Chapter 3
Stem Cell Niche

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Abstract The adult stem cells, or tissue-specific stem cells, are essential for maintaining tissue homeostasis and commonly reside in specific local microenvironment named niche. The niche keeps stem cells in multipotent/unipotent state and prevents them from precocious differentiation, and in some cases, aligns them and promotes asymmetric division to produce differentiated progenies for tissue regeneration. The niches employ a variety of factors including cell adhesion molecules, extra cellular matrix, growth factors and cytokines in a tissue-specific manner to regulate the resident stem cells. Stem cells in turn may also contribute to niche integrity and function. Continuous elucidation of stem cell niche regulation at the cellular and molecular level would help understanding tissue homeostasis and disease mechanisms, and may also provide useful strategies for therapeutic application of stem cells.

3.1 Introduction

Unlike embryonic stem cells, which possess the innate self-replicating capacity (Ying et al. 2008), the maintenance of most adult stem cells, if not all, requires stimuli from specialized local microenvironment, or niche. Dynamic interactions between niches and stem cells govern tissue homeostasis and repair under physiological and pathological conditions throughout life. Deregulation of the stem cell niches has been implicated in many diseases, including aging, cancer and degenerative diseases (Voog and Jones 2010).

The stem cell niche hypothesis was initially put forward by Schofield, who proposed that the maintenance of stem cells requires association with a complement

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of cells, a 'niche' (Schofield 1978). However, it was not fully appreciated until studies in the model organisms, *Caenorhabditis elegans* and *Drosophila melanoganster*, demonstrated that the supporting stromal cells are important for the maintenance and self-renewal of germline stem cells. Subsequently, as new techniques and tools for charactering stem cells in vivo are accessible, the stem cell niches are accompanyingly identified and characterized in many mammalian tissues. Because stem cells are usually regulated by both cellular niche cells and non-cellular components, the stem cell niche is currently defined as the local tissue microenvironment that houses and maintains stem cells (Morrison and Spradling 2008).

Studies on both invertebrate and vertebrate stem cell niches in a variety of tissues revealed some principles of their functions. The stem niche controls stem cell self-renewal and prevent their precocious differentiation by secreting signaling molecules or cell-surface ligands, and anchors stem cells in place by utilizing cell adhesion molecules or the extracellular matrix. The niche also frequently positions stem cells in a way facilitating their asymmetric cell divisions, so that after each cell division, one daughter will remain aside the niche to continue self-renewing, while the other daughter will leave the niche and differentiate. Because of the intimate relationships between stem cells and their niches, mimicking the in vivo microenvironment could also help stem cell with in vitro expansion and functional integration into damaged tissues for future stem cell-based therapies. Thus, a comprehensive understanding of the molecular mechanism underlying the niche function not only contributes to our understanding of tissue homeostasis control and diseases, but also helps to put a step forward for the clinical application of stem cells.

Owing to advantages in simple tissue structure and availability of sophisticated genetic tools, studies in simple model organisms such as *Drosophila melanoganster* have pioneered our understanding of the niche, with clear demonstration of cellular composition and molecular basis of physical interaction and signaling regulation in the stem niches. Although adult stem cells in mammals are usually difficult to identify due to tissue complexity, with the identification of more reliable stem cell markers and endeavors of many researchers, tremendous progresses have also been made for adult stem cell niches in mammals. In the following parts, some examples of the best studied stem cell niches from invertebrates to mammals are introduced, with emphases on the structural composition and molecular functions. Subsequently we summarize the general features of the stem cell niche and discuss future challenges and clinical perspective on the stem cell niche.

3.2 *C. elegans* Germline Stem Cell Niche

The principle of cell-cell interaction in controlling stem cell behavior was first described in the worm gonad in early 1980s. In the *C. elegans* hermaphrodite gonad, there is one somatic cell at the distal end known as the distal tip cell (DTC). Germline stem cells (GSCs) are localized within the mitotic germ cell region close to the DTC tip (Fig. 3.1a). Moving along the distal-proximal axis, germ cells gradually switch

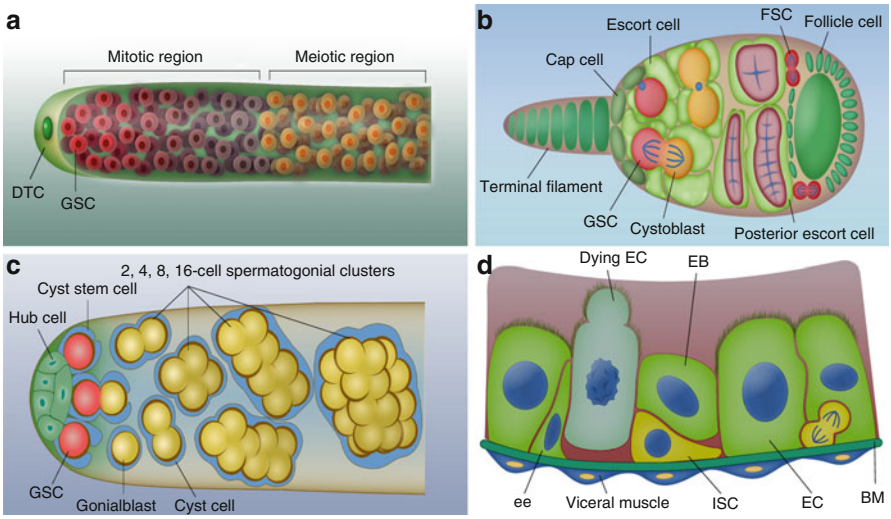

Fig. 3.1 The anatomy of *C. elegans* and *Drosophila* stem cell niches. (**a**) *C. elegans* germline stem cell (GSC) niche. GSCs are located in the mitotic region (*red*). The distal tip cell (DTC) (*green*) provides both physical support and signaling instructions to maintain GSCs. (**b**) *Drosophila* ovarian GSC and follicle stem cell (FSC) niches. Cap cells together with terminal filament and escort cells constitute the ovarian GSC niche. Cap cells anchor the GSCs by forming adherens junctions, and produce instructive signals to maintain GSCs. Daughter cells of GSCs positioned outside the GSC niche are differentiating cystoblasts. Two FSCs at the mid region of the germarium are responsible for the generation of the follicle cells that encapsulating the developing germline cysts. FSCs are in contact with the neighboring posterior escort cells and underlying basal lamina. (**c**) *Drosophila* male GSC niche. The male GSC niche is composed of hub cells and cyst stem cells. Similar with the ovarian counterparts, male GSC daughter cells positioned outside the niche become differentiating gonialblasts, which subsequently undergo four rounds of transit amplifying divisions with incomplete cytokinesis, generating 16-cell spermatogonial clusters. (**d**) *Drosophila* intestinal stem cell (ISC) niche. ISCs in the midgut are directly associated with a thin layer of basement membrane. The underlying visceral muscle secretes multiple signaling molecules to regulate ISC maintenance. The dying ECs may produce mitogens to stimulate ISC proliferation in response to various damage. *EB* enteroblast, *EC* enterocyte, *ee* enteroendocrine cell. Art works in this and subsequent figures are provided by Ning Yang

from mitosis to meiosis and subsequently develop into functional gametes (Kimble and Crittenden 2007). DTC is crucial for maintaining GSCs, because laser ablation of DTC causes GSC elimination, as GSCs are switched from mitosis to meiosis and subsequently differentiate. Also, when the location of male DTC was genetically manipulated, the axis of the gonad was disrupted and ectopic mitotic germ cells were formed around the mislocalized DTC (Kimble and White 1981). These data demonstrate that DTC is both necessary and sufficient for the maintenance of GSCs. Interestingly, the DTC sends short processes to encapsulate distal-most germ cells and long processes extending as many as 30 germ cells (Crittenden et al. 2006), which might provide a unique physical environment to support a pool of stem cells by a single niche cell.

