterations of the *RUNX1* gene are found in approximately 30% of all human acute myeloid leukemia (AML). These alterations arise because of either chromosomal translocations, resulting in dominant negative fusion proteins with RUNX1, or mutations in the *RUNX1* gene itself [Osato et al., 1999; Osato, 2004]. Generally, the common underlying mechanism of RUNX1-related leukemia, or RUNX leukemia, is considered to be loss-of-function of RUNX1. However, a series of mouse models designed to recapitulate genetic alterations in human leukemias such as *RUNX1-ETO* conditional knock-in mice or majority of the *Runx1* conditional knockout mice models failed to develop spontaneous leukemia [Higuchi et al., 2002; Ichikawa et al., 2004; Growney et al., 2005]. Moreover, chimeric genes involving *RUNX1* are detected even in healthy volunteers [Basecke et al., 2002; Mori et al., 2002].

The above results suggest that Runx1-deficient cells, though abnormal, are not fully malignant by themselves and require additional genetic changes, or "second hits" for full-blown leukemia. The most interesting observation from the studies of *Runx1* conditional knockout mice is the increase in HSC/progenitor cell compartment soon after the induced excision of the *Runx1* gene. This expansion could be due to increased expression of critical genes such as *Bmi1*, involved in self-renewal of stem cells, and *Bcl-2*, an anti-apoptotic factor. Thus, Runx1-deficient immature cells accumulate and have enhanced self-renewal properties and greater resistance to apoptosis than wild-type cells [Motoda et al., 2007]. Such alterations may increase susceptibility of Runx1-deficient cells to leukemia development. However, as mentioned repeatedly, these properties are not sufficient and there is no spontaneous leukemia development. Furthermore, surprisingly, despite the initial increase in stem/progenitor cell fraction in *Runx1* conditional knockout mice, the immature cell numbers reduce progressively, and after a certain period of time, the number of Runx1-deficient stem/progenitor cells is much lower than control wild-type cells. This progressive decline in stem/progenitor cell numbers is considered to be stem cell exhaustion.

Preliminary results suggest that stem cell exhaustion of Runx1-deficient cells results from compromised interaction of the HSC with its niche, due to reduced expression of niche-interacting factors. The interaction between HSC and its niche is essential for maintaining the functional integrity of stem cells, in particular, quiescence. In an adult mouse, 60–70% of the BM stem cells are shown to be in G_0 phase by Ki-67 staining [Calvi et al., 2003]. Detachment from the niche due to weakened interaction would result in loss of quiescence and hence loss of long-term self-renewal capacity of Runx1-deficient HSC, resulting in an increase of short-term stem cells. Furthermore, during homeostasis, HSC leave their original niche and enter the circulation. The circulating Runx1-deficient HSC may be compromised in homing to and interacting appropriately with another niche. In the initial stage of leukemia, since the circulating stem cells are mainly made up of wild-type HSC, a minor clone of Runx1-deficient HSC could be outcompeted in establishing adequate interaction with the niche. Thus, problems in niche interaction could be a critical issue in leukemia development at the initial step and the leukemia-initiating clone with *Runx1* alteration has to overcome this selective disadvantage for leukemia progression.

Since the number of circulating stem cells is a small fraction of the actual stem cell pool, and a few of the Runx1-deficient stem cells may still be able to reestablish niche interaction, the decrease in number of Runx1-deficient stem cells is gradual and not easily noticeable. In this regard, it is interesting to note that the evidence for stem cell exhaustion mainly comes from BM transplantation assay. In the competitive transplantation experiments done by Growney et al. [2005], Runx1-deficient cells are shown to be outcompeted by wild-type cells. This highlights the importance of BM transplantation experiments for easy visualization of the exhaustion process. The stress due to transplantation may enhance stem cell exhaustion, making the phenomenon obvious, rather than examination of *Runx1* knockout mice per se which may have to be aged very long before they display significant exhaustion. The experimental setting of transplantation could also be more suitable to assess leukemogenesis where most of the surrounding cells are normal wild-type cells. In actual leukemia development, presumably, a single cell acquires a genetic alteration resulting in loss-of-function of RUNX1 and this cell turns into a possible leukemia-initiating clone. This setting is recapitulated by transplantation of *Runx1* knockout cells into wild-type recipients.

STEM CELL EXHAUSTION IS A COMMON PHENOMENON IN CANCER DEVELOPMENT

The initial phase of HSC/progenitor increase in *Runx1* knockout mice is consistent with the fact that loss-of-function alterations of *RUNX1* are frequently seen in human leukemia. However, the progressive stem cell exhaustion in *Runx1* knockout mice seems paradoxical as it results in the lower number of HSC/progenitor cells (Fig. 1A). Surprisingly, this is not the first instance of a genetic alteration involved in cancer leading to detrimental effects such as stem cell exhaustion. Table I summarizes the current literature on genetic alterations that lead to HSC exhaustion in mice. It is not surprising that knockout of genes such as *Bmi1* and *Gfi1* lead to HSC exhaustion as these genes are essential for maintaining the integrity of stem cells. This type of exhaustion does not result in a tumorigenic status. *Bmi1* knockout mice even show the resistance to cancer. On the other hand, it is paradoxical that validated tumorigenic alterations such as knockout of *p21* [Cheng et al., 2000], *Pten* [Zhang et al., 2006], and activation of *β-catenin* [Scheller et al., 2006] and hedgehog signaling [Trowbridge et al., 2006] also cause stem cell exhaustion. The oncogenic potential of the above alterations could be attributed to the preceding HSC expansion, which serves as an increased target cell pool for leukemia development. Furthermore, as has been recently reported in a *Pten* conditional knockout model [Guo et al., 2008], the clone carrying

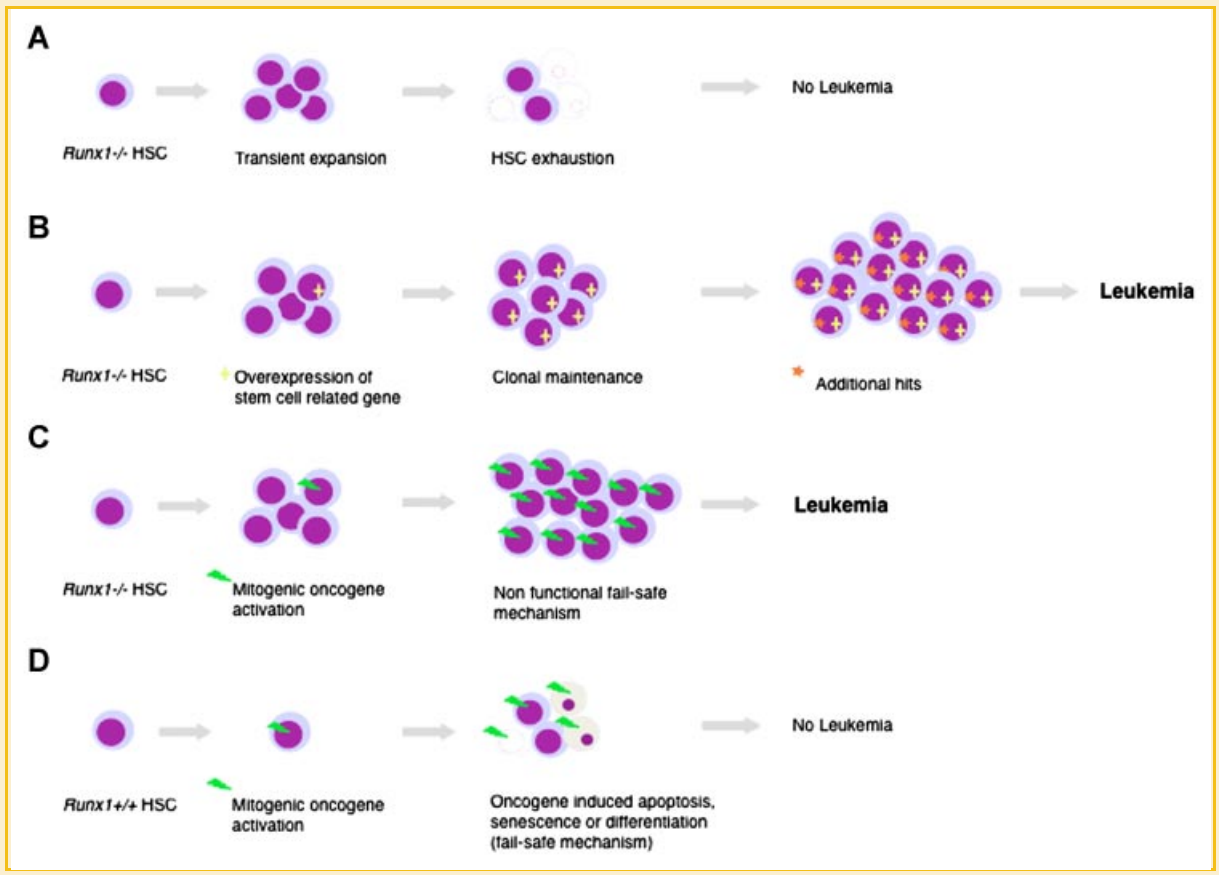

Fig. 1. Schematic representation of (A) stem cell expansion followed by exhaustion due to Runx1 deficiency and (D) cellular fail–safe mechanism induced by mitogenic oncogenes in Runx1+/+ healthy cells; leukemia development by cooperation between *Runx1* knockout status and (B) overexpression of stem cell-related genes or (C) mitogenic oncogene activation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the oncogenic alteration probably acquires further genetic changes that sustain and enhance their leukemogenic potential before stem cell exhaustion occurs. The above two mechanisms, namely the preceding HSC expansion and cooperative genetic hits, could explain the paradox of stem cell exhaustion seen in potentially tumorigenic genetic alterations. There is also another category of alterations including knockout of *Cdc42*, *Cxcr4*, and *Ship1*, which show HSC expansion followed by exhaustion. These alterations have not been implicated in cancer yet. However, since the alterations result in the initial stem cell expansion, the expanded pool of cells could be a target for further hits. Hence, the involvement of these alterations in leukemia may be uncovered in the future.

We also hypothesize that higher incidence of *RUNX1* mutations in human leukemia compared to the other genes points to the exhaustion time window as a critical factor in leukemia development. Exhaustion of Runx1-deficient stem cells is a very gradual process as a result of which these aberrant cells persist in the body for long periods of time and there is adequate time for a rescuing second hit to occur, probably in a stemness-related gene, before complete stem cell exhaustion takes place. On the other hand, deficiency of Pten, which is a well-known tumor suppressor, leads to rapid stem cell exhaustion after expansion of HSC, which may be the reason why mutations in this gene are very rare in human leukemias even though leukemias are seen in the mouse models [Guo et al., 2008]. The aberrant cells lacking Pten may not have enough time to acquire additional cooperating genetic alterations before they are completely exhausted and eradicated from the human body, despite the initial stem cell expansion. Time window for stem cell exhaustion may govern the incidence of individual cancers in humans.

MULTISTEP LEUKEMOGENESIS

Stem cell exhaustion should be rescued by other hits in cancer development. We propose two mechanisms whereby additional hits can overcome stem cell exhaustion. Firstly, alteration of a gene with function in stem cell maintenance is expected to prevent stem cell exhaustion. Overexpression of a cooperating stem cell-related gene may result in spontaneous leukemia development from Runx1-deficient stem cells or at least maintenance of the expanded pool of abnormal cells in the body till they acquire further oncogenic hits and become leukemic (Fig. 1B). Thus, stem cell-related genes may constitute a new class of oncogenes, which maintain the leukemia-initiating clone in the body. Secondly, a strong mitogenic stimulus can cooperate with Runx1-deficient status in rapid, spontaneous

TABLE I. Classification of Genetic Alterations That Lead to HSC Exhaustion in Mice

Gene[a]	KO/ mutated	Function[b]		Leukemogenic/ oncogenic?[c]	Preceding HSC/P expansion	HSC exhaustion	Mechanism	References
p21	KO	Cell cycle regulator	Tumor suppressor	Oncogenic	+	+	Increased cycling	Cheng et al. [2000]
Rb	KO	Cell cycle regulator	Tumor suppressor	Leukemogenic/oncogenic	+	+	Niche defect	Viatour et al. [2008], Walkley et al. [2007]
Atm	KO	DNA repair	Tumor suppressor	Oncogenic	–	+	Niche interaction defect, increased ROS	Ito et al. [2004]
Pten	KO	PI3 kinase signaling	Tumor suppressor	Oncogenic[e]	+	+	Niche interaction defect, increased cycling	Zhang et al. [2006], Guo et al. [2008]
Ptch1	KO	Hedgehog signaling	Tumor suppressor	Oncogenic	+	+	Increased cycling	Trowbridge et al. [2006]
Ctnnb Ex3[d]	KO	Wnt signaling[d]	Oncogene	Oncogenic[d]	+	+	Increased cycling	Scheller et al. [2006]
APC	KO	Wnt signaling	Tumor suppressor	Oncogenic	–	+	Increased cycling	Qian et al. [2008]
Runx1	KO	Transcription factor	Tumor suppressor	Leukemogenic	+	+	Niche interaction defect	Jacob et al., unpublished work; Growney et al. [2005]
Pu.1	KO	Transcription factor	Tumor suppressor	Leukemogenic	–	+	Not known	Iwasaki et al. [2005], Metcalf et al. [2006]
Pml	KO	Transcription factor	Tumor suppressor	Leukemogenic	+	+	Increased cycling	Ito et al. [2008]
Mll	KO	Transcription factor (Polycomb)	–	Leukemogenic	–	+	Not known	McMahon et al. [2007]
Bmi1	KO	Transcription repressor (Polycomb)	Oncogene	Resistant to cancer	–	+	Loss of self renewal	Park et al. [2003]
Gfi1	KO	Transcription factor	Oncogene	No	–	+	Increased cycling	Hock et al. [2004]
Lig4	Inactivation	DNA repair	–	No	–	+	DNA repair defect, increased cycling	Nijnik et al. [2007]
Terc	KO	Telomere maintenance	Oncogene	No	–	+	Niche interaction defect	Ju et al. [2007]
FoxO	KO	Transcription factor	–	No	–	+	Increased cycling and ROS	Tothova et al. [2007], Miyamoto et al. [2007]
Id1	KO	Transcription factor	–	No	–	+	Increased cycling	Jankovic et al. [2007]
RARγ	KO	Nuclear receptor	–	No	–	+	Niche interaction defect	Purton et al. [2006]
Zfx	KO	Transcription factor	–	No	–	+	Increased apoptosis	Galan-Caridad et al. [2007]
cKit	KO	Cytokine receptor	Oncogene	No	–	+	Increased cycling, increased apoptosis	Thoren et al. [2008]
Tpo	KO	Cytokine	–	No	–	+	Niche interaction defect, increased cycling	Qian et al. [2007]
Cxcr4	KO	Chemokine receptor	–	NA	+	+	Niche interaction defect, increased cycling	Sugiyama et al. [2006]
Cdc42	KO	Cell cycle, migration	–	NA	+	+	Increased cycling	Yang et al. [2007]
Ship1	KO	PI3 kinase signaling	–	NA	+	+	Niche interaction defect	Desponts et al. [2006]

HSC/P, hematopoietic stem cell/progenitor; ROS, reactive oxygen species; NA, not available.
[a]Gene involved in the alteration, which is knocked out (KO) or mutated.
[b]Function of the gene in normal cells and in cancer.
[c]Involvement of the genetic alteration in leukemia and other cancers.
[d]Knockout of exon 3 of *Ctnnb* leads to constitutive activation of beta catenin and hence is oncogenic.
[e]*Pten* knockout in mice leads to leukemia though *PTEN* is rarely involved in human leukemias.

development of leukemia before the Runx1-deficient stem cells undergo exhaustion (Fig. 1C). Towards leukemia caused by mitogenic oncogene activation, Runx1 deficiency is considered to be an ideal cooperative genetic hit. A mitogenic stimulus in a cell results primarily in hyperproliferation, but can also induce detrimental effects such as oncogene-induced apoptosis, senescence, and differentiation which are currently well recognized as a vital fail–safe mechanism to restrict the malignant transformation of cells (Fig. 1D). Runx1 insufficiency is shown to impair fail–safe mechanism, particularly in the stem/progenitor cells, thereby supporting the clonal maintenance of leukemia-initiating cells expressing the activated oncogene [Motoda et al., 2007].

NICHE THERAPY FOR CANCER TREATMENT

The reported reasons for stem cell exhaustion in various models include one or a combination of the following—increased cycling of HSC, defective niche interaction, high reactive oxygen species, increased apoptosis, and DNA damage (Table I). These defects should be rescued, to some extent, by cooperative genetic changes in the development of cancer. However, these weak points might still be seen in full-blown clinical cancers and could be targeted by therapeutic drugs. The fact that stem cell exhaustion due to defective niche interaction is frequently caused by oncogenic alterations leads us to propose a new therapeutic direction, namely niche therapy. HSC have a defined spatial organization in the BM cavity, with the most primitive cells being located in stem cell niche near the endosteum of the bone—the layer of connective tissue that lines the medullary cavity of a bone. The endosteum is lined with osteoblasts, which are thought to secrete or activate a variety of factors such as angiopoietin-1 and CXCL12 (CXC chemokine ligand 12) that regulate the maintenance and numbers of HSC in the BM [Arai et al., 2004; Calvi et al., 2003; Zhang et al., 2003]. Quiescent leukemic stem cells (LSC), similar to normal quiescent HSC, are shown to reside in the endosteal niche, are dormant and highly resistant to chemotherapeutic agents that target proliferating or cycling cells [Ishikawa et al., 2007; Trumpp and Wiestler, 2008]. Hence, there is a high incidence of relapse whereby LSC give rise to new leukemic cells after treatment. Specific targeting of LSC and their niche interaction could enhance the egress from the niche. Once mobilized, they may lose their quiescence and enter the cell cycle, which sensitizes them to chemotherapeutic agents. It is well known that patients with AML with t(8;21) have good prognosis [Asou, 2003]. One of the reasons for this chemosensitivity could be the defective niche interaction of Runx1-deficient LSC as Runx1 regulates expression of key niche-related factors.

Known agents for stem cell mobilization such as granulocyte colony-stimulating factor (G-CSF) can be utilized for releasing stem cells from the niche. However, since G-CSF could stimulate proliferation of leukemic cells, the application of G-CSF alone seems limited. Other strategies such as Cxcr4 inhibition have also been explored [Trumpp and Wiestler, 2008]. We would like to propose the use of parathyroid hormone (PTH), frequently used for the treatment of osteoporosis, as a novel option to mobilize stem cells during niche therapy. Pharmacologic use of PTH has been shown to increase the number of HSC mobilized into peripheral blood as it causes bone turnover, resulting in forceful release of HSC from the primitive endosteal niche [Adams et al., 2007]. Therefore, PTH holds promise in its function in the mobilization of LSC besides HSC. The advantage of niche therapy to eliminate LSC is still restricted as long as specific targeting of LSC cannot be achieved. Careful examination of LSC and their niche-interacting factors needs to be done to identify abnormally upregulated niche-interacting factors that could be targeted by drugs. For example, chronic myeloid leukemic cells upregulate CD44, which facilitates the homing and migration of leukemic cells to the niche [Jin et al., 2006]. Combining a CD44 antagonist with PTH might result in faster release of CML stem cells from the niche, rather than healthy stem cells. Similarly, in RUNX leukemias, if cooperating stem cell-related genetic hits, which rescue niche interaction are identified, specific targeting of these factors or their downstream molecules may result in efficient release of LSC from the niche. Thus, niche therapy seems to be a good option to eradicate residual LSC left in the niche after conventional treatment.

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