Stem Cell Niche: Microenvironment and Beyond*

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The multipotentiality and self-renewal ability of stem cells are controlled by intrinsic genetic pathways that are subject to regulation by extrinsic signals emanating from the stem cell niche. The stem cell niche provides a microenvironment composed of cellular structures or extracellular matrix in which stem cells are maintained as undifferentiated (1−6). The concept of “the stem cell niche” was first proposed in studies of the HSC2 (7); however, in vivo evidence of its existence was first shown in the Drosophila GSC (8−10). Over the past several years, there has been much progress made in identifying stem cell niches in different mammal tissues, including nerves, hair follicles, intestines, teeth, and BM (11−16). In this review, our focus is on comparing Drosophila GSC niches and mouse HSC niches (two of the best characterized niches). By such comparison, we hope to provide some common principles of stem cell niches that will be useful in other tissue stem cell niche studies.

In recent years, remarkable progress has been made in the identification and characterization of the stem cell niches in invertebrate systems (10, 17−22). In studies of GSCs in Drosophila, the ovary and testis provide attractive models for stem cell niche studies. In Drosophila ovary, cap cells, a specific type of somatic cell located at the tip of the germarium in the ovary, function as the niche for GSCs. An E-cadherin/β-catenin-formed cell-cell adhesion junction mediates the physical interaction between GSCs and their niche cells (23, 46), ensuring stem cell control by niche signals. Normally, asymmetric division of stem cells results in two daughter cells with different fates: one daughter cell attaches to the niche and is maintained as a stem cell (self-renewal), whereas the other daughter cell leaves the niche and develops into a cystoblast (1, 4). CBs undergo four incomplete cell divisions to form an interconnected 2−16-cells germ line cyst. In addition to the GSC, another type of cell called the ESC also attaches to cap cells interspersed between the GSCs. Together with cap cells, ESCs encapsulate GSCs to separate GSCs from their differentiated daughter cells. When the GSCs divide and detach from the niche to produce CBs and cyst cells, the ESCs also proliferate and differentiate to produce more escort cells. The escort cells expand and continue to encapsulate CBs and cysts during the process of cyst formation and are finally replaced by follicle cells after the 16-cells germ line cyst stage (Fig. 1a). Interestingly, the newly formed cysts (prior to the eight-cell cyst stage, while still encapsulated by the escort cell) can revert to the stem cell state under certain circumstances, suggesting that they may still retain (albeit limited) stem cell properties (47).

A similar niche is also found in Drosophila testis and is composed of hub cells located at the end of the testis (48). Male GSCs surrounding the hub cells are interspersed with CPs (which are counterparts of ESCs in the ovary). Both GSCs and CPs are attached to the hub cells through an adhesion junction. Similar to ESCs in the ovary, proliferation and differentiation of CPs always accompany proliferation and differentiation of GSCs. CPs in the testis produce cyst cells, which encapsulate the gonialblasts (the immediate daughters of male GSCs) during spermatogonium formation (Fig. 1b). The newly formed gonialblasts can also revert to stem cells under certain circumstances, resembling their counterpart cells (CBs and cysts) in the ovary (49).

The role of cap and hub cells in GSC regulation has been well studied, but the contribution of ESCs and their offspring in the ovary and CPs and their progeny in the testis to GSC self-renewal, proliferation, and differentiation is largely unknown. However, it is known that a dialogue between the two types of cells is required for the coordination during organogenesis (30). Cap and hub cells provide an attachment point for anchoring GSCs to the niche; they also produce signals that inhibit differentiation but promote self-renewal of stem cells (17).