The DTC controls GSC self-renewal via GLP-1/Notch signaling pathway (Crittenden et al. 1994; Henderson et al. 1994). The two DSL ligands LAG-2 and APX-1 are expressed in the DTC (Nadarajan et al. 2009), while the Notch-like receptor GLP-1 is expressed in germ cells in the mitotic region. Disruption of GLP-1/Notch signaling results in stem cell loss, whereas GLP-1 gain-of-function mutation leads to GSC overproliferation (Austin and Kimble 1987; Berry et al. 1997; Lambie and Kimble 1991). Activation of GLP-1/Notch signaling in GSC leads to the expression of downstream target fbf-2, which in turn represses the expression of differentiation-promoting genes including GLD-1, 2 and 3 (Byrd and Kimble 2009; Crittenden et al. 2002; Eckmann et al. 2004; Kimble and Crittenden 2007; Suh et al. 2009).

A body of knowledge has been acquired regarding the mechanisms regulating the DTC formation and maintenance. Briefly, the DTC is descended from somatic gonadal progenitor cell (SGP) through asymmetric division. The Wnt/β-catenin asymmetric (WβA) pathway plays central role in DTC specification. Activation of WβA pathway promotes the DTC fate through upregulating the expression of its direct target *ceh-22* (Lam et al. 2006). By contrast, NHR-25 represses the DTC fate by antagonizing WβA pathway (Asahina et al. 2006). In addition, the HLH-2/daughterless transcription factor is implicated in the DTC specification as well as maintenance (Chesney et al. 2009; Karp and Greenwald 2004). Of note, both WβA pathway and *ceh-22* are required and sufficient to specify the DTC fate. Loss of WβA pathway or *ceh-22* results in loss of the DTC, while over-activation of WβA pathway or *ceh-22* produces extra DTCs (Kidd et al. 2005; Lam et al. 2006; Siegfried et al. 2004; Siegfried and Kimble 2002).

3.3 Stem Cell Niches in *Drosophila*

3.3.1 Germline Stem Cell Niche in the Drosophila Ovary

The anatomic structure of the *Drosophila* gonad is well defined. The female and male GSCs can be reliably identified in vivo by their localization and by specific cellular markers, and remain accessible to sophisticated genetic manipulations. Consequently, they serve as excellent model systems to study niche regulation of stem cells. In fact, the molecular mechanisms of *Drosophila* GSC-niche regulation are among the best studied and have provided a conceptual framework for the niche study in mammalian systems.

In the Drosophila ovary, GSCs can be identified by their anterior-most location in the germarium and the presence of a unique organelle named spectrosome. In each germarium, five to ten terminal filament (TF) cells, four to six cap cells and GSC-contacting escort cells constitute the female GSC niche that houses two or three GSCs (Fig. 3.1b). Normally, GSCs undergo asymmetric divisions. Upon each division, one daughter remains within the niche and adopts the GSC fate, while the other daughter is positioned outside the niche and invariably differentiates into a cystoblast (CB), which will commit four rounds of incomplete mitosis to generate a 16-cell cyst and ultimately a new oocyte.

The cap cells are the principal component of the GSC niche (Xie and Spradling 2000), which anchor GSCs by forming DE-cadherin-mediated adherens junctions between the GSCs and the cap cells (Song et al. 2002). Loss of this adhesion would cause GSCs to leave their niche and differentiate. In addition to the role in physical support, the cap cells also provide signals that are essential for GSC maintenance. They secrete BMP family ligands Dpp and Gbb, which locally activate receptors on GSCs and suppress the expression of a differentiation-promoting gene, *bag of marbles* (*bam*). In cystoblasts, the BMP signaling activity diminishes, which results in the release of *bam* repression and the initiation of differentiation. BMP signaling is required for GSC maintenance, as compromised BMP signaling pathway transduction in GSCs causes their precocious differentiation. Dpp overexpression is also sufficient to stimulate GSC self-renewal and block GSC differentiation, leading to the accumulation of GSC-like cells in the ovariole (Chen and McKearin 2003; Song et al. 2004; Xie and Spradling 1998). GSC-contacting escort cells are also an important component of GSC niche, as blockade of JAK/STAT signaling in escort cells results in loss of GSCs (Decotto and Spradling 2005). In addition, unpaired (Upd) produced from TF cells activates JAK/STAT signaling in cap cells and escort cells, leading to augmented expression of Dpp (Lopez-Onieva et al. 2008; Wang et al. 2008). Therefore, TF cells also contribute to the niche.

Much progress has been made in understanding how niche controlled BMP signaling activity is restricted to GSCs. That has been reviewed somewhere else (Chen et al. 2011; Losick et al. 2011). Briefly, JAK/STAT signaling seems to be necessary and sufficient for dpp expression in cap cells, while Lsd1 inhibits dpp expression in escort cells, as knockdown of Lsd1 in escort cells augments dpp transcription (Eliazer et al. 2011). In addition, the heparin sulfate glycoprotein Dally, and the type IV collagen Viking are required to restrict diffusion of Dpp outside the niche (Guo and Wang 2009; Hayashi et al. 2009; Wang et al. 2008). Moreover, the serine/threonine kinase Fused, together with the E3 ligase Smurf direct the degradation of BMP receptor Thickvein (Tkv) in CBs, allowing for CB differentiation (Xia et al. 2010).

The niche function also requires Yb and Piwi, which are required in the somatic niche cells to maintain GSCs (Cox et al. 1998; King and Lin 1999). GSCs also send signals to the niche to regulate niche function. Delta, the ligand for the Notch pathway, is specifically expressed in the germ cells, and activates Notch in the niche cells for their specification during the development for their maintenance during adulthood (Song et al. 2007; Ward et al. 2006).

3.3.2 Follicle Stem Cell Niche in the Drosophila Ovary

In each germarium, two follicle stem cells (FSCs), which generate follicle cells to envelop the developing germ cells, are located near the boundary between the 2A and 2B regions (Nystul and Spradling 2007) (Fig. 3.1b). So far there is no reliable cellular marker to identify FSCs. It has been suggested multiple signal molecules produced from the TF/cap cells, including Hedgehog (Hh), Wingless (Wg) and Dpp, are all required for the long-term maintenance of FSCs, indicating that these

signaling pathways function cooperatively to regulate FSC behavior (Forbes et al. 1996; Kirilly et al. 2005; Song and Xie 2003; Zhang and Kalderon 2001). Therefore, the GSC niche also functions as a part of the niche for FSCs.

Apart from that, FSC-contacting posterior escort cells located near the region 2A/2B border could be an essential component of the FSC niche as well. Escort cells do not turn over regularly and do not move along with cysts at the junction of 2A and 2B region (Morris and Spradling 2011). In addition, E-cad and Armadillo/β-catenin enriched at the junctions between FSCs and its adjacent cells are required for the maintenance of FSCs (Song and Xie 2002), suggesting adherens junctions anchor FSCs to the escort niche cell. Besides, integrin-mediated FSC anchoring to the basal lamina is also required for the long-term maintenance of FSCs (O'Reilly et al. 2008), suggesting that extracellular matrix is a critical component of the FSC niche.

