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Adhesion in the stem cell niche: biological roles and regulation

Shuyi Chen[1], Michelle Lewallen[1] and Ting Xie[1,2,*]

Summary

Stem cell self-renewal is tightly controlled by the concerted action of stem cell-intrinsic factors and signals within the niche. Niche signals often function within a short range, allowing cells in the niche to self-renew while their daughters outside the niche differentiate. Thus, in order for stem cells to continuously self-renew, they are often anchored in the niche via adhesion molecules. In addition to niche anchoring, however, recent studies have revealed other important roles for adhesion molecules in the regulation of stem cell function, and it is clear that stem cell-niche adhesion is crucial for stem cell self-renewal and is dynamically regulated. Here, we highlight recent progress in understanding adhesion between stem cells and their niche and how this adhesion is regulated.

KEY WORDS: Niche, Adhesion, Cadherin, Integrin, Stem cell

Introduction

Various populations of adult stem cells reside in the body and undergo continuous self-renewal throughout an organism's lifespan. The complex milieu composed of cells and extracellular matrix (ECM), as well as the signaling molecules associated with each population of stem cells, is collectively referred to as the stem cell niche (Spradling et al., 2001). The physical structure of the niche varies between organisms and between stem cell types, its composition ranging from a single cell or cell type to many cells of varying cell types. In the *C. elegans* hermaphrodite gonad, for example (Fig. 1A), a single cell, known as the distal tip cell, functions as the niche for germline stem cells (GSCs) (Byrd and Kimble, 2009; Kimble and Crittenden, 2007). By contrast, in the *Drosophila* ovary (Fig. 1B) and testis (Fig. 1C), two or three somatic cell types form the niche for GSCs: the female GSC niche is composed of terminal filament cells, cap cells and GSC-contacting escort cells, whereas the male niche consists of hub cells and cyst stem cells (CySCs) (de Cuevas and Matunis, 2011; Xie, 2012). Sometimes, two different stem cell types in the same tissue share a common niche cell component. For example, cap cells in the *Drosophila* ovary serve as a component of both the GSC and follicular stem cell (FSC) niches, whereas hub cells of the testis function as the common niche component that regulates GSCs and CySCs (de Cuevas and Matunis, 2011; Xie, 2012). Mammalian stem cell niches are generally more complex. The hematopoietic stem cell (HSC) niche contains at least four different cell types (Fig. 1D), including osteoblasts, vascular cells, mesenchymal stem cells and neuron-Schwann cells (Wang and Wagers, 2011). In addition to specialized cell types, the ECM is a crucial component of the stem cell niche; many stem cell types, such as mammalian spermatogonial stem cells (SSCs), epidermal stem cells and neural stem cells (NSCs) (Fig. 1E), express high levels of integrins and directly contact the ECM, highlighting the role of ECM as an integral part of the stem cell niche (Kanatsu-Shinohara et al., 2008; Kazanis et al., 2010; Shen et al., 2008; Watt, 2002). This complex nature of the stem cell niche allows the formation of distinct and specialized niche structures for different stem cell types in the same organism or for the same stem cell type in different organisms.

Individual stem cell niches also use distinct combinations of signaling molecules to control stem cell self-renewal and proliferation. For some stem cell types, the activation of a single signaling pathway by the niche is sufficient for promoting stem cell self-renewal. For example, bone morphogenetic protein (BMP) in the *Drosophila* female GSC niche is necessary and sufficient for GSC self-renewal (Xie, 2012). This is also true for Notch in the *C. elegans* GSC niche (Byrd and Kimble, 2009; Kimble and Crittenden, 2007). However, for most stem cell types, the simultaneous activation of several pathways is needed for continuous stem cell self-renewal. For example, the fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF) and sonic hedgehog (Shh) signaling pathways are needed for long-term mammalian NSC self-renewal *in vivo* (Zhao et al., 2008). Although specific signals or combinations of signals are needed by different niches to control stem cell self-renewal, many of them appear to function as short-range signals. Thus, stem cells must stay inside the niche in order to maintain long-term self-renewal. One of the most convenient, and arguably the most reliable, methods is to anchor stem cells in their niche using adhesion molecules. In this Review, we summarize recent progress in understanding how stem cells are maintained in their niche, and we highlight how adhesion molecules contribute to cell-cell adhesion and cell-niche anchorage as well as to other aspects of stem cell regulation.