The Complexity of HSC Niches in Mammals

The stem cell niches in mammals are very complicated because of the complexity of cellular components of mammalian tissues, even though the mechanism for niche regulation is conserved from invertebrate to vertebrate. In mammals, BM tissue is composed of more than eight different

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2 The abbreviations used are: HSC, hematopoietic stem cell; GSC, germ line stem cell; BM, bone marrow; CAR, CXCL12-abundant reticular; CBs, cystoblasts; ESC, escort stem cell; CPs, cyst progenitors; Ang-1, angiopoietin-1.
The Osteoblastic Niche on the Endosteal Surface

Accumulated evidence supports the existence of an osteoblastic niche in BM. The concurrence of hematopoiesis and osteogenesis in BM suggests a close relationship between bone-forming cells and hematopoietic cells. The two HSC niches, osteoblastic (34–41, 50–52) and vascular (41, 53), are found in adult BM tissue. The vascular niche might also exist in fetal hematopoietic tissues such as yolk sac, aorta-gonad-mesonephros region, placenta, liver, and spleen (54–56) as well as in adult spleen and liver. Both niches may be critical for HSC self-renewal. Cooperation between these two niches might be required for maintaining normal hematopoietic homeostasis and re-establishing hematopoiesis after injury.

It is worth pointing out that not all osteoblasts function as the HSC niche; we found that a subset of osteoblasts that express a high level of N-cadherin function as a key component of the osteoblastic niche (34). This is further supported by long-term in vitro functional studies demonstrating that an increase in N-cadherin expression in OP9 cells (a BM stromal cell line) by either retrovirus-transduced overexpression or Ang-1 induction enhances the ability of OP9 cells to support HSCs (36). The existence of an N-cadherin+ osteoblastic niche has also been confirmed in several other studies. Wilson and co-workers (70) found that the majority of HSCs/progenitors lodge in the endosteum and attach to N-cadherin+ osteoblastic cells 15 h post-transplantation. By analyzing inducible c-Myc knockout mice, they further demonstrated that HSCs with c-Myc mutation have increased N-cadherin expression and enhanced adhesion to osteoblastic niche cells and hence fail to release from the restrictive osteoblastic niche for activation, proliferation, and differentiation (70). HSCs residing in the osteoblastic niche are relatively stable during normal homeostasis but can be altered as a result of bone remodeling (72) or stress (71).

Recently, Kiel et al. (73) showed that HSCs lack N-cadherin expression. Our data show, however, that not only is N-cadherin expressed by HSCs, its expression is dynamically changed when HSCs are in different states. We found that HSCs expressing intermediate to low levels of N-cadherin can successfully reconstitute the entire hematopoietic system in irradiated mice but with the latter more robust. In addition, osteoclasts, which function in bone remodeling, have also been shown to regulate HSC migration and maintenance by clearing osteoblasts (72). However, depending on the severity of the mutation, a reduction in the number of osteoblasts may not compromise HSC function given that fewer HSCs would result in a reduced demand for osteoblastic niches. Nonetheless, mouse models such as UDP-galactose:ceramide galactosyltransferase-deficient (Cgt−/−) mice and Rb- and retinoic acid receptor-γ-deficient mice (71, 74, 75) have demonstrated that severe reduction in trabecular bone or osteoblast number does compromise HSC function, resulting in a reduced number of HSCs in BM (in Cgt−/− and Rb−/− mice) and/or mobilization and extramedullary hematopoiesis in the spleen, where the vascular niche is dominant, and thus setting the stage for developing into myeloid proliferative disorders (retinoic acid receptor-γ−/− and Rb−/− mice).

In BM, some of the molecules produced in the intercellular matrix of the osteoblastic niche may also be important for HSC lodging and maintenance. Osteopontin, a matrix glycoprotein produced by osteoblasts, is a negative regulator of the HSC osteoblastic niche that limits the size of the stem cell pool and may provide a mechanism for restricting excess stem cell expansion under conditions of niche stimulation (76). In addition, the high concentration of Ca2+ in the BM endosteal region mediates HSC homing and lodging in the osteoblastic niche by attracting HSCs through a calcium sensor receptor that is highly expressed in HSCs (52).

Sinusoidal endothelial cells isolated from hematopoietic tissue more strongly support hematopoiesis compared with microvasculature endothelial cells from other organs (53, 77–79). Kiel et al. (41) have demonstrated that HSCs attach to the endothelium of sinusoids in BM and spleen in mice after HSC mobilization and also attach to BM sinusoids during homeostasis. The discovery of a large number of long-term self-renewable HSCs in the vascular niche indicates that the BM and spleen vasculature provides a specific niche for HSC/progenitor expansion, proliferation, and differentiation under both homeostatic and stress conditions. The observation that quiescent HSCs detach from the osteoblastic niche and migrate toward the center of BM to the vascular region, where they re-establish hematopoiesis during hematopoietic regeneration after myeloablation (43, 53, 80), also suggests the existence of a vascular BM HSC niche. However, the fact that deletion of osteoblasts results in a reduction of HSCs in BM and extramedullary hematopoiesis (68) suggests that the vascular niche may provide a stimulator environment (81).

CAR Cells, a New Component of the HSC Niche Shared by Both Osteoblastic and Vascular Niches

CXCL12 is a chemokine that induces cell migration through interaction with its receptor CXCR4, which is expressed by the responsive cells. CXCL12/CXCR4 signaling plays multiple essential roles in embryonic development, including HSC colonization in BM during the transition of hematopoiesis from fetal liver to BM, B cell and myeloid cell development, and blood vessel formation, as demonstrated by mutant mice with targeted gene disruption (82). CXCL12/CXCR4 signaling plays important roles in HSC trafficking and HSC mobilization. Studies of CXCL12/green fluorescent protein knock-in mice (the green fluorescent protein gene knocked into the Cxcl12 locus) demonstrate that CXCL12 is expressed by a population of stromal cells scattered throughout BM (42, 44, 82). Among them, there is a population of CAR cells scattered throughout the trabecular spaces in the BM cavity. Interestingly, the majority of Slam-coded HSCs (CD155+CD48−CD41+) near the sinusoidal endothelium are in direct contact with CAR cells instead of endothelial cells on the extravascular surface. Moreover, HSCs located in the endosteal region near the bone surface are also found attached between CAR cells and osteoblastic cells (Fig. 2). The observation that HSCs attach to CAR cells in the endosteal and sinusoidal regions suggests that CAR cells might be an important component of both the osteoblastic and vascular niches in adult BM (42).

CXCL12/CXCR4 signaling also mediates the migration, homing, and growth of other hematopoietic progenitors as well as leukemia cells. Prepro-B (early B cell progenitor) cells are maintained in BM by adhesion to CXCL12-expressing cells (morphologically indistinguishable from CAR cells) and move away from these cells as they mature. Interestingly, after further development in the spleen, end-stage B cells (plasma cells) colonize BM and attach to CXCL12-expressing cells, as did their ancestral prepro-B cells. CXCL12/CXCR4 signaling is also required for megakaryocyte development and platelet production by inducing vascular accumulation of HSCs or progenitors (43, 44). CAR cells may also contribute to this process. The physical relationship of CAR cells with other hematopoietic progenitors, including multipotent, common myeloid, common lymphoid, and myeloid/erythroid progenitors, requires further investigation. However, CXCL12/CXCR4 signaling is also involved in BM metastasis and engraftment of malignant cells, including leukemia cells (83–86), myeloma cells (87), and tumor cells in other tissues (88–90). Thus, CAR cells and CAR cell-derived CXCL12/CXCR4 signaling are not HSC-specific.

Cooperation between the Osteoblastic and Vascular Niches Is Critical for Both Normal Homeostasis and Reconstitution of the Hematopoietic System after Injury

HSCs migrate between the osteoblastic and vascular niches in both normal and stress situations. Osteoblastic niche cells express several cell-signaling molecules such as BMP4, Jagged1, and Ang-1, which are important for HSC self-renewal, survival, and maintenance (35, 36). Effective osteoblastic niche signaling on HSCs is ensured by the close physical interaction of HSCs and osteoblastic cells. The N-cadherin/β-catenin adhesive junction and integrin-mediated cell adhesion facilitate HSC attachment to N-cadherin+ osteoblastic cells (34, 36), keeping HSCs in an environment with high concentrations of BMP4, Jagged1, and Ang-1, thus protecting HSCs from exposure to differentiation signals.

In the vascular niche, sinusoidal endothelial cells are known to support survival, proliferation, and differentiation of myeloid and megakaryocytic progenitors (43). HSCs in the vascular niche are exposed to blood-borne factors from peripheral blood such as hormones, growth factors, oxygen, and nutrients. Thus, HSCs in the vascular niche constantly sense signals and stimulation from the peripheral circulation and accordingly change constantly in self-renewal, proliferation, and/or differentiation.
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Therefore, the osteoblastic niche might serve as a reservoir for HSC storage, whereas the vascular niche provides an environment for HSC proliferation and differentiation to produce progenitors and mature blood cells.

Under normal homeostatic conditions, a portion of HSCs routinely mobilize and home (53, 91, 92); therefore, HSCs travel back and forth between the osteoblastic and vascular niches. We speculate that CAR cells might mediate this migration of HSCs between the two niches (81). In response to stress in BM, the number of mobilized HSCs dramatically increases and results in extramedullary hematopoiesis to fulfill the increased demand for blood cells. After recovery from stress, some HSCs in the vascular niche (such as in the spleen) may travel back to BM to restore the HSC pools in both the osteoblastic and vascular niches (92–94).

Future Prospects

The data from Drosophila GSC studies demonstrate that two types of stem cells are attached to the same niche cells. These two types of stem cells intimately interact with each other in the niche and maintain contact during proliferation and differentiation. This phenomenon, sharing of the same niche by two different types of stem cells, might also exist in mammals. In mouse hair follicles, both hair follicle epithelial stem cells and melanocyte stem cells are located in the bulge region of the hair follicle and accompany each other during the process of proliferation and maturation (95–97). The fact that two types of cells maintain close interaction throughout their development suggests the importance of cell coordination to facilitate organogenesis.

The role of CAR cells in HSC regulation is still not clear. Insight from the Drosophila GSC niche model is helpful in understanding HSC niches. We speculate that CAR cells might resemble escort cells in Drosophila ovary. CAR cells may play a critical role in mediating migration of HSCs between the osteoblastic and vascular niches. They may accompany HSCs and possibly some progenitors during proliferation and differentiation (Fig. 2). We still do not know the origin of CAR cells. Based on their morphology, CAR cells are more like the progeny of mesenchymal stem cells. Mesenchymal stem cells and their progeny have also been found to accompany HSCs during all stages of hematopoietic development, including aorta-gonad-mesonephros, fetal liver, and BM hematopoiesis (98, 99). It is highly possible that mesenchymal stem cells may also share the same niche with HSCs in BM. To better understand the roles of CAR cells in hematopoietic regulation, the following questions need to be addressed. 1) Are other hematopoietic progenitors (such as common lymphoid, common myeloid, granulocyte/macrophage, and myeloid/erythroid progenitors) also attached to CAR cells? 2) Do CAR cells form a relatively stable network through which HSCs and progenitors migrate, or do they dynamically change their location by accompanying the proliferating and differentiating HSCs and progenitors? 3) What happens to CAR cells upon HSC mobilization by growth factors (such as granulocyte colony-stimulating factor) or in response to stress (such as myeloablation)? It has been proposed that reduction of CXCL12 expression is one of the mechanisms leading to HSC mobilization after granulocyte colony-stimulating factor treatment and in stress situations (100, 101). Does applying a mobilization agent only induce reduction of CXCL12 expression, without affecting the other properties of the CAR cells, or does it also induce apoptosis or mobilization of CAR cells? 4) Besides CXCL12 expression, what other molecules expressed by CAR cells are involved in regulation of HSCs and progenitor cell properties? 5) Do leukemia stem cells also attach to CAR cells?

REFERENCES