Although it is poorly understood how these extrinsic niche signals act on FSCs to regulate their self-renewal, some intrinsic factors have been identified to be involved in this process. The ATP-dependent remodeling factor Domino (DOM) is required for FSC self-renewal (Xi and Xie 2005), while two polycomb genes Psc and Su(z)2 function redundantly and necessarily in FSCs for their differentiation. Loss of Psc and Su(z)2 ultimately leads to neoplastic tumor (Li et al. 2010). Further studies would provide more profound insights into the fundamental yet intricate mechanisms by which the niche signals link to intrinsic factors for the control of FSC self-renewal.

3.3.3 Germline Stem Cell Niche in the Drosophila Testis

The male GSC niche is also well-studied in Drosophila. A cluster of somatic cells (which form a hub) are located at the anterior tip of the testis and serve as the niche for both GSCs and the cyst stem cells (CySCs, or cyst progenitor cells) (Fig. 3.1c). About 8–10 GSCs reside around each hub, and each GSC is encapsulated by two CySCs. After each asymmetric division, the GSC produces a new GSC that remains in contact with the hub and a differentiating daughter namely gonialblast, which is positioned outside the niche and subsequently undergoes four rounds of transit amplifying divisions with incomplete cytokinesis, generating a 16-cell spermatogonial cluster. Spermatogonia further differentiate into spermatocytes which undergo meiosis and ultimately produce sperms. GSCs and gonialblasts contain a spectrosome as their counterparts in the ovary, while differentiated germ cell clusters have a branched fusome. The CySC divides coordinately with GSC division to produce a pair of cyst cells which enclose the differentiating gonialblast.

The activation of JAK/STAT signaling by the hub cells secreted ligand Upd was initially suggested to be necessary and sufficient for both GSCs and CySCs self-renewal (Kiger et al. 2001; Tulina and Matunis 2001). However, intrinsic activation of JAK/STAT signaling pathway in GSC alone stimulates the expression of DE-cadherin, which mediates GSC adhesion to hub cells, but is not sufficient to promote GSC self-renewal (Leatherman and Dinardo 2010). It turns out that activation of JAK/STAT signaling in CySCs induces the expression of zfh-1, which

stimulates the expression of BMP ligands Dpp and Gbb. BMP signaling activation in GSCs represses the transcription of differentiation-promoting factor *bam* and ultimately leads to GSCs self-renewal away from the hub cells (Kawase et al. 2004; Leatherman and Dinardo 2008). Therefore, in addition to the hub, CySCs may also be important components of the male GSC niche.

Like the ovarian counterpart cap cells, hub cells also express BMP ligand Gbb and Dpp. In addition, the male GSC niche also utilizes ECM to restrict BMP ligands diffusion. Dally-like instead of Dally is involved in this process (Hayashi et al. 2009).

The hub is derived from somatic gonadal precursors (SGPs) in the embryonic gonad. Notch and EGFR signaling have been implicated in hub cell specification. Notch signaling promotes hub specification, while EGFR signaling acts antagonistically with Notch to suppress hub differentiation (Kitadate and Kobayashi 2010). Interestingly, CySCs shares a common precursor with hub cells and can contribute to hub replenishment under certain circumstances, highlighting the dynamic nature of stem cell-niche relationship (Dinardo et al. 2011; Voog et al. 2008).

Studies in the male GSC niche also provide insights into the mechanisms of spindle orientation for asymmetric division of stem cells. The centrosome is replicated during interphase, and during mitosis, the mitotic spindle is mostly perpendicular to the hub-GSC interface. DE-cadherins could act through membrane-bound β-catenin and adenomatous polyposis coli (APC) to anchor the spindle pole (Yamashita et al. 2003). Interestingly, the mother and daughter centrosomes are asymmetrically inherited after mitosis by the two daughters of one stem cell, as the mother centrosome is always inherited by the daughter retaining stem cell fate (Yamashita et al. 2007).

3.3.4 Intestinal Stem Cell Niche in the Drosophila Midgut

The *Drosophila* gastrointestinal tract shows a high similarity to the mammalian intestine in development, cell composition and physiological function. In addition, the *Drosophila* intestinal epithelium is also maintained by multipotent intestinal stem cells (ISCs) (Micchelli and Perrimon 2006; Ohlstein and Spradling 2006). The epithelium is composed of a layer of cells projecting to the gut lumen, with highly organized apical-basal polarity. The ISCs, the only epithelial cells that are competent to undergo mitosis, reside at the basal surface of the epithelium and directly contact with the basement membrane (BM) composed of ECM, which separates the gut epithelium with the surrounding visceral muscles. An ISC undergoes asymmetric division to produce two daughters with one retaining ISC fate and the other undergoing differentiation. The differentiated daughter, named enteroblast (EB) will differentiate into either an absorptive enterocyte (EC) or a secretory enteroendocrine (ee) cell (Fig. 3.1d). Notch signaling plays a critical role in the cell fate determination of intestinal cell linage (Micchelli and Perrimon 2006; Ohlstein and Spradling 2006). ISCs specifically express a Notch ligand Delta (Dl), which actives Notch in the EBs and promotes them to differentiate into ECs or ee cells. The expression level of Dl in the ISCs is variable from one ISC to another. It is believed

that the high Dl level activates Notch at a high level in EB to promote its differentiation towards EC fate, whereas the low Dl level activates Notch at a low level to allow EB to differentiate toward ee fate (Ohlstein and Spradling 2007).

ISCs do not directly contact with any fixed stromal cells. The underlying visceral muscle is proposed to be a major component of the ISC niche. Wingless is the first identified molecule produced by the niche, which is able to traverse through the BM and activates the canonical Wnt signaling pathway in ISCs to regulate their long-term maintenance and proliferation (Lin et al. 2008). The visceral muscle also expresses Unpaired (Lin et al. 2009), the ligand of JAK/STAT pathway, and Vein (Biteau and Jasper 2011; Buchon et al. 2010; Jiang et al. 2010; Xu et al. 2011), the ligand for EGFR, which respectively activate JAK/STAT and EGFR/Ras signaling in ISCs to regulate ISC maintenance and proliferation. Recently, the Drosophila insulin-like peptides, dILP3, was found to be produced by the visceral muscle cells as well, which activates ISCs and expands ISC population to promote adaptive growth of intestine in response to nutrition availability (O'Brien et al. 2011). It is noteworthy that activation of any one of Wingless, JAK/STAT or EGFR signaling pathway alone in ISCs is not sufficient to completely block ISC differentiation (Lee et al. 2009; Lin et al. 2009; Xu et al. 2011). Therefore, the self-renewal of ISCs is likely controlled by a cooperative action of multiple signaling pathways. Several JAK/STAT and EGFR ligands, such as Upd3, Spitz and Karen, could also be detected in epithelial cells, including ISCs, progenitor cells and ECs (Beebe et al. 2009; Biteau and Jasper 2011; Jiang et al. 2009, 2010; Lin et al. 2009; Liu et al. 2010; Xu et al. 2011), especially under stress conditions (Buchon et al. 2009, 2010; Jiang et al. 2010), suggesting that non-stem cells in the intestinal epithelium could also contribute to niche function. The diverse and dynamic expression of those maintenance signals suggest that the niche function can be dynamically regulated in co-ordinance with environmental changes.

3.4 Stem Cell Niches in Mammals

Increasing evidence suggests that adult stem cells in mammals are also housed and maintained by the niches, although most of the tissue-specific stem cell niches have not been rigorously verified largely due to their associated tissue complexity. In addition to the common scenarios regarding the functional relationships between the stem cells and the stem cell niches, there could be distinct mechanisms uniquely exploited in mammalian stem cells but not stem cells in invertebrate. For example, the invertebrate stem cells are usually mitotically active. In contrast, the mammalian adult stem cells are often in a relatively quiescent state. In many cases, there seems to be two populations of stem cells with distinct niche locations: quiescent and active stem cells. In the following parts, some examples of the best studied mammalian stem cells and their associated niches are described and discussed, focusing on the physical composition and signaling interactions within the stem cell niches.

3.4.1 The Hematopoietic Stem Cell Niche

As mentioned before, the niche hypothesis was first proposed based on studies on the rodent hematopoietic stem cell (HSC) system several decades ago, although the exact location of the HSCs in the bone marrow (BM) had been a mystery. Until recent years, considerable progresses have been made to understand the HSC niche in the BM. The current view is that there are two HSC niches within the BM, the osteoblastic niche on the endosteal surface and the vascular niche of sinusoid endothelial cells (Fig. 3.2a).

3.4.1.1 The Osteoblastic Niche

Before the in vivo HSC niche was characterized, a series of in vitro studies showed that osteoblastic cell lines were capable of supporting primitive hematopoietic cells for a long term in ex vivo culture systems (Taichman et al. 1996). These observations provided an important hint for finding the HSCs niche in the BM. Osteoblastic cells were first demonstrated to participate in HSC regulation in vivo by two simultaneous studies working with different engineered mouse models (Calvi 2003; Zhang 2003). Both cases of genetic manipulation of the mouse models induced an increase in the number of osteoblasts and trabecular bone, and the number of HSCs increased accompanyingly. Consistently, ablation of osteoblasts by expression thymidine kinase specific in the osteoblasts leads to a decrease of primitive hematopoietic cells in the BM and an increase of extrameduallary hematopoiesis (Visnjic et al. 2004). It is noteworthy that only N-cadherin+ osteoblasts are associated with HSCs (Zhang 2003). However, N-cadherin is not required for HSC maintenance as loss of N-cadherin does not lead to HSC depletion or defective hematopoiesis (Kiel et al. 2009).

There are additional molecules produced by the osteoblasts that have been implicated in the regulation of HSCs, such as Angiopoietin-1, Thrombopoietin, Osteopontin (Opn), and CXCL12 (also called SDF-1). Angiopoietin-1 and Thrombopoietin interact with their receptors (Tie-2 and MP1 respectively) expressed on the HSCs to maintain HSC quiescence (Arai et al. 2004; Yoshihara et al. 2007). Opn, a glycoprotein, negatively regulate HSC proliferation and the size of the HSC pool, perhaps via interaction with integrins and CD44 (Nilsson et al. 2005; Stier et al. 2005). CXCL12, a chemokine that activates the receptor CXCR4 in HSCs, is also important for HSC quiescence and maintenance in the BM (Nie et al. 2008; Sugiyama et al. 2006a). CXCL12 is also expressed in other non-osteoblast cells, including endothelial cells, and a subset of reticular cells scattered in the BM. Thus, these cells may also play a role in the BM niche (Sugiyama et al. 2006a). The Wnt signaling may also regulate HSC quiescence, as osteoblast-specific overexpression of the canonical Wnt inhibitor Dikkopf1 (Dkk1) results in HSC activation (Fleming et al. 2008), although the requirement of Wnt signaling has not been directly demonstrated.

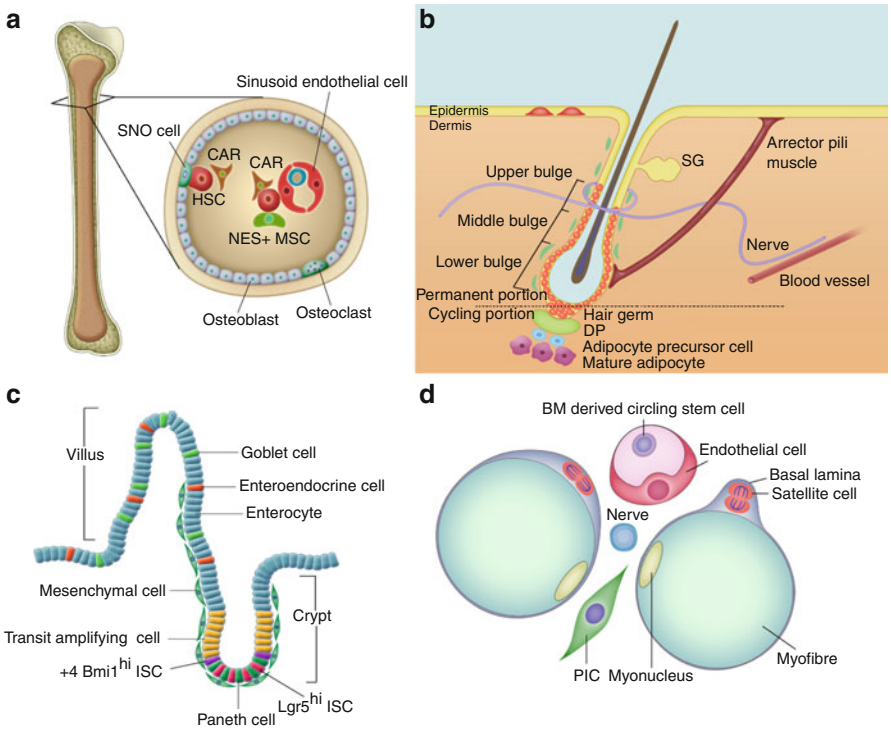

Fig. 3.2 The anatomy of mammalian stem cell niches. (**a**) Hematopoietic stem cell (HSC) niche. HSCs in the bone marrow reside in two niche locations: at the endosteal surface associate with spindle-shaped N-cadherin^{+}CD45^{-} osteoblastic (SNO) cells, and at the microvasculature associated with sinusoid endothelial cells and mesenchymal stem cells (MSCs) expressing Nestin. HSCs at both regions are frequently associated with CXCL12-abundent reticular (CAR) cells. (**b**) Stem cell niches in skin. A diagram of hair follicle (HF) in telogen. In the epidermis, stem/progenitor cells are located in the basal layer and differentiate into suprabasal cells. The basement membrane separates basal layer from the underlying dermis. The HFSCs reside in the bulge region below the sebaceous gland (SG). The mesenchymal dermal papilla (DP) and adipocyte lineages are crucial for follicle stem cells maintenance and activation. The upper bulge is wrapped by sensory nerve fibers, which release Sonic hedgehog (Shh) to induce Gli1 expression in adjacent upper stem cells. The activation of Hh pathway is essential for the upper stem cells to gain the potential to become epidermal stem cells during wound healing. (**c**) Intestinal stem cell (ISC) niche in the small intestine. Bmi1^{hi} ISCs are located at the +4 position from the crypt bottom and contact with paneth cells and transit amplifying cells. Lgr5^{hi} ISCs are located at the crypt bottom and surrounded by paneth cells which form the niche for Lgr5^{hi} ISCs. A hierarchy between Bmi1^{hi} ISCs and Lgr5^{hi} ISCs has been suggested recently. (**d**) Muscle stem cell niche. Two types of muscle-resident stem cells have been described. Satellite cells are located beneath the basal lamina and are in contact with myofibers. They could undergo planar symmetric divisions and apical-basal asymmetric divisions. The recently identified muscle stem cells – PW1+Pax7- interstitial cells (PICs) are located between myofibers. Both PICs and bone marrow-derived cells are able to generate functional satellite cells during regeneration