Classes of adhesion molecules that mediate stem cell-niche interactions

The cadherin family of adhesion proteins

Classical cadherin molecules mediate cell-cell adhesion via homophilic interactions between the extracellular domains of cadherins on adjacent cells and via interactions of cadherin intracellular domains with cytoskeleton-associated proteins. The intracellular domains of cadherins can interact with β-catenin and α-catenin, which are scaffold proteins that connect cadherins to the cytoskeletal network in order to cluster cadherin molecules and form stable adherens junctions (AJs) (Gates and Peifer, 2005; Leckband and Sivasankar, 2012; Meng and Takeichi, 2009). The best-studied molecule involved in stem cell-niche adhesion is E-cadherin. In the *Drosophila* ovary, E-cadherin was first shown to accumulate at the junction between GSCs and their niche cells (the

[1]Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA. [2]Department of Cell Biology and Anatomy, University of Kansas School of Medicine, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA.

*Author for correspondence (tgx@stowers.org)

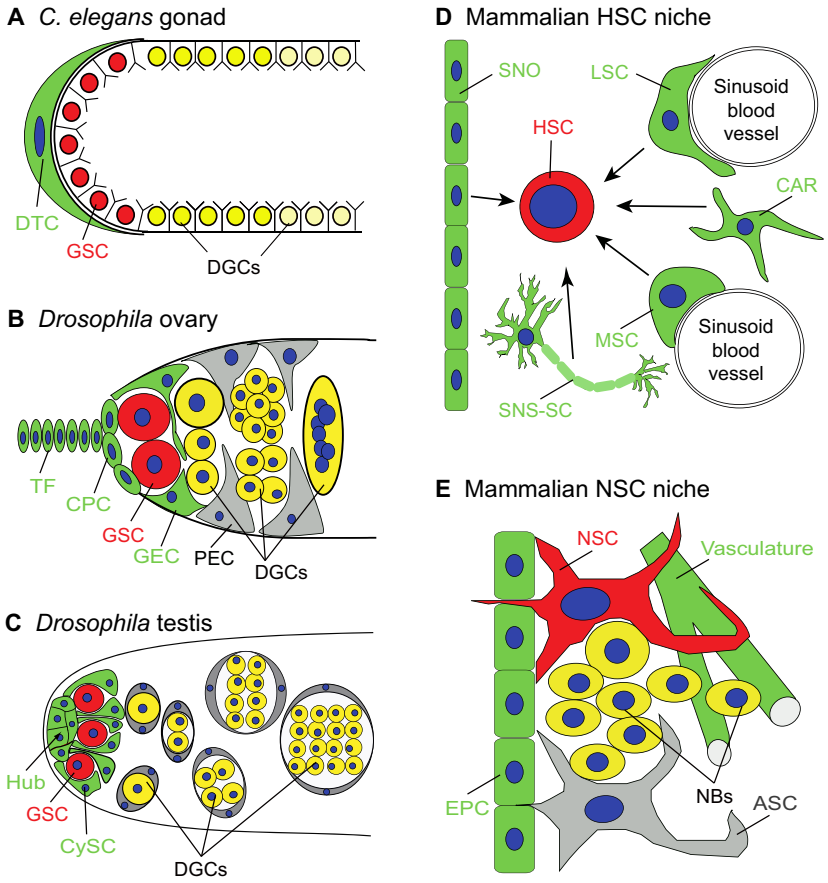

Fig. 1. Stem cell niches. Niche cells and stem cells are depicted in green and red, respectively. Differentiated stem cell progeny and their surrounding somatic cells are shown in yellow and gray, respectively. (**A**) The GSC niche in the *C. elegans* hermaphrodite gonad. (**B**) The GSC niche in the *Drosophila* ovary. (**C**) The GSC niche in the *Drosophila* testis. (**D**) The mammalian HSC niche. (**E**) The NSC niche in the mammalian subventricular zone. ASC, astrocyte; CAR, CXCL12-abundant reticular cell; CPC, cap cell; CySC, cyst stem cell; DGCs, differentiated germ cells; DTC, distal tip cell; EPC, ependymal cell; GEC, GSC-contacting escort cell; GSC, germline stem cell; HSC, hematopoietic stem cell; LSC, leptin receptor$^+$ perivascular stromal cell; MSC, mesenchymal stem cell; NBs, neuroblasts; NSC, neural stem cell; PEC, posterior escort cell; SNC-SC, sympathetic neuronal cell-Schwann cell; SNO, spindle-shaped N-cadherin$^+$ osteoblast; TF, terminal filament.

