Hematopoietic Stem Cells and Their Niche

Hiroko Iwasaki and Toshio Suda

Abstract The stem cells’ major capabilities (i.e., the pluripotency and the self-renewal) are the keys to sustain the lifelong functionality of the organ. Stem cells reside in the special microenvironment called niche. The niche and stem cells adhere to each other via adhesion molecules and exchange the molecular signals that maintain the stem cell features. It has been suggested that tumor tissue also contains such type of cells.

In this chapter, the hematopoietic system is exemplified to show the interaction between the stem cell and its niche, which is the most intensively studied and archetypical stem cell system. Different kinds of niche and the regulatory mechanisms are explained. Furthermore, the niche involvement in cancer stem regulation, tumor invasion, and metastasis, as well as the novel therapeutic approaches in association with the cancer stem cell niche are also discussed.

Introduction

Virtually every organ has stem cells. These stem cells possess two major roles: the pluripotency that gives rise to the mature cells to compose the specific organ or tissue, and the self-renewal capability to supply enough cells to maintain the organ’s function. These stem cells tend to be found in specific areas in the organ, where special microenvironment maintains the stem cell functions. This special

H. Iwasaki (✉)
Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582 Japan
e-mail: hiroko-ko@umin.ac.jp

T. Suda
Director and Professor, Center for Integrated Medical Research, The Sakaguchi Laboratory of Developmental Biology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582 Japan
e-mail: sudato@sc.itc.keio.ac.jp
microenvironment is called the niche. The stem cell and the niche cells adhere to
each other via adhesion molecules and exchange the molecular signals that main-
tain the stem cell features.

The concept of the stem cell niche was proposed by R. Schofield in 1978 for the
hematopoietic stem cell (HSC) in bone marrow (Schofield 1978). Schofield stated
“the cellular environment which retains the stem cell I shall call a stem cell ‘niche.’
As long as the stem cell remains fixed its further maturation is prevented and it
continues indefinitely to replicate as a stem cell, i.e., it exhibits immortality.” Since
this initial proposal, not only the HSCs but various other kinds of stem cells have
also been identified, and their niches and the molecular mechanisms for stemness
maintenance have been revealed.

The stem cells are not only present in healthy normal organs or tissues. Recent
findings suggest that a group of cells exist in tumors that behave in a similar way
to the normal stem cells. Such cells are called cancer stem cells and are considered
to be deeply involved in the tumor proliferation, invasion, and metastasis. Their
niche plays a regulatory role for the cancer stem cell maintenance.

As of today, the most advanced studies in the stem cell niche field can be found
in the hematopoietic system. In this chapter, the interaction between the HSC and
its niche will be discussed. Furthermore, the niche involvement in the cancer stem
regulation, tumor invasion, and metastasis and the novel therapeutic approaches in
association with the cancer stem cell niche will also be approached.

**Stem Cell and Niche**

The stem cell research, in terms of the maintenance of the pluripotency and self-
renew capacity, was greatly advanced through the experiment using *Drosophila* and
*Caenorhabditis elegans*. In 1997, the group of W. Deng and H. Lin demonstrated
that the female *Drosophila* has “cap cells” at the tip of the ovary, to which the germ
line stem cells adhere (Deng and Lin 1997). In mitosis, the daughter stem cells that
divide to the direction in which they no longer adhere to the cap cells started the
differentiation and grew into the cystoblast. In contrast, those that kept the adhesion
to the cap cells after the cell division remained as the germ line stem cells. These
results suggested that the cap cells provide the special environment for the germ
line stem cell and play a role as the stem cell niche in the *Drosophila* ovary.

T. Xie and A. C. Spradling demonstrated in 2000 that the newly introduced cells
to the *Drosophila* ovary performed as the germ line stem cell as long as they adhere
to the cap cells, thus proving that the cap cells function as the true stem cell niche
(Xie and Spradling 2000). They showed that the germ line stem cells require a
signal mediated by dpp, a member of the TGF-β (transforming growth factor beta)
super family, in order to maintain the adhesion to the cap cells (to maintain the stem
cell) and control the frequency of the cell division. Additional signals, such as
Hedgehog, Wingless, or Armadillo, were also suggested to be involved in the regu-
lation of the germ line stem cells.
Interaction Between Hematopoietic Stem Cell and Niche

Osteoblastic Niche

In human hematopoiesis in bone marrow, the osteoblasts offer the microenvironment as the niche (i.e., osteoblastic niche) for the HSCs. HSC and the osteoblast bind each other via the adhesion molecules, such as N-cadherin (Fig. 1). The two independent research groups, led by L. Li and D. T. Scadden, respectively, revealed this new function of the osteoblast as the HSC niche in 2003. Both groups approached the issue in the investigation of the regulatory system for the number of the HSCs. Li’s group reported that bone morphogenetic protein (BMP) signaling pathway through BMP receptor type IA (BMPRIA) expressed in osteoblasts controls the number of HSCs by regulating the niche size (Zhang et al. 2003). Scadden et al. showed that the osteoblastic cells stimulated by activated PTH (parathyroid hormone)/PTHrP(parathyroid hormone-related peptide) receptors (PPRs) and increased in number produced high levels of the Notch ligand jagged 1 and supported the increase in the number of HSCs (Calvi et al. 2003).

It is widely known that the stem cell in general is in a quiescent state (G0 phase in cell cycle) and that this quiescence prevents the stem cells from entering into the cell cycle and differentiation. In this regard, the evidence for the importance of Tie2 signaling in HSC–niche interaction has been demonstrated by Puri and Berstein by using chimeric mice generated between normal embryonic cells and cells lacking Tie family receptors (Tie1 and Tie2) (Puri and Berstein 2003). Although Tie receptors

Fig. 1 Schematic model for hematopoietic stem cell (HSC) and osteoblastic niche. Osteoblastic niche lodges HSC via adhesion molecules, such as N-cadherin. Osteoblast and HSC exchange various signaling in order to maintain the stem cell functions
were not required for fetal hematopoiesis, including emergence of definitive HSCs, relocation to fetal liver, and differentiation, HSCs lacking these receptors were not maintained in the adult bone marrow microenvironment. Since Tie1-deficient cells, which express normal levels of Tie2, contribute to hematopoiesis, these findings indicate that Tie2 is required for postnatal bone marrow hematopoiesis. In addition, they analyzed chimeric mice generated between Tie1/Tie2 deficient embryonic cells and Rag2-deficient morula as the host, which does not produce mature lymphocytes, with a result that Tie-deficient cells could fully contribute to lymphopoiesis when no wild-type competing cells coexist. These findings indicated that Tie1 and Tie2 deficiency in HSCs leads not to the defect in differentiation but to survival in the microenvironment in adult bone marrow.

Arai et al. directly observed that angiopoietin-1 (Ang1) expressed in osteoblast interacts with Tie2, a type of receptor tyrosine kinase expressed in hematopoietic stem cell in bone marrow, and demonstrated that Tie2/Ang1 interaction activates β1-integrin and N-cadherin. This enhanced adhesion between the niche cell and the stem cell contributes to the maintenance of the quiescence of the stem cell and self-renew (Arai et al. 2004). Further studies are needed to elucidate the functional relationship between cell adhesion and cell cycle regulation.

Osteopontin (Opn), expressed by osteoblast, is also reported as a key component in HSC niche (Nilsson et al. 2005). Nilsson et al. demonstrated that the LSK (lineage-Sca-1+c-Kit+) cells that were isolated from bone marrow of Opn knockout mouse after the continuous 4-week BrdU treatment were 100% BrdU positive, meaning that the HSCs divided at least once in 4 weeks in bone marrow of the Opn knockout mice. On the other hand, approximately 60% of the collected LSK cells were BrdU+, as in the case of wild-type mice. They also showed that CD34+ human hematopoietic progenitors were inhibited to bind to Opn when treated with the specific β1 integrin-blocking antibody prior to the cell culture. Also, the LSK cells were observed to be rather randomly distributed within bone marrow after transplanted into Opn knockout mice, whereas they were likely to be located at the endosteal region, where osteoblasts reside, in the case of wild-type mice. These data suggested that Opn expressed by osteoblast contributes to the adhesion between the HSC and osteoblastic niche and negatively regulates HSC proliferation, contributing to the maintenance of the stem cell quiescence.

Thrombopoietin (TPO) and its ligand Mpl, the regulator of megakaryopoiesis, are also critical regulators in HSC maintenance in osteoblastic niche, as reported by two independent groups (Yoshihara et al. 2007; Qian et al. 2007). Yoshihara et al. found that the majority of Mpl+ LSK cells were found in side population (SP), the most concentrated fraction of quiescent HSCs. Mpl+ HSCs were observed in close contact with TPO-producing osteoblastic cells at the endosteal surface in trabecular bone area. On the other hand, Quin et al. demonstrated that TPO/Mpl signaling is required for postnatal HSC expansion but not in the prenatal phase, using adult Thpo−/− mice. In the knockout mice, the number of HSCs was 150-fold reduced, and p57kip2, a cell cycle regulator specifically expressed in the quiescent population of LT-HSC, was dramatically downregulated in HSCs. Meanwhile, Yoshihara et al. found that p57kip2 was upregulated by stimulation with TPO and downregulated by
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AMM2, an anti-Mpl neutralizing antibody. These data consistently indicate that the Mpl/TPO signaling regulates cell cycle of adult quiescent HSCs to maintain the pool at the physiologically reasonable level.

c-Myc is another important regulator of the HSC fate. Wilson et al. demonstrated, using conditional c-Myc deficient mice, that the number of HSCs in bone marrow was significantly higher, and that the HSC differentiation was prevented due to the upregulated adhesion molecules between HSC and the niche, such as N-cadherin and various integrins, resulting in the severe cytopenia. Conversely, overexpression of c-Myc led to loss of self-renewal activity and augmented differentiation of HSCs. The endogenous c-Myc mRNA level appeared to differ consistently with these results (i.e., the lower expression level in the self-renewing long-term HSCs and higher level in the differentiating short-term HSCs). c-Myc controls the balance between the HSC self-renewal and differentiation by adjusting the adhesion between HSCs and the niche (Wilson et al. 2004).

ECM Contribution to Osteoblastic Niche

Aside from the factors expressed in osteoblasts, various extracellular matrix (ECM) proteins in bone marrow are also confirmed to be involved in the regulation of the osteoblastic niche environment. Glycosaminoglycan hyaluronic acid (HA), for example, is one of the key components of the niche regulators. HA is the major component of the ECM and is found ubiquitously, including in bone marrow. Its receptor, CD44, is a multifunctional transmembrane protein expressed by a wide variety of cells, including the hematopoietic stem cells and progenitors. Matrosova et al. reported that the 5-fluorouracil (5-FU)-treated mice for bone marrow suppression exhibited quicker recovery in terms of the white blood cell and platelet counts when administered with HA during the recovery period, compared to phosphate buffered saline as control (Matrosova et al. 2004). In order to examine the location of injected HA in vivo, the fluorescein isothiocyanate (FITC)-labeled HA was traced and found concentrated at bone marrow, binding to CD44. This suggests that HA plays a role as a regulator of supportive function of niche in bone marrow for hematopoietic stem cells.

Another report demonstrated that the xenografting of human CD34+ hematopoietic stem/progenitor cells into the bone marrow of nonobese diabetic severe combined immunodeficient (NOD/SCID) mice was significantly impaired by masking the HSCs’ cell surface with anti-CD44 antibody or HA (Avigdor et al. 2004), indicating that CD44-HA interaction is an important factor for homing and engraftment of the HSC to the niche.

Because the osteoblasts are located at the endosteal area, where higher calcium concentration is expected due to its active bone modeling/remodeling, Adams et al. hypothesized that the sensor for calcium must be expressed on HSCs and contributes to their homing and lodgment to bone marrow. They revealed that calcium-sensing receptor, CaR, was expressed on normal HSCs and that CaR-deficient mice had a
normal number of primitive hematopoietic cells in the circulation and spleen but few of them were found in bone marrow. HSCs isolated from fetal liver of CaR-deficient mice could not localize at the endosteal niche in wild-type recipient mice because of the defective adhesion to the collagen I, one of the major components of the ECM secreted by osteoblast. Thus, it was suggested that the HSCs preferentially localize the calcium-rich endosteum and lodged to the niche via adhesion to the ECM surrounding the osteoblast (Adams et al. 2006).

**Vascular Niche**

As described above, the adhesion molecules and their signaling play a fundamental role in the HSC maintenance for the osteoblastic niche. There has been another kind of niche recently found along the endothelial cells of the sinusoidal vessels in bone marrow or spleen. This microenvironment is called the vascular niche. This new type of niche was revealed through a recent study to distinguish the stem and the progenitor cells in bone marrow using the SLAM family receptors. The most purified HSCs were fractioned as CD150\(^+\)CD244\(^-\)CD48\(^-\) cells, and the majority of these cells were found associated with sinusoidal endothelium (Kiel et al. 2005).

Sugiyama et al. showed that the HSCs were found specifically adjacent to the cells expressing a high level of CXCL12 (also called stromal cell–derived factor-1 or SDF-1) and surrounding the sinusoidal endothelial cells. They named these cells CXCL12-abundant reticular (CAR) cells and demonstrated that the depletion of CXCR4 led to the reduction of the HSC pool and a poor survival rate after 5-FU-induced bone marrow suppression, thus suggesting that CXCL12-CXCR4 chemokine signaling plays an essential role in maintaining the quiescent HSC pool (Sugiyama et al. 2006). The connection between CAR cells and HSCs is also observed at the endosteum. It appears to be the universal component of the hematopoietic stem cell niche.

The homing and lodgment mechanism to the vascular niche, the integrin very late antigen 4 (VLA-4) and its ligand vascular cell adhesion molecule 1 (VCAM-1), was proposed to be the regulator (Papayannopoulou et al. 1995). The pretreatment of donor cells with either anti-VLA-4 or VCAM-1 antibody prior to the transplantation into the recipient mice resulted in impaired lodgment of HSCs to bone marrow and increased the number of hematopoietic progenitors in circulating blood and the spleen.

Taking advantage of the VLA-4-mediated interaction between HSCs and vascular niche in bone marrow, natalizumab, a human anti-VLA-4 antibody, has been suggested for treating patients with poor response to granulocyte colony-stimulating factor (G-CSF)-based protocols for the peripheral blood stem cell transplantation (Zohren et al. 2008).

Human anti-VLA-4 antibody natalizumab was originally developed and introduced for the treatment of autoimmune diseases, such as multiple sclerosis (MS) and Crohn’s disease. The authors examined peripheral blood of patients with MS receiving natalizumab and found that the number of CD34\(^+\) primitive hematopoietic cells, including
HSCs, circulating in peripheral blood was significantly higher in natalizumab-treated MS patients, compared to those in untreated MS patients or healthy volunteers. The increase in the number of CD34+ cells was observed as soon as 1 hour after the infusion of natalizumab, and the colony-forming unit assay demonstrated that progenitors mobilized into peripheral blood formed significantly higher numbers of BFU-E and CFU-G/M/GM colonies compared to before infusion. The increase of CD34+ cells in peripheral blood was the result of both mobilization effect and inhibited homing by natalizumab. The latter effect lasted longer than the half-life of natalizumab. Thus, anti-VLA-4 antibody natalizumab with mobilizing ability may have the potential to become an alternative in peripheral blood stem cell transplantation.

The functional difference between the osteoblastic and vascular niches is yet to be elucidated; however, it may be reasonable to approach this issue from a physiological aspect. One of the major differences of these two microenvironments is the oxygen level. In vascular niche, a higher oxygen level is expected than in osteoblastic niche. Under such a microenvironment, the cell cycle of the stem cell would resume (Parmar et al. 2007). Thus a model of the dynamics of the hematopoiesis can be hypothesized: the HSCs in G0 state located at the osteoblastic niche under the regional hypoxia would move to the vascular niche at a certain time, undergo differentiation, and supply the required pool of the mature cells into the peripheral bloodstream (Heissig et al. 2002). Once the supply fulfills the necessary mature cells, the HSCs at the vascular niche would move back to the osteoblastic niche, where they are maintained in the G0 state again. The logistics of the HSCs shuttled between these two types of niches may be the key for well-balanced hematopoiesis (Fig. 2).

The endogenous oxidative metabolism in HSCs was studied by Piccoli et al., using human CD34+ HSCs forced to mobilize from bone marrow to peripheral blood in the G-CSF infusion regimen (Piccoli et al. 2005). The mitochondrial oxygen consumption level in HSCs was significantly reduced to approximately 10%, compared to regular types of cells, and it was found not because of inhibitory control but relatively poor content to mitochondrial cytochromes. The mitochondrial and extramitochondrial respiration was assessed by treatment with electron transport inhibitors, such as potassium cyanide, or enzymatic scavengers of reactive oxygen species (ROS), such as superoxide dismutase. The ratio of mitochondrial versus extramitochondrial respiratory activity was approximately 60:40, and by combining both treatments the endogenous respiratory activity was completely blocked. The major ROS producer in this cell is known to be cell membrane–bound NADPH oxidase, and the authors verified this by immunoprecipitation to show that NADPH oxidase exists on HSCs’ membrane, forming the fully assembled functional complex coordinated with p22phox, p67phox, and p47phox.

The amount of mitochondria was directly measured by confocal microscopy analysis using the fluorescent dye specifically accumulated in membrane potential-generating mitochondria, costained with CD34, an antigen expressed in HSCs or early hematopoietic progenitor cells. In general, the higher the expression is, the more primitive the cell is. The density and distribution of stained mitochondria have high variation among observed cells. Interestingly, the higher the CD34 staining signal density, the lower the mitochondria staining signal density.
Collectively, a HSCs oxygen metabolism model was proposed, suggesting that primitive HSCs that have a low amount of mitochondria use membrane-bounded NADPH oxidase complex as oxygen sensor and/or ROS source, contributing to signaling inducing mitochondriogenesis, differentiation, or cell survival.

**External Oxidative Stress and HSC**

The evidence of the oxidative stress impact on the long-term HSCs’ capability was demonstrated by Ito et al. (Ito et al. 2006). Treatment of the immature hematopoietic cells of LSK fraction with buthionine sulfoximine (BSO), even at low levels, affected the repopulation capacity of HSCs after transplantation, but not the number of colonies formed in culture. This result indicated that elevation of ROS induced by BSO did not have an impact on the colony-forming progenitors in LSK fraction but specifically lead to the defect of the HSC function. The elevation of ROS upregulated tumor suppressors *p16^{ink4a}* and *p19^{arf}* specifically in HSCs, and
treatment with p38 MAPK inhibitor or an antioxidant N-acetyl-L-cysteine (NAC) blocked this ROS-induced increase of p16\(^{ink4a}\) and p19\(^{Arf}\). This suggested that oxidative stress induces the HSC-specific phosphorylation of p38 MAPK, and this activation of p38 MAPK lead to the defect in the maintenance of HSC self-renewal capacity.

Ito et al. also investigated the ROS effect \textit{in vivo} using the ataxia telangiectasia mutated (Atm) knockout mice, which exhibit the defect in oxidative stress regulation. In Atm\(^{−/−}\) mice, ROS elevation and defective HSCs’ self-renewal were observed (Ito et al. 2004) and, therefore, the activation of p38 MAPK was expected. After 2 days of incubation, p16\(^{ink4a}\) and p19\(^{Arf}\) were upregulated in Atm\(^{−/−}\) LSK cells as ROS level elevated. As predicted, p38 MAPK was activated only in LSK cells, and treatment with p38 MAPK inhibitor blocked this upregulation. Furthermore, prolonged treatment with NAC or p38 MAPK inhibitor extended the lifespan of the wild-type HSCs in serial transplantation. Collectively, the inactivation of p38 MAPK played a role as a protector for HSC against the loss of self-renewal capacity through ROS elevation.

Tothova et al. demonstrated that the Forkhead O family members (FoxOs) are also mediators of HSCs resistance against oxidative stress by generating FoxO1, FoxO3, and FoxO4 knockout mice. (Tothova et al. 2007) The FoxOs play an important role in various physiological responses, such as induction of cell cycle arrest, stress resistance, or apoptosis. The FoxO-deficient mice exhibited a significant decrease in LSK size, myeloid lineage expansion, and lymphoid developmental abnormalities. The HSCs from FoxO-deficient bone marrow had defective self-renewal capacity accompanied by increased cell cycling and apoptotic HSCs as well as increased ROS level specifically observed in HSCs. On the other hand, NAC treatment rescued the LSK size, HSC cell cycling profile, and apoptosis, leading to the rescue of long-term HSC function in the cobblestone forming cell assay. Thus, FoxOs mediate quiescence and survival of HSCs by detoxification of ROS.

\textbf{Seed or Soil? Niche Disruption and Disease}

If a stem cell comes out of the niche, its differentiation program resumes and the mature cells are supplied to the organ. If the stem cell niche become corrupted for some reason, more stem cells would be forced to leave. This may lead to the ideal situation for the organ, at least temporarily, given plenty of healthy mature cells to sustain the function. However, what if the majority of the stem cell niche is permanently destroyed? If a disease has developed, is it because the niche is bad or because the stem cell itself is no longer healthy due to the niche malfunction?

Walkley et al. answered this question by introducing the retinoic acid receptor \(\gamma\) (RAR\(\gamma\)) deficient mice (Walkley et al. 2007a). The mice exhibited myeloproliferative syndrome (MPS) with significantly increased granulocyte/macrophage progenitors and granulocytes in bone marrow, peripheral blood, and spleen. The phenotypes became more severe with age. A significant reduction of trabecular bone was observed, resulting in the osteoblasts depletion, and thus the niche was destroyed.
In order to determine whether this disease is caused by the bad seed (HSCs) or soil (niche), the transplantation of the wild-type bone marrow cells into lethally irradiated RAR\(\gamma\) overexpressed and deficient mice was performed, revealing the result that the onset of MPS occurred exclusively in RAR\(\gamma\) deficient mice. These data clearly proved that the MPS was not due to the altered HSC but the malfunctioning niche.

At the same time, they also investigated the effect of the Rb gene, a major cell cycle regulator, using the interferon-inducible Mx-Cre transgene and pRb\(^{fl/fl}\) mice (Walkley et al. 2007b). When Rb was widely inactivated in the transgenic mice, including the bone marrow and HSCs, the mice exhibited profound myeloproliferation, loss of HSC from bone marrow, differentiation, and extramedullary hematopoiesis. The substantial loss of trabecular bone was also observed after the Rb inactivation. However, when either the transplanted HSC or recipient is the wild type, no such phenotype was observed.

The effect of Rb deletion was also examined using lysozyme-M-Cre mice, by which the gene deletion specific to the myeloid lineage (i.e., granulocytes, macrophages, and osteoclasts) is made possible. The myeloproliferation occurred when the Rb-inactivated hematopoietic cells, including the unaffected HSCs harvested from the lysozyme-M-Cre mice, were transplanted into the widely Rb-inactivated mice, but not when transplanted into the wild-type mice. Collectively, this Rb deletion model concluded that myeloproliferative disease is observed only when Rb-inactivated myeloid-derived cells interacted with Rb-inactivated microenvironment, regardless of the condition of the HSCs.

Both of these studies exhibited the loss of the osteoblastic niche and myeloproliferation, however, the causes were not identical. The status of the residual microenvironment, recovery system for the lost niche, or the relevance of the gene to hematopoiesis and microenvironment may influence the differences of the observation between these two cases. The bottom line is that the disruption of the niche or microenvironment can trigger the system disorder, albeit even with healthy stem cells. Knowing the pathological involvement of the niche can bring the novel therapeutic strategy with enhanced effectiveness, and further studies in this area are awaited.

Is There a Niche for Cancer Stem Cell?

Recently it has been reported by various studies that there is a functional microenvironment that supports cancer stem cells. This should also be considered as a niche, thus called cancer stem cell niche. A representative example is acute myeloid leukemia (AML) and its niche in bone marrow. Jin et al. showed that the anti-CD44 antibody-treated NOD/SCID mice transplanted with AML cells exhibited significantly lower rate of the disease onset (Jin et al. 2006). Also, Krause et al. showed impaired induction of chronic myeloid leukemia (CML)-like myeloproliferative disease among the recipient mice when transplanted with BCR-ABL1–transduced CML progenitors from CD44-mutant donors (Krause et al. 2006). These results indicate that, in both AML and CML cases, CD44 is essential for the homing and engraftment of the cancer stem cells to the niche. In another words, the
CD44-expressing leukemic stem cell adhere to the niche, binding to its ligand hyaluronic acid expressed by the cells on the surface of sinusoidal endothelium or endostium in bone marrow, which is crucial for the niche maintenance of the stem cells. Interestingly, this molecular mechanism resembles that of healthy HSC and the vascular niche described earlier. Cancer and normal stem cells have much in common in the maintenance system in the niche.

Interestingly, cancer stem cell can evolve according to the microenvironment and initiate different type of cancer. Barabe et al. demonstrated that human hematopoietic cells infected with a retrovirus encoding mixed-lineage leukemia (MLL)-eleven-nineteen leukemia (ENL) fusion gene caused acute lymphoid leukemia (ALL) when transplanted into sublethally irradiated immunodeficient mice, but acute myeloid leukemia (AML) when cultured in a myeloid-promoting culture condition prior to transplantation for a prolonged period (Barabe et al. 2007). This indicates that the cancer stem cell with MLL fusion gene can initiate either ALL, AML, or both, depending on the microenvironment.

In contrast to the hematopoietic system, the stem cell research in the solid cancer field is relatively new. Among them, the stem cells study for brain tumors has made significant progress lately. Calabrese et al. showed brain tumor cells coexpressing Nestin and CD133, the fraction believed to contain the cancer stem cell, located closely to the capillaries in the brain tumor (Calabrese et al. 2007). When these cells were cocultured, the cancer stem cells selectively adhered to the endothelial cells. These suggested that the endothelial cells secreted factors necessary to maintain the cancer stem cells. Furthermore, the CD133-positive cells derived from human medulloblastoma developed brain tumor only when xenografted to the brain of the recipient nude mouse with endothelial cells. These data collectively suggest that the cancer stem cells of the brain tumor rely on the endothelial cells, which forms the vascular niche, for the maintenance of their self-renewal, differentiation, and proliferative capacity.

Roles of Niche Against Development, Maintenance, and Proliferation of Cancer

As described above, niche has the ability to control the pool, function, and even the fate of the stem cells. It is indeed an important aspect of niche to maintain the stem cells in a quiescent state; however, it also has to drive the adequate number of stem cells into the proliferation and differentiation path at the same time as far as the maintenance of the organ is concerned. This two-way signal of the niche against stem cell must be carefully regulated (Fig. 3). For example, in osteoblastic niche for hematopoietic stem cell in bone marrow, Tie2/Ang1 adhesion or BMP (bone morphogenetic protein), a member of TGF-β super family, are involved in maintaining the quiescence and proliferation suppression, while Wnt or Notch signaling promote the self-renewal, proliferation, and differentiation (Fig. 4) (Wilson and Trumpp 2006).
It is obvious that cancer tissue is highly proliferative. Does the stem cell regulatory system in the niche for cancer lean more toward the proliferative side than those for normal stem cells? Li and Neaves approached this problem via the dependence of stem cell on niche. They hypothesized that the behavior of cancer and normal stem cell are regulated by niche at different degrees by niche (Li & Neaves 2006). Cancer stem cells are derived through intrinsic mutation that leads to its highly proliferative activity. This highly proliferative state itself alters the signaling balance between niche and the stem cell. Namely, the niche function of quiescence maintenance becomes relatively ineffective, thus the function to support proliferation and differentiation become more dominant (Fig. 3). This model is supported by some clinical symptoms, one of which is the blast crisis of the CML. The Wnt/β-catenin signal, a causative pathway for self-renew, differentiation, and proliferation, is enhanced in the mutated nucleus of granulocyte-macrophage progenitors in the CML patient, due to the higher concentration of the β-catenin and irregularly activated TCF/LEF (T-cell factor/lymphocyte-enhancement factor) transcription activity (Jamieson et al. 2004). The blast crisis is thought to be triggered by this irregular TCF/LEF activity,
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leading to the excess transcription of Wnt target gene products. Similarly, relevance of irregular Wnt/β-catenin activation has been reported in the cases of colon cancer or melanoma, in which the β-catenin itself has mutation. It is important to note that many signaling pathways involved in the normal stem cell and niche interaction are also found between the cancer stem cell and its niche, playing a role as the promoter of tumorigenesis and cancer proliferation. The identical set of proteins in a slightly different condition can deliver totally different results.

Mechanism of Cancer Metastasis Regulated by Niche

Niche not only cradles existing cancer stem cells, but also the future incoming cancer stem cell. Rather, it passively sends the inviting signal to the remote cancer stem cells.

MMPs (matrix metalloproteinases) are factors known not only for their contribution to the repair of inflammation or wounds but also for their involvement in cancer invasion and metastasis. A model of the molecular mechanism for remote metastasis and invasion in association with MMPs has been proposed for the lung (Hiratsuka et al. 2002). In this model, vascular endothelial growth factor (VGEF) secreted by primary cancer cells induces the MMP9 expression specifically in lung endothelial cells and macrophages via vascular endothelial growth factor receptor (VEGFR) tyrosine kinase,
resulting in the formation of the cancer stem cell niche. This means that the cancer cells can produce their own favorable microenvironment, the future cancer stem cell niche, from a distance by secreting factors to influence the protein composition at that site.

This niche creation mechanism by cancer cells themselves has been verified for various other kinds of metastasis as well: the bone metastasis of prostate cancer has been shown to be supported by urokinase-type plasminogen activator (uPA) or prostate-specific antigen (PSA) secreted by the prostate cancer cells through altering the growth factors at the bone microenvironment, thus enhancing the proliferation of the osteoblasts that serve as the cancer stem cell niche (Logothetis and Lin 2005). Lung metastasis of breast cancer via secreted protein acidic and rich in cysteine (SPARC), osteonectin, or MMP2 is also found based on this mechanism (Minn et al. 2005).

Cells are not the only component of niche. The factors existing in the ECM can also be a part of the cancer mediator. In cancer stem cell in hypoxia, the oncogene MET is upregulated. MET binds to its ligand hepatocyte growth factor (HGF), which is found in the ECM, and enhances the transcription of plasminogen activator inhibitor type 1 (PAI-1) and cyclooxygenase 2 (COX2). This leads to enhanced blood coagulation and fibrin deposition. The fibrin deposition (fibrin nest) induces the vasculogenesis in the surrounding area, supporting the homing of cancer stem cell and its proliferation, thus serving as the cancer stem cell niche. The migration of the vascular vessel triggered by the fibrin nest also facilitates the metastasis to other sites (Boccaccio et al. 2005).

Kaplan et al. recently reported on a new type of cancer stem cell niche created by a third party. They demonstrated this mechanism using the lethally irradiated mice, transplanted with bone marrow-derived cell (BMDCs), followed by the lung cancer or melanoma cells. The transplanted BMDCs expressing VEGFR1 gathered at a site, such as lung, creating a group of cells. This microenvironment is highly receptive for cancer metastasis, and they call this microenvironment the “premetastatic niche” (Kaplan et al. 2005). The formation of premetastatic niche has been found for breast cancer, lung cancer, and gastrointestinal cancer in humans. The BMDCs creating the premetastatic niche are a type of hematopoietic progenitor expressing VLA-4 (integrin α4β1) and/or Id3 (inhibitor of DNA binding 3). The primary cancer cells secrete factors (such as cytokines) that positively regulate the expression of fibronectin (a ligand of VLA-4) of fibroblasts located at the site of future premetastatic niche, inducing the homing of VEGFR1-expressing BMDCs. The mice transplanted with VEGFR1-depleted BMDCs or treated with anti-VEGFR1 antibody did not show the formation of premetastatic niche or metastasis, proving the theory of this new metastatic mechanism induced by cancer cells involving the third party.

**Novel Cancer Therapy Targeting Cancer Stem Cell and Its Niche**

Most of the current anticancer drugs act as inhibitors of DNA synthesis or cell division of the cancer cell, consequently suppressing the growth of tumor. Therefore, the cancer cells must be in the cell cycle, not in quiescence, in order to
see the positive effect of chemotherapy. The cancer stem cells as well as normal stem cells are supposed to be in the quiescent state. Even if the tumor size is decreased after chemotherapy, it is simply because the regular cell-cycling cancer cells have been killed. One must expect the quiescent, tumorigenic cancer stem cells to survive the chemotherapy.

A report by Graham et al. proved the cancer stem cells’ insensitivity through the demonstration that most of the BCR-ABL expressing blood cells of patients with chronic myeloid leukemia arrested after the cell culture with addictive of STI571 (Gleevec®), except those in the quiescent state, namely the leukemic stem cells, survived and remained in quiescence (Graham et al. 2002). This suggests that degenerating the quiescent cancer stem cell is one of the most promising therapeutic methods in the next generation cancer treatments aimed at complete cure. Various attempts based on this concept have been investigated, including the alteration of cancer stem cell characteristics, manipulation of the niche environment to induce a resumption of the cell cycle, and differentiation of cancer stem cells. From the viewpoint of cancer stem cell control, complete inhibition of cell division of cancer stem cells, even if only minimally, may also have possibility.

HSC transplantation is routinely performed for patients with blood diseases, bone marrow diseases, or some types of cancer, such as leukemia. In transplantation, purified healthy HSCs derived from bone marrow, peripheral blood, or cord blood are injected intravenously into patient’s bloodstream. These HSCs are then homed to niche in bone marrow and resume the healthy hematopoiesis. Prior to transplantation, patients undergo conditioning with aggressive anticancer drugs and/or irradiation, which destroy their own hematopoietic system, including HSCs, and suppress the immune reactions. This way, the newly introduced healthy HSC can home to the empty niche in bone marrow and successfully reconstitute the recipient patient’s hematopoietic system. This conditioning regimen, however, is highly toxic and life-threatening, and patients who do not respond to anticancer drug and/or irradiation or with low tolerance due to poor general status should be excluded from this treatment.

Recently, Czechowicz et al. reported that treatment with an antibody that blocks the HSC function led to the clearance of niche, suggesting the possibility of mild but effective conditioning regimen (Czechowicz et al. 2007). They demonstrated that administration of ACK2, an anti-c-Kit antibody, led to rapid and transient removal of almost all endogenous HSCs in mice, and subsequent transplantation of purified HSCs led to as high as 90% of donor chimerism. Without conditioning, typical donor chimerism in recipient mice was 3%, perhaps due to the limited number of empty niche available for engrafting donor HSCs. Moreover, the HSCs derived from ACK2-treated mice showed significantly (>90%) reduced engraftment capability, compared to control. The another anti-c-Kit antibody, 2B8, did not show the effect observed with ACK2. Thus, the mechanism of HSC clearance was considered as the complete inhibition of c-Kit signaling in HSC by ACK2.

Unlike the case of human anti-VLA-4 antibody natalizumab, as described earlier, ACK2 does not have the mobilization effect, which was confirmed by the lack of HSC detected in spleen, liver, and peripheral blood after infusion. Instead, ACK2...
provides a short time window to allow donor HSC to graft by depleting the host HSC. This regimen may be an attractive alternative to the conventional conditioning regimen not only for those who should otherwise have been excluded in this medical procedure but also for tolerant patients.

Parthenolide (PTL) is a sesquiterpene lactone and major component in the herbal remedy feverfew. Recently, PTL was found to have a unique characteristic against tumors, including inhibition of DNA synthesis, cancer cell proliferation, and nuclear factor kappa B (NF-κB) activation, and increase intracellular reactive oxygen species (ROS). Guzman et al. successfully demonstrated that PTL induces stem cell–specific apoptosis among leukemic cells in primary acute myeloid leukemia (AML) and blast crisis chronic myeloid leukemia (bcCML) without affecting the normal hematopoietic cells (Guzman et al. 2005). This action was identified as a result of multiple effects of PTL, such as inhibition of upregulated NF-κB expression in tumor cells, proapoptotic activation of p53, and increased intracellular ROS.

Glioblastoma is considered one of the most malignant cancers, extremely proliferative, and resistant not only to chemotherapy but also to radiotherapy. The prognosis is very poor with the average survival duration less than a year. Piccirillo et al. showed a successful result for glioblastoma in attempting the novel therapy based on the above-mentioned concept. They discovered that the BMPs, especially BMP4, can induce the differentiation of the CD133+ glioblastoma cells, which are considered to be the cancer stem cells, and ultimately decrease of cancer stem cell pool (Piccirillo et al. 2006). Moreover, xenograft of human glioblastoma cells to mice showed significant suppression of the tumor growth and improvement of the survival rate when treated along with BMP4, which would have been 100% lethal otherwise. In this case, BMP4 or the other BMPs did not kill the glioblastoma cells but let them extinct through the induced temporary differentiation and proliferation by altering the stem cell characteristics. This positive experimental result should stimulate the exploration of the novel therapeutic method targeting a cancer stem cell or its niche, showing an approach for a breakthrough in treatment of otherwise abandoned cancer patients.

For the niche-targeting therapy, Calabrese et al. suggested that alternation of vascular niche is effective to brain tumor treatment. Medulloblastoma cells express high levels of erythroblastic leukemia viral oncogenic homolog 2 (ERBB2), and also VEGF, consequently. ERBB2-overexpressing mice bearing brain tumor were treated with either ERBB2 inhibitor erlotinib (Tarceva®) or anti-VEGF antibody bevacizumab (Avastin®). The treatment significantly inhibited the tumor vasculature and suppressed tumor growth. The cancer stem cell expressing Nestin and CD133, the self-renewing cancer cells, were not found in the mice after either treatment. Similar treatment was applied to mice with glioma, and the results showed a significant suppression of vasculogenesis and tumor proliferation, as well as the decrease of the Nestin+CD133+ cancer stem cell pool. The small number of surviving Nestin+CD133+ cancer stem cells were all found in association with the tumor vessels. These data collectively suggest that the suppression of the vascular niche by inhibiting the vasculogenesis has a potential for novel medulloblastoma or glioma treatment.
Conclusion

Stem cell and its niche influence each other to maintain their capabilities, control the fate, and ultimately sustain the healthy organ or tissue. Besides the interactions between stem cell and niche, some intrinsic factors of stem cell have been also revealed as a regulator of stem cell capacity. A good example is Bmi-1, a member of polycomb group of genes involved in maintenance of transcriptional repression of target genes. HSCs derived from Bmi-1-deficient mice exhibited defective self-renewal phenotype, while maintaining the multilineage differentiation potential. The microenvironment in Bmi-1-deficient mice was found intact. The forced expression of Bmi-1 led to the enhanced repopulation capacity in vivo and augmented expansion of the progenitors ex vivo. These data collectively suggest that the self-renewal capacity of HSCs is controlled by the intrinsic expression level of Bmi-1 (Iwama et al. 2005).

Stem cell regulation and maintenance, whether intrinsic or extrinsic, are not simple single-factor phenomena, and the large part of detailed molecular mechanism is yet to be elucidated. Understanding the regulatory system could lead to a novel approach for the effective therapeutic methods that directly target stem cells (normal or cancer) or niche. As described in this chapter, the latest drugs targeting the cancer stem cell or its microenvironment employ this state-of-the-art strategy and are expected to minimize the complications and improve the patients’ quality of life at the same time. The biology of stem cell and its niche is one of the most promising research fields of the next few decades, from both scientific and clinical viewpoints.

References


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