3.4.1.2 The Vascular Niche

Increasing evidence indicates that the vasculature in the BM may also serve as the HSC niche. Multiple cell types have been reported to make up the HSC vascular niche. A simple combination of three SLAM family receptors is found to be able to specifically distinguish the stem and progenitor cells and thus make it possible to detect the HSC niche in tissue section (Kiel et al. 2005). With the help of these new markers, many of the hematopoietic stem/progenitor cells (HSPCs) were found to be mainly located in the perivascular region. Consistently, an in vivo imaging study revealed that after transplantation, the labeled primitive hematopoietic cells could home to SDF-1–rich subdomains of microvessels in the bone marrow, where they persisted and increased in number over time (Sipkins et al. 2005). These studies suggest the perivascular region could serve as the HSC niche. VEGFR2 and VEGFR3 are expressed in sinusoidal endothelial cells (SECs), but not smooth-muscle-invested arterioles or osteoblasts. VEGFR2 is not required for normal HSC homeostasis. However, upon severe myelosuppressive damage, VEGFR2-mediated SEC regeneration is critical for HSC engraftment and reconstitution (Hooper et al. 2009).

Recently, a population of nestin-expressing (NES+) mesenchymal stem cells (MSCs), which are exclusively distributed in perivascular region, has been identified to act as an unique niche of bone marrow HSC. NES+ cells are physically associated with HSCs and express multiple HSC maintenance genes including CXCL12 and Angiopoietin-1. In vivo ablation of NES+ MSC cells leads to significant reduction of long term HSCs (LT-HSCs) number (Mendez-Ferrer et al. 2010).

Additionally, CXCL12-abundant reticular (CAR) cells are the major source of CXCL12. And most HSCs near endosteum or the sinusoidal endothelium, if not all, are in contact with CAR cells (Sugiyama et al. 2006b). Selective ablation of CAR cells cause reduction of HSCs number by approximately 50% and HSCs become more quiescent, suggestive of CAR cells as an essential HSC niche component (Omatsu et al. 2010). Both CAR cells and NES+ MSCs are competent to differentiate into adipocytes and osteoblasts, suggesting that there may be some overlap between these two cell types.

Therefore, the HSC pool in the BM could be divided into two subpopulations or states: the quiescent population, which is inactive and functions as a potent reservoir for the long-term maintenance of HSCs, and the active population, which is highly proliferative and responsible for the daily regeneration. The HSCs in the osteoblastic niche are BrdU retaining cells, and the signals from the osteoblastic niche usually regulate the quiescence of the HSCs. In contrast, the majority of HSCs identified by the SLAM markers are mitotically active (Kiel et al. 2005). These observations lead to a proposal that the osteoblastic niche and the vascular niche could function to support quiescent (reserved) and activated HSCs, respectively (Zhang and Li 2008).

3.4.2 Skin Stem Cell Niche

The mammalian skin, which is under constant turnover, serves as a physical barrier to protect the body from many environmental stresses such as bacteria infection, dehydration and UV-irradiation. The epidermis appendages such as hair follicles, nail, oil and sweat glands endow additional sophisticated functions to the body. The epidermis is comprised of stratified layers of progenitors and differentiated cells, and the stem cells or progenitors are believed to reside in the basal layer above the dermis (Fuchs 2009; Watt 1998) (Fig. 3.2b). Attached to the BM that separates epidermis from dermis, the basal cells can undergo asymmetric division to generate suprabasal spinous cells, which subsequently move upward and became enucleated and finally shed from the body. Notch signaling, p63 and microRNAs are important for the basal-to-suprabasal switch of the progenitor cell (Blanpain and Fuchs 2006; Moriyama et al. 2008; Yi et al. 2008).

The skin with hair can be divided into the following structural units: each with a hair follicle (HF), sebaceous gland (SG) and interfollicular epidermis (IFE). Sequentially down from the SG is the bulge where stem cells reside, outer root sheath, inner root sheath, hair shaft, transit amplifying matrix cells that envelop a group of mesenchymal cells, and dermal papilla (DP) (Fig. 3.2b). The adult HF constantly undergoes rounds of degeneration (catagen), rest (telogen) and growth (anagen), known as hair cycle. HF stem cells (HFSCs) provide the source of proliferation during anagen. In the destructive catagen phase, the matrix cells undergo programmed cell death and bring up the DP to the position that is underneath the (secondary) hair germ, the early progenies of bulge stem cells. The DP plays an inductive role in maintaining HFSCs in quiescent state and competent for the next cycle of growth (Blanpain and Fuchs 2006) (Fig. 3.2b). Normally, HFSCs do not contribute to the maintenance of SG and IFE. However, during the repairing process after wounding, they can regenerate the damaged epidermis and SG. HFSCs can be divided into two populations based on their location with the basal lamina: basal and supra-basal populations. These cells differ in their expression signatures, but both populations are able to self-renew in vitro and share the same differentiation potential (Blanpain et al. 2004).

The epithelial-mesenchymal interactions are important to regulate HFSCs (Blanpain and Fuchs 2009). Among the signaling pathways, Wnt and BMP are the most intensively studied. From embryonic HF initiation to adult stem cell self-renewal and differentiation, Wnt signaling plays multiple important roles during these processes. Loss of β-catenin, which complexes with TCF/LEF transcription factors to activate Wnt-response genes, completely blocks HF formation, while overexpression of an activated form leads to de novo HF morphogenesis (Gat et al. 1998; Huelsken et al. 2001). Elegant genetic and mathematical modeling show that Wnt ligands and the inhibitor Dkks pattern the HF spacing by a reaction–diffusion mechanism (Sick et al. 2006). In adult HF, β-catenin nuclear accumulation correlates with the transition from telogen to anagen, indicating the important roles of Wnt signaling in regulating stem cell self-renewal (Lowry et al. 2005). Wnt/beta-catenin signaling activities are also detected during matrix cell differentiation towards hair shaft (DasGupta and Fuchs 1999), and LEF1 rather than TCF3 in the bulge are required

for matrix cell differentiation. Despite these prominent roles, the source of Wnt ligands is difficult to probe, as there are dozens of Wnts in mammals with some expressed in the epithelium, yet others in the mesenchyme (Reddy et al. 2001). The BMP pathway has long been known for its inhibitory effects on HF morphogenesis and adult HFSC proliferation (Blessing et al. 1993; Botchkarev et al. 1999). The mesenchyme produces a balanced level of BMP ligands and the antagonist noggin (Blanpain and Fuchs 2009). In activating the BMP receptor BMPR1a in HF epithelium leads to enhanced cycling of HFSCs and impaired differentiation (Kobielak et al. 2007). Other signaling pathways such as hedgehog and Notch are also involved in either regulating HF proliferation or differentiation (Blanpain and Fuchs 2009).

Recently, it has been found that sensory nerves regulate stem cell function in the upper bulge by producing Sonic hedgehog (Shh), which induces expression of Gli1 expression in adjacent stem cells. Gli1+ cells have the potential to become epidermal stem cells during wound healing. And the activity of these cells depends on Shh released from the perineural niche (Brownell et al. 2011). It is also worth additional attention that adipocyte precursor cells positively regulate follicle stem cell activity by producing platelet-derived growth factors (PDGFs). Lack of adipocyte precursor cells due to the inhibition of adipogenesis at early developmental phase in Efb1 knockout mice leads to defects in stem cell activation. And injection of WT adipocyte precursor cells into $Efb1^{-/-}$ skin at P21 is able to activate stem cell and rescue the hair cycling defects. A recent study further demonstrate that adipocyte precursor cells are sufficient to activate follicle stem cells (Festa et al. 2011).

3.4.3 Intestinal Stem Cell Niche

The mammalian intestinal epithelium turns over every 3–5 days, making it one of the most rapid self-renewing tissues in adult. In the small intestine of mouse, the gut epithelium is organized into numerous crypt/villi units, with the invaginations known as crypts and protrusions termed villi, surrounded by pericryptal fibroblasts and mesenchyme. The intestinal stem cells (ISCs) reside in the crypt and give rise to transit amplifying cells, which move upward and differentiate into absorptive enterocytes, mucos-secreting goblet cells and hormone-secretive enteroendocrine cells in the villi. Upon reaching the tip of villi, these cells undergo programmed cell death before shedding into the lumen. The ISCs also generate bactericidal Paneth cells, which are located in the bottom of the crypt (van der Flier and Clevers 2009) (Fig. 3.2c).

Two populations of stem cells have been identified with compelling evidence. Conventional long-term BrdU label retaining assay based on the "immortal strand" hypothesis suggests that ISCs are located just above the paneth cells at the +4 position from the crypt bottom. The polycomb group gene *Bmi1* is found to be specifically expressed in the cells located at the +4 position. Genetic lineage tracing mediated by Bmi1-CreER demonstrates that the *Bmi*-1 expressing cells can populate the whole epithelium 12 months after tamoxifen induction, further supporting that the $Bmi1^{+}$ cells at the +4 position behave as intestinal stem cells (Sangiorgi and Capecchi 2008). +4 position ISCs can be marked by mouse telomerase reverse transcriptase

(*mTert*)-GFP as well. Similar lineage tracing mediated by *mTert* -CreER further confirms that cells at +4 position give rise to all differentiated intestinal cell types (Breault 2008; Montgomery 2011).

Similar genetic tracing studies done by the Clevers group identify the crypt base columnar (CBC) cells which express a Wnt target gene Lgr5 and are interspersed among the paneth cells as bona fide ISCs. The Lgr5-expressing cells can regenerate the vili-crypt unit within 2 months after induction (Barker 2007). Interestingly, a single isolated $Lgr5^+$ stem cell could regenerate the intact crypt-villus organoid in vitro without the long postulated mesenchymal niche, suggesting that ISCs have an innate and robust self-organizing ability to direct the formation of a functional epithelium (Sato 2009). The identification of CBC as intestinal stem cells is further sustained by lineage tracing studies conducted with *Prominin 1* (Zhu 2009). Most recently, Clevers and colleges have shown that paneth cells constitute the niche for $Lgr5^+$ stem cells. Co-culture of sorted $Lgr5^+$ cells with paneth cells significantly promote the crypt-villus organoid formation. Additionally, selective ablation of paneth cells in vivo leads to loss of $Lgr5^+$ stem cells coincidently (Sato et al. 2011). Notably, $Lgr5^+$ stem cells divide symmetrically in their niche. They undergo "neutral competition" for niche occupation and the loser is expelled from the niche to undergo differentiation (Lopez-Garcia et al. 2010; Snippert et al. 2010).

Until most recently, the relationship between +4 position ISCs and $Lgr5^+$ ISCs was unclear. Interestingly, mTert-expressing ISCs have been reported to be able to give rise to $Lgr5^+$ ISCs, suggestive of a hierarchy between the slow-cycling and fast-cycling ISCs (Montgomery 2011). However, the $Lgr5^+$ ISCs also display significant telomerase activity (Schepers et al. 2011). Therefore it requires reconsideration whether mTert-expressing ISCs overlap with $Lgr5^+$ ISCs. Interestingly, a recent study shows that complete loss of $Lgr5^+$ ISCs by genetic ablation does not perturb the architecture and homeostasis of the intestinal epithelium, suggesting other stem cell pools can compensate for the loss of $Lgr5^+$ ISCs. Lineage tracing studies suggest that $Bmi1^+$ ISCs can replenish the fast-cycling $Lgr5^+$ ISCs both under normal condition and after injury (Tian et al. 2011), further supporting the existence of slow-cycling and fast-cycling ISCs, which can be marked by Bmi1 and Lgr5, respectively.

Multiple signaling pathways participate in the regulation of the gut homeostasis, including Wnt, BMP, Notch, Hedgehog, EphB and Ras pathways, and each of them have different roles in regulating cell proliferation, differentiation and migration. The Wnt/β-catenin pathway is the major pathway controlling ISC maintenance and self-renewal. High levels of nuclear β-catenin are found in the epithelial cells at the crypt bottom, but not in the epithelial cells in the villus. Disrupting Wnt pathway activity causes crypt loss, indicating that Wnt signaling is essential for ISC maintenance (Korinek et al. 1998). On the other hand, Wnt pathway activation by the loss of APC, a negative regulator of Wnt signaling, produces giant crypts because of hyperproliferation of intestinal progenitor cells (Andreu et al. 2005; Sansom et al. 2004). The source of the active Wnt ligand remains elusive. In situ results show that several Wnts are expressed in the crypt bottom, while several other Wnts are expressed in the mesenchymal cells (Girgenrath et al. 2006). BMP signaling

activated by the BMP ligands produced from the mesenchymal cells functions to restrict ISC proliferation and facilitate differentiation, as loss of Bmpr1a or expression of noggin inhibitor in intestine epithelium leads to intestinal polyposis (Haramis et al. 2004; He et al. 2004). Hedgehog signaling inhibits ISC proliferation and promotes their differentiation by inducing the expression of BMP ligands in the mesenchymal cells (Madison et al. 2005; van den Brink et al. 2004). These observations also indicate that the mesenchyme beneath the crypt has important role in regulating ISC behavior and could be an important constitute of the ISC niche.

3.4.4 Muscle Stem Cell Niche

Satellite cells, the best understood muscle-resident stem cells, are believed to be crucial for postnatal skeletal muscle growth and regeneration after injury. They are located between the plasma membrane of muscle fiber and basement membrane surrounding the muscle fiber (Fig. 3.1d). After injury, satellite cells are activated to generate myogenic precursor cells, which undergo transit amplification and differentiation before finally fuse to form multinucleated myofibers. Recent studies demonstrate that satellite cells are heterogeneous populations consisting of slow-cycling stem cells and fast-cycling progenitor cells. Both stem cells and progenitor cells express Pax7, but only progenitor cells express Myf5. $Pax7^{+}$ $Myf5^{-}$ satellite cells can undergo planar division (usually symmetric) and apical-basal division (usually asymmetric). There is a strong correlation between the fate and location of their daughter cells upon division. The daughter cell attached to basement membrane remains a self-renewing stem cell, and the other daughter positioned away from basement membrane becomes a committed myogenic cell (Kuang et al. 2007).

The host muscle fiber, extracellular matrix, microvasculature and interstitial cells constitute the niche for satellite cells (Kuang et al. 2008). Mice lacking the ECM component Laminin-α2 show defects in muscle growth and regeneration (Miyagoe et al. 1997), indicating a critical role of ECM in satellite cell function. Injured muscles could release HGF to activate the quiescent satellite cells, and the macrophage could release the TNF ligand TWEAK to promote muscle progenitors regeneration (Girgenrath et al. 2006; Tatsumi et al. 1998). Other growth factors and cytokines such as bFGF, IGF, BNDF, VEGF, PDGF, IL-6 and LIF could also regulate satellite cell proliferation and differentiation (Kuang et al. 2008). The Delta/Notch signaling pathway plays an important role for maintaining muscle stem cells (Conboy and Rando 2002). The ligand Delta-1 enriched in $Pax7^{+}Myf5^{+}$ progenitor cell is assumed to activate Notch signaling to promote self-renewal of the adjoining $Pax7^{+}Myf5^{-}$ stem cell. Blockage of Notch signaling leads to reduced stem cell self-renewal and regeneration ability (Conboy et al. 2003; Kuang et al. 2007). Intriguingly, crosstalk between Wnt and Notch signaling via GSK3β has been shown to be involved in the cell fate choices of activated satellite cells.

Over-activation of Wnt signaling pathway leads to premature muscle differentiation while its inactivation prevents muscle differentiation. The defects in muscle differentiation caused by enhancement of Notch signaling can be rescued by enhancement of Wnt signaling (Brack et al. 2008).

Emerging evidence suggest that non-satellite cells may contribute to myogenesis in response to injury. Transplanted adult bone marrow-derived cells (BMDC) can be converted to functional satellite cells following irradiation-induced damage (LaBarge and Blau 2002). Recently, a population of $PW1^{+}Pax7^{-}$ interstitial cells (PICs) have been identified to be able to generate satellite cells during regeneration, suggesting a hierarchy between these two muscle stem cell populations (Mitchell et al. 2010). The potential niche for PICs remains to be defined.

3.5 Key Components of the Stem Cell Niche

As described above, niche structure varies greatly from tissues to tissues and in different organisms. In terms of physical composition, some niches are relatively simple, composed of a single type of stromal cell, but some are rather complex, composed of multiple types of stromal cells and also non-cellular components. In terms of the stem cell types they host, some niches specifically host a single type of stem cells, and some rather simultaneously control more than one type of stem cells. However, all of these relatively well-characterized niches share certain common components, which are summarized as the following.

1. Physical support. The residence of stem cells within specific anatomic locations requires particular physical support including association with supportive stromal cells or basement membrane or both. The physical support keeps stem cells from being exposed to detrimental environment and prevents them from undergoing precocious differentiation. On the basis of physical association between stem cells and niches, two general types of niche -stromal niches and epithelial niches have been proposed (see below) (Morrison and Spradling 2008).
2. Secreted signals. The stromal cells in the niche commonly produce secreted signal molecules to directly regulate stem cell maintenance and self-renewal. Some niches require one principal signal for this function, whereas some niches require the cooperative function of multiple signals. These signaling activities often function to prevent the initiation of differentiation programs, thereby keeping stem cells in the undifferentiated states. The niche signaling also frequently regulates stem cell activity by promoting or inhibiting their division, therefore controls stem cell quiescence and activation.
3. Cell adhesion molecules. Stem cells commonly produce cell adhesion molecules for their anchorage to the niche. Cadherin-mediated cell-to-cell adhesion between the stem cells and the niche cells and integrin-mediated cell-to-ECM adhesion between the stem cells and the basement membrane are two general types of cell

adhesion utilized in the stem cell niches. In addition to the role of adhesion molecules in anchoring stem cells, they also participate in regulating stem cell division by anchoring and orientating mitotic spindles and regulating signaling cascades (Marthiens et al. 2010; Xi 2009).

3.6 Classification of Stem Cell Niches: Stromal Versus Epidermal

Based on the comparison of physical structures among these well-characterized stem cell niches in simple organisms, the niche can be categorized into two general types, stromal niche and epidermal niche (Morrison and Spradling 2008), which may also be applicable to the stem cell niches in mammals.

The stromal niche is best exemplified by the GSC niches in *Drosophila*. The stromal niche is constituted of fixed stromal cells. For example, cap cells or hub cells constitute the female and male GSC niches, respectively. In the stromal niche, the stem cells are usually anchored to the niche cells by forming cadherin-mediated adherens junctions. The junctional structure at the stem cell-niche interface may be utilized for spindle pole anchorage for asymmetric stem cell division. In the stromal niche, short range self-renewal signals from the niche cells are critical for stem cell self-renewal, such that stem cells that are out of the niche could not receive self-renewal signals and will commit differentiation. On the other hand, stem cells could also send signals back to the niche cells to maintain their fate and function (Fig. 3.3a).

In the epidermal niche, exemplified by the FSC niche in the *Drosophila* ovary and the ISC niche in the *Drosophila* midgut, stem cells do not directly contact any fixed stromal cells but are constantly associated with the basement membrane composed of ECM. In addition, both stem cells and their differentiating daughter cells are exposed to seemingly similar surrounding environments without apparently distinctive compartalization. Stem cell anchorage and self-renewal mechanisms are different from that utilized in the stromal niche, and may be diverse from one system to another (Fig. 3.3b). In the FSC niche, stem cells are anchored in a fixed location by integrin-mediated cell adhesion between the stem cell and the ECM. Stem cells receive multiple signals produced from a relative distant source at the anterior tip for their self-renewal. There is no evidence for a specific composition of ECM at the stem cell location and the location of the FSC is probably controlled by both the levels of self-renewal signaling activity and communications between the stem cells and nearby non-stem cells and ECM. In the single-layered *Drosophila* midgut epithelium, ISCs are lining along the basement membrane that separates the epithelial layer with the muscular niche. The non-stem epithelial cells including enterocytes and enteroendocrine cells are also in direct contact with the basement membrane, and Wingless and Unpaired self-renewal signals are expressed in the muscle cells along the length of the midgut. Thus, it seems that in addition to ISCs, non-stem epithelial cells are also exposed to the niche microenvironment. It is therefore

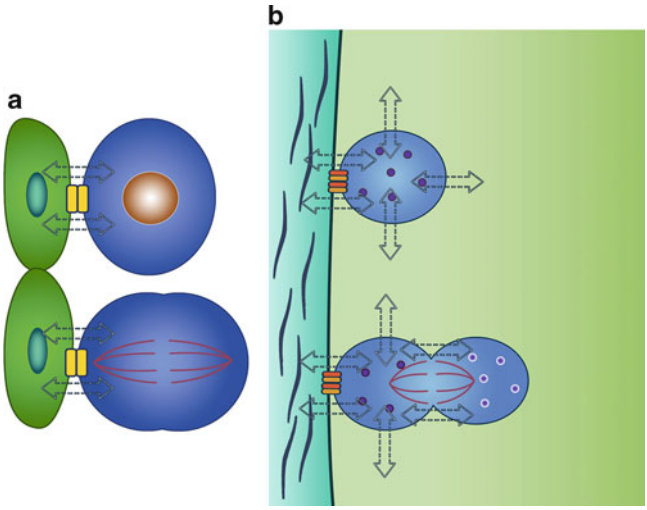

Fig. 3.3 Classification of stem cell niches based on cellular and structural composition. (**a**) A stromal niche. In the stromal niche, stem cells are anchored in the niche cells by forming cadherin-mediated cell-to-cell adhesion between the stem cells and the niche cells. Signaling between the niche cells and the stem cells is critical for stem cell maintenance and self-renewal. (**b**) An epidermal niche. In the epidermal niche, stem cells are anchored in the niche by forming integrin-mediated cell-to-ECM adhesion between the stem cells and the basement membrane. Signaling interactions between the stem cells and the niche environment, including the ECM, the neighboring cells and the immediate daughters may cooperatively regulate stem cell fate or symmetric or asymmetric segregation of cell fate determinants

possible that stem cell self-renewal could be controlled by additional mechanisms in addition to the instructive signals from the muscular niche. Delta expressing ISC could direct daughter cell fate by activation of Notch in the differentiating daughter cells, and Delta-Notch mediated lateral inhibition may further reinforce each other's cell fate. Thus, stem cell self-renewal in the epidermal niche is possibly controlled by both the instructive communications between the stem cells and the niche, and the instructive communications between the stem cells and neighboring differentiated cells, including the differentiating daughter cells (Fig. 3.3b).

3.7 Stem Cell Self-renewal in the Niche: Division Asymmetry Versus Population Asymmetry

As the ultimate defense for tissue homeostasis, stem cells have to accomplish two tasks throughout adult life: one is to generate more stem cells (self-renewal), the other is to produce committed cells (differentiation). And these two tasks must be tightly coordinated. Accumulating data from studies in invertebrates together with vertebrates point out two plausible strategies used by stem cells to interpret how the balance between

self-renewal and differentiation is achieved. Stem cells can adopt either division asymmetry or population asymmetry strategy to maintain tissue homeostasis (Morrison and Kimble 2006; Simons and Clevers 2011; Watt and Hogan 2000).

Division asymmetry refers to that each individual stem cell divides to produce two daughters with distinct fates: one remains as a new stem cell and the other commits differentiation. Asymmetric division can be achieved either through asymmetric segregation of cell fate determinants, such as for Drosophila neuroblasts (Knoblich 2008), or through cues from the niche. The well-characterized *Drosophila* GSCs in the ovary and testis use the latter strategy. In this scenario, the highly asymmetric niche architecture directs and facilitates the outcome of stem cell division: the daughter cell remained in the niche will self-renewal, while the daughter cell positioned away from the niche will differentiate.

In population asymmetry, each stem cell gives rise to two daughter cells upon division, the fate of which is unpredictable and depends on the extrinsic input. Some stem cells may be lost through differentiation and some stem cells can expand to replace the lost stem cells. And the replacement rate is comparable to the loss rate. Therefore, the net effect of population asymmetry is the same as division asymmetry. The total number of stem cells remains constant at the level of stem cell population. Stem cells in many mammalian tissues adopt this strategy to achieve homeostasis. For instance, the $Lgr5^{hi}$ ISCs in mouse intestine divide symmetrically to generate two daughter cells, which subsequently undergo "neutral competition" for contact with Paneth cells with the neighboring stem cells. And the loser cells in the competition are squeezed out of the niche to initiate the differentiation program (Lopez-Garcia et al. 2010; Snippert et al. 2010). Besides, the GSCs in mammalian testis and epidermal stem cells in mouse interfollicle epithelium might fall into this category as well.

3.8 Stem Cell Behavior Within the Niche

Studies on the *Drosophila* GSC niche have also revealed several interesting stem cell behaviors that may be important for stem cell long-term maintenance and function, and those phenomena have enriched our understanding of the stem cell niche concept. Here are some examples.

1. Stem cell replacement. It is evident that adult stem cells have limited half-life. They turn over regularly, but the stem cell number within each niche could remain relatively constant. This is probably due to a phenomenon named stem cell replacement. One example is the GSC in the *Drosophila* ovary. When one GSC is depleted from the niche, the other GSCs could undergo symmetric division to supplement the lost GSC (Xie and Spradling 2000). This indicates that the niche has the capability to sustain a stable number of GSCs by controlling symmetric and asymmetric division of GSCs.
2. Stem cell dedifferentiation in the niche. This represents another potentially important mechanism for maintaining constant stem cell number in the niche.

When GSCs in the *Drosophila* ovary and testis are forced to differentiate, the early differentiating germ cells could be dedifferentiated into functional GSCs and reoccupy the niche, if they again receive the niche signaling. This reveals the plasticity of progenitor cells and a dominant role of niche in determining stem cell fate (Brawley and Matunis 2004; Kai and Spradling 2004).

3. Stem cell competition. The regular turn-over of stem cells and replacement by the neighboring stem cells may also indicate that these stem cells within the same niche may constantly compete with each other for niche occupation. Studies of GSCs with different genetic background in the same niche have shown that cell adhesion molecules are involved in stem cell competition (Jin et al. 2008). Stem cell competition may be important for the quality control of stem cells, and for coordinating the functions of different types of stem cells that share a single niche (Rhiner et al. 2009). It is also possible that cancer stem cells could potentially make more devastating damages by utilizing this mechanism to hijack the niche and eliminate the normal stem cells.

3.9 Future Perspective

The study of the stem cells and their niches has provided important implications on the relationships between dysregulation of the stem cell niche and human diseases and aging, and may provide useful strategies for clinical applications. Increasing evidence suggests that many cancers are stem cell diseases, in which a rare population of cancer stem cells is responsible for the initiation and recurrence of cancers (Clarke and Fuller 2006). Understanding stem cell self-renewal mechanisms could help to provide novel therapeutic strategy to treat cancers. For example, the CD44 adhesion receptor, which is known to mediate Osteopontin signaling from the niche, could be a therapeutic target of acute myeloid leukemia (AML) cancer stem cells, as administration of CD44 antibody efficiently eliminates leukemia stem cells in the mouse model of human AML (Jin et al. 2006). In addition, abnormalities in the niche, rather than stem cells themselves, may also lead to the development of cancers. For example, increasing evidence suggests that leukemia could be contributed by both cell autonomous abnormalities and dysfunction of the microenvironment in the bone marrow (Lane et al. 2009). Microenvironmental deletion of retinoic acid gamma receptor (RARγ) or retinoblastoma leads to a phenotype reminiscent of myeloproliferative disease in mouse, which raises the possibility that some leukemia may result from disorder of the microenvironment (Walkley et al. 2007). Therefore, targeting abnormal niche function could be another therapeutic strategy to treat cancers.

Understanding of the stem cell and niche regulation may also lead to improved methods for stem cell manipulation in vivo and in vitro to facilitate replacement therapies in the future. For example, osteoblastic cells, the niche cells for HSCs, can be manipulated by PTH in mouse models of clinical use of HSCs. PTH administration

can increase stem cell harvest, protect HSC from chemotherapy and promote HSC function in transplant recipients (Adams et al. 2007).

The ability of adult stem cells to regenerate tissue declines with age and this phenomenon, regarded as stem cell aging, is contributed by the changes in the niche microenvironment, systemic environment and intrinsically within the stem cells, although the contribution of each factor could vary greatly in different tissues and organisms. For example, in the *Drosophila* testis and ovary, the GSC activity declines greatly with age, largely due to the functional decay of niche signaling (Boyle et al. 2007; Pan et al. 2007; Zhao et al. 2008). In mouse satellite stem cell niche, systemic change-induced Wnt signaling activation has been linked to the decline of regeneration potential in aged satellite stem cells (Brack et al. 2007; Carlson et al. 2008). Therefore, modulating stem cell niche function could also be a useful strategy to delay the development of aging and promote tissue regeneration and damage repair.

Aside from these promising clinical prospective, there are still a lot of mysteries about the stem cells and their associated niches. The identification and characterization of these less understood mammalian stem cell niches would be an urgent task. How the extrinsic signals integrate with intrinsic circuitries to maintain the stemness and how stem cell self-renewal and differentiation are precisely balanced only begins to be understood. Again, studies on simpler genetic model systems would certainly continue to pioneer our understanding of stem cells and their niches.

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