cap cells) and form AJs (Song et al., 2002). In addition, E-cadherin also accumulates between FSCs and their niche cells (Song and Xie, 2002). The removal of E-cadherin from GSCs or FSCs leads to rapid GSC or FSC departure from the niche, indicating that E-cadherin-mediated cell adhesion is crucial for maintaining GSCs and FSCs in the niche for long-term self-renewal (Song and Xie, 2002; Song et al., 2002). Similarly, in the *Drosophila* testis, E-cadherin accumulates at the interface between GSCs and their niche cells (the hub cells) and is also important for GSC maintenance (Inaba et al., 2010; Yamashita et al., 2003). These findings have solidified a role for E-cadherin in mediating stem cell-niche interactions (Fig. 2A).

Cadherin molecules are also expressed in a variety of mammalian stem cells, where they similarly appear to play important roles in regulating stem cell adhesion and self-renewal. In the adult brain, the subventricular NSC niche has been suggested to consist of at least ependymal cells on the ventricular surface and endothelial cells in adjacent blood vessels. E-cadherin is expressed by ependymal cells and NSCs, and it forms AJs between the two cell types on their apical sides and is required for NSC self-renewal *in vitro* and *in vivo* (Karpowicz et al., 2009). In the HSC niche, HSCs and osteoblasts express N-cadherin (Zhang et al., 2003), and multiple studies have suggested that N-cadherin is required for maintaining HSCs in the niche for long-term self-renewal (Haug et al., 2008; Hosokawa et al., 2010a; Hosokawa et al., 2010b). However, other independent studies have disputed the role of N-cadherin in controlling HSC self-renewal (Bromberg et al., 2012; Greenbaum et al., 2012; Kiel et al., 2009). The contradictory conclusions concerning the role of N-cadherin in the regulation of HSC self-renewal could arise from different experimental methods.

The role of cadherins in other stem cells is yet to be examined. Satellite cells, which serve as stem cells in skeletal muscle (Seale and Rudnicki, 2000), are situated between the basal membrane and the mature muscle fiber, which collectively function as the niche. M-cadherin is localized to the side of the satellite cell facing the muscle fiber, but its role in regulating satellite stem cells remains to be determined (Kuang et al., 2008). Since E-cadherin, N-cadherin and other classic cadherin molecules are widely expressed in different adult tissues, it would not be surprising if they are also required for stem cell-niche interactions in other systems.

The integrin family of adhesion molecules

Integrins are heterodimeric (consisting of α and β subunits) transmembrane molecules that mediate cell-ECM interactions. The extracellular domains of integrins can bind directly to ECM proteins such as laminin, collagen and fibronectin (Barczyk et al., 2010; Hynes, 2002). In addition to ECM components, integrins can also bind to other cell-surface adhesion molecules such as intercellular adhesion molecule 1 (Icam1, also known as CD54) and vascular cell adhesion molecule 1 (Vcam1, also known as CD106), which are known to be present in some stem cell niches (Barczyk et al., 2010). In the *Drosophila* ovary, integrin-mediated interaction between FSCs and the basal lamina is required for anchoring FSCs in the niche to allow self-renewal and asymmetric cell division (O'Reilly et al., 2008). In the *Drosophila* testis, integrins are required for positioning the GSC niche in the apical tip of the testis, but are dispensable for GSC or CySC anchorage to the niche (Tanentzapf et al., 2007).

Many mammalian stem cell types also express integrin molecules and directly contact the ECM or the ECM-rich basal

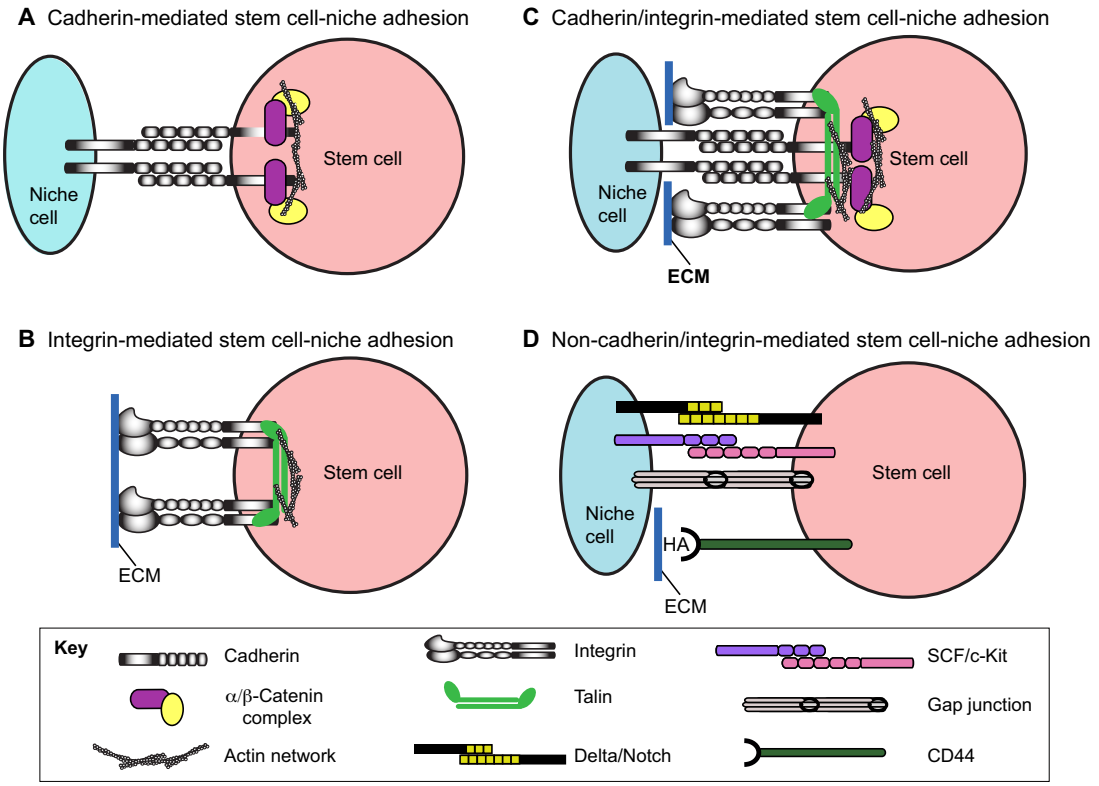

Fig. 2. Cadherin- and integrin-mediated stem cell-niche adhesion. (**A**) Classical cadherin-mediated physical cell-cell adhesion helps anchor stem cells to their niche. α-catenin and β-catenin, which associate with the intracellular domain of cadherins, help to cluster cadherin molecules and form adherens junctions (AJs). (**B**) Integrin-mediated cell-extracellular matrix (ECM) interactions help anchor stem cells to the niche, which often contains a number of ECM components. The intracellular domains of integrins interact with the actin cytoskeleton network through talin proteins to cluster integrin molecules together. (**C**) Cadherin-mediated cell interactions and integrin-mediated cell-ECM interactions can work together to anchor stem cells to the niche. (**D**) In addition to cadherins and integrins, other molecules, such as Delta/Notch, SCF/c-Kit, CD44/hyaluronic acid (HA) and gap junction components, are also involved in stem cell-niche adhesion.

membrane. SSCs in the mouse testis, for example, directly contact the basal membrane and express high levels of α6β1 integrin, a receptor for the ECM protein laminin (Shinohara et al., 1999). In addition, anti-α6 and anti-β1 integrin antibodies can purify and enrich SSCs from dissociated testicular cells (Shinohara et al., 1999), confirming that these integrins are also expressed on the surface of SSCs. Indeed, β1 integrin is required to enable SSCs to home to the testicular niche (Kanatsu-Shinohara et al., 2008), indicating that integrins are required in SSCs for their interaction with the basal membrane. Interestingly, α6β1 integrin is also highly expressed in developing and adult subventricular NSCs and in cultured NSCs, and laminin chains α2 and α4 are rich in the subventricular zone (Lathia et al., 2007; Shen et al., 2008). Using neutralizing antibodies, it was demonstrated that α6β1 integrin helps NSCs adhere to endothelial cells in the NSC vascular niche (Shen et al., 2008). HSCs also express high levels of α4, α6, α7, α9 and β1 integrins (Grassinger et al., 2009; Potocnik et al., 2000; Schreiber et al., 2009; Voura et al., 1997). Adult HSCs deficient for β1 integrin show a severe defect in homing to the bone marrow niche after the elimination of endogenous HSCs by radiation, indicating that β1 integrin is required for HSCs to interact with the niche (Potocnik et al., 2000). Similarly, α4, α6 and α9 integrins are also crucial for HSC-niche interaction (Grassinger et al., 2009; Qian et al., 2006; Schreiber et al., 2009). In the skin, stem cells attach to the basal membrane via integrins α6, β1 and β4 (Watt, 2002), whereas in skeletal muscle α7β1 integrin is localized on the side of satellite cells facing the basal membrane component of the stem cell niche (Kuang et al., 2008).

Taken together, it is evident that many different stem cell types use integrins to interact with ECM proteins or with other adhesion molecules on the surface of niche cells (Fig. 2B). Furthermore, some stem cells, including FSCs, HSCs and NSCs, appear to use both cadherin and integrin adhesion molecules to interact with their niche (Fig. 2C).

Other classes of adhesion molecules

In addition to cadherins and integrins, many other families of adhesion molecules are capable of mediating cell-cell interactions, and stem cells do indeed utilize other adhesion molecules for anchorage or communication with their local environments. The interaction between Notch receptors and their transmembrane ligands has long been proposed to be important for cell-cell physical interactions as well as for cell signaling (Watt et al., 2008), although it is difficult to distinguish between adhesion-mediated versus signaling-mediated roles performed by Notch. The best example of stem cell adhesion mediated by Notch signaling is the clustering of epidermal stem cells in the skin by delta-like 1-mediated Notch interactions (Estrach et al., 2007). Furthermore, in the *C. elegans* gonad, GSCs expressing the Notch receptor GLP-1 are always physically tethered to the distal tip cell, which expresses the Notch ligand LAG-2 (Kimble and Crittenden, 2007). However,

it remains to be demonstrated whether, in this case, Notch has any direct role in keeping stem cells in the niche (Fig. 2D).

Gap junctions, which are specialized intercellular protein channels that connect two adjacent cells, are formed by the connexin family of proteins (White and Paul, 1999). Because the two cells are physically connected via protein complexes, gap junctions are thought to have an adhesive role in addition to facilitating direct electrical and chemical communication and small molecule transfer between the cells. Connexin 43 (also known as gap junction protein alpha 1) was first shown to be required in stromal cells to support mouse HSCs in culture (Cancelas et al., 2000), and was later shown to be important for CXCL12 secretion from the stromal cells to control HSC self-renewal (Schajnovitz et al., 2011). Most recently, connexin 43 was shown to prevent HSC senescence by transferring reactive oxygen species into the supporting stromal cells, thus reducing their damage to HSCs (Taniguchi Ishikawa et al., 2012). In the mouse subventricular NSC niche, the gap junction-mediated calcium transfer between supporting astrocytes and NSCs is important for NSC proliferation (Lacar et al., 2011). In *Drosophila* ovary and testis, Zero population growth (Zpg), a gap junction protein, is required for GSC maintenance and differentiation (Gilboa et al., 2003; Tazuke et al., 2002). In all these cases, it remains to be demonstrated whether the function of gap junctions in the regulation of stem cell maintenance derives from their adhesion role, intercellular molecule transfer or both (Fig. 2D).

HSCs and their niche cells also are known to express many other cell membrane receptors, including c-Kit, CD44 and Vcam1 (Imai et al., 1999; Osawa et al., 1996; Uchida et al., 1998). c-Kit is a transmembrane receptor tyrosine kinase, and its ligand, stem cell factor (SCF, also known as Kitl), has two forms, secreted and membrane-bound, which are produced by alternative mRNA splicing (Toksoz et al., 1992). Genetic studies have shown that the membrane-bound form of SCF is particularly important for HSC maintenance and steady hematopoiesis (Barker, 1997). Interestingly, when SCF is specifically inactivated in both endothelial and perivascular niche cells, HSCs are rapidly depleted, indicating that the niche cell-stem cell interaction mediated by SCF-c-Kit might help to keep HSCs in the perivascular niche (Ding et al., 2012). CD44 is a cell adhesion molecule that belongs to the family of hyaluronic acid (HA)-binding proteins, and an anti-CD44 monoclonal antibody or free HA can block the homing of transplanted HSCs to the niche (Avigdor et al., 2004). Because sinusoidal blood vessels, which have been suggested to serve as one of the HSC niche sites, express abundant HA, CD44 might help anchor HSCs in the perivascular niche. Interestingly, Vcam1 is also expressed in NSCs, and its functional disruption leads to loss of the pinwheel niche architecture and ultimately to NSC loss (Kokovay et al., 2012). Together, these results suggest that other classes of adhesion molecules in addition to cadherins and integrins are also involved in the regulation of stem cell-niche interactions (Fig. 2D).

Biological functions of adhesion molecules in stem cell regulation

Cadherins, integrins and other adhesion molecules are involved in the regulation of not only cell-cell physical interactions but also other cellular events, such as cell signaling and cell polarity. Therefore, it is difficult, particularly for Notch and integrins, to pinpoint the extent to which their role in adhesion, rather than in signaling, plays a role in stem cell maintenance. Nonetheless, a number of experimental approaches (see Box 1) have been employed in an attempt to characterize the precise roles played by adhesion molecules in regulating stem cell biology. These studies have shown that adhesion molecules can function in a variety of ways to influence stem cell maintenance.

Box 1. Experimental approaches for studying adhesion molecule function in stem cells

The molecules involved in stem cell-niche adhesion have been identified and characterized using a variety of approaches. In many cases, the importance of adhesion molecules in stem cell-niche physical interaction has simply been inferred based on their expression patterns and their requirement for stem cell maintenance. The expression of adhesion molecules in either stem cells or niche cells can be determined, for example, by antibody staining and by gene expression profiling of purified cells. The function of adhesion molecules in stem cells or niche cells is normally investigated by standard gene knockout approaches, by cell type-specific knockout, or via the application of neutralizing antibodies against their extracellular domains; if the stem cell-niche interaction mediated by a particular adhesion molecule is essential for stem cell anchorage, for example, its inactivation should result in rapid stem cell departure from the niche. Successful homing of stem cells to the niche, by contrast, can be tested experimentally by monitoring transplanted stem cells. This method has been particularly useful for studies of HSCs in the bone marrow and of SSCs in the mouse testis to test the function of a given gene in stem cell homing to the niche. Although the transplantation assay does not directly demonstrate the role of an adhesion molecule in the niche anchorage of stem cells, it does provide important information about its potential involvement in stem cell-niche interaction; its role in stem cell-niche anchorage must be directly confirmed by stem cell-specific or niche-specific conditional inactivation of its function.

Niche anchorage and homing

For GSCs in the *Drosophila* ovary, the primary role of E-cadherin in regulating stem cell maintenance is to anchor GSCs to the niche. E-cadherin is required for GSCs to compete for niche occupancy (Jin et al., 2008). When one of the GSCs in a niche is deficient for E-cadherin, it is lost rapidly from the niche due to competition (Song et al., 2002). Interestingly, a wild-type GSC expressing more E-cadherin can push the other wild-type GSC expressing less E-cadherin out of the niche, suggesting that the E-cadherin-deficient GSC departs from the niche due to competition for the niche space (Jin et al., 2008). Cadherin-mediated stem cell anchorage in the niche might also be used in other systems, but requires further direct experimental demonstration.

In combination with gene knockout or blocking antibodies, homing assays (see Box 1) have helped demonstrate that integrins and CD44 are required for transplanted HSCs to home to the niche (Avigdor et al., 2004; Grassinger et al., 2009; Potocnik et al., 2000; Schreiber et al., 2009). In combination with Cre-mediated deletion, it has also been demonstrated that β1 integrin, but not E-cadherin, is required for SSCs to home to the testicular niche (Kanatsu-Shinohara et al., 2008). Transplantation approaches have also demonstrated the essential roles of E-cadherin, integrins and Vcam1 in mediating NSC-niche interactions in the subventricular zone (Karpowicz et al., 2009; Kokovay et al., 2012; Shen et al., 2008). Similarly, the interactions of SCF and c-Kit, and thrombin-cleaved osteopontin (also known as secreted phosphoprotein 1) and its integrin receptor, are required for mediating HSC-niche interactions in the bone marrow (Ding et al., 2012; Grassinger et al., 2009). The adhesion molecules that have been demonstrated to

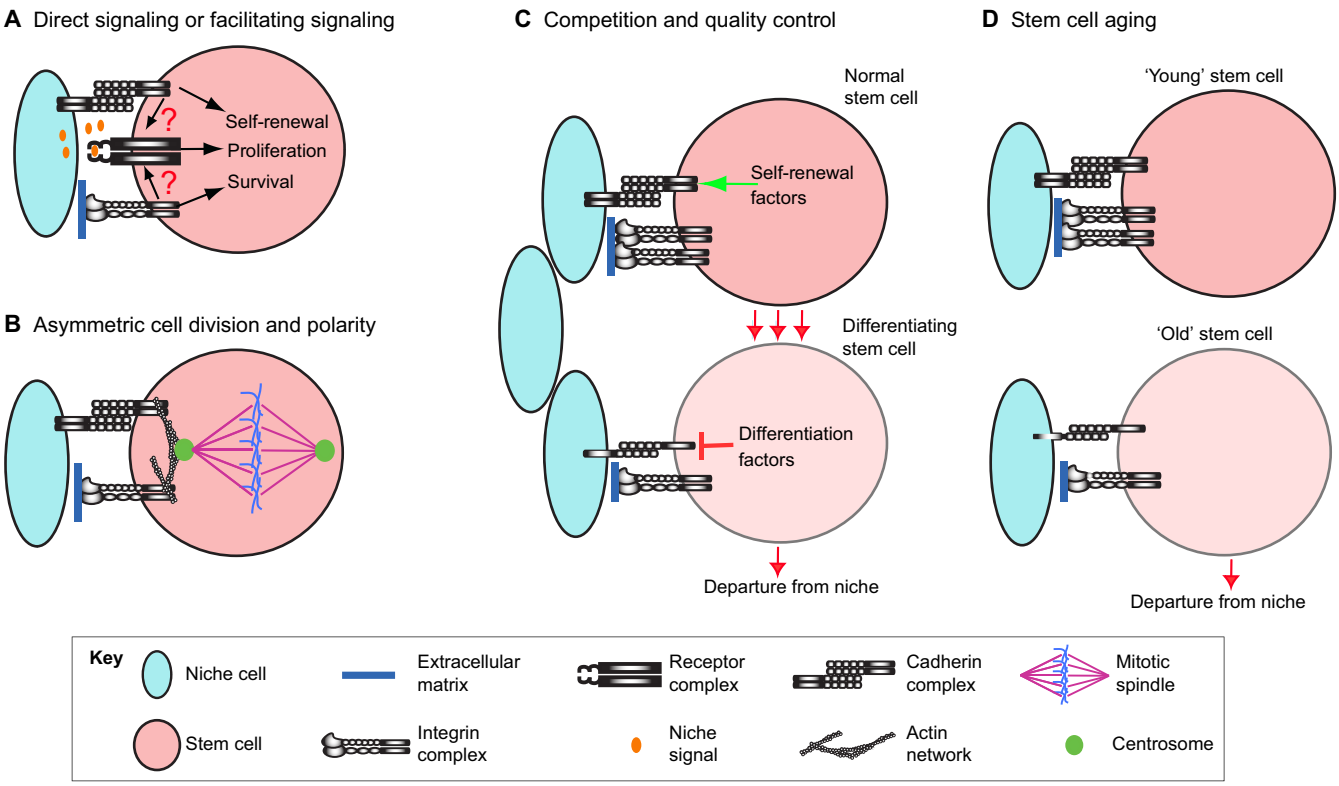

Fig. 3. Adhesion molecules have other roles in stem cell regulation besides stem cell-niche anchorage. (**A**) Cadherin and integrin complexes can signal directly downstream or facilitate receptor-mediated niche signaling to regulate stem cell self-renewal, proliferation and survival. (**B**) Cadherin and integrin molecules are required to regulate asymmetric cell division and possibly to maintain cell polarity; the actin cytoskeleton network associated with cadherins or integrins helps anchor one centrosome to the apical side of the cell to ensure that its mitotic spindle is always orientated perpendicular to the niche surface. (**C**) Stem cells utilize the strength with which they are anchored to the niche to regulate their relative competitiveness for niche occupancy. The expression levels or functions of adhesion molecules are often regulated by self-renewal and differentiation factors. Stem cell-niche adhesion thus serves as a quality control mechanism that ensures that stem cells are retained in the niche whereas differentiating cells are lost. (**D**) The expression levels or functions of adhesion molecules in stem cells and niche cells are affected by aging. The adhesion between stem cells and their niche is, therefore, also affected by aging.

be important for stem cell homing are not necessarily essential for stem cell anchorage in the niche under physiological conditions, and instead they might be important for transplanted stem cells to find their way to niches.

Controlling stem cell self-renewal via signaling

As mentioned above, Notch can mediate stem cell-niche interactions in epidermal stem cells and in *C. elegans* GSCs. However, aside from its potential role in adhesion, Notch signaling is well known for maintaining stem cell self-renewal and controlling cell differentiation by regulating target gene expression (Bigas et al., 2012; Gude and Sussman, 2012; Iglesias-Bartolome and Gutkind, 2011). Notch signaling is necessary and sufficient for maintaining *C. elegans* GSC self-renewal (Kimble and Crittenden, 2007). In mammalian stem cell systems, Notch signaling controls the maintenance and differentiation of many different stem cell types, including NSCs and epidermal stem cells (Ables et al., 2011; Iglesias-Bartolome and Gutkind, 2011). In addition, Notch signaling was recently shown to control the homing of skeletal muscle progenitor cells into the satellite cell niche by modulating the expression of ECM proteins and integrins (Brohl et al., 2012). In all of these cases, the relative contributions of Notch signaling-mediated adhesion and gene expression to stem cell regulation are difficult to separate experimentally.

Integrins can regulate downstream signaling via focal adhesion kinases (FAKs) and phosphoinositide 3-kinase (PI3K), which are important for stem cell self-renewal and proliferation (Buitenhuis, 2011; Legate et al., 2009; Lu et al., 2012). NSCs are embedded in the laminin-rich ECM and also express various integrin molecules (Kazanis et al., 2010). Blocking the function of specific integrins in NSCs using neutralizing monoclonal antibodies enhances precursor proliferation and migration and promotes the depletion of NSCs from the niche (Loulier et al., 2009). The laminin-integrin-mediated interaction also contributes to the development of the NSC niche and the maintenance of its integrity (Loulier et al., 2009). In addition, netrin 4 secreted by glial fibrillary acidic protein (GFAP)-positive astrocytes and laminin secreted by NSCs themselves can activate the α6β1 integrin-mediated signaling pathway to control NSC proliferation (Staquicini et al., 2009). Integrins can also promote NSC self-renewal by facilitating Notch and EGFR signaling (Campos et al., 2006), and integrins have recently been shown to regulate thrombopoietin-mediated HSC maintenance (Umemoto et al., 2012). Taken together, these findings demonstrate that, in addition to adhesion, integrins can regulate stem cell function via direct or indirect participation in cellular signaling (Fig. 3A).

Cadherin molecules also have the capacity to participate in intracellular signaling and to mediate gene expression via

regulation of their interacting proteins, such as p120 (δ1-catenin), β-catenin and plakoglobin (Cavallaro and Dejana, 2011). Additionally, N-cadherin has been shown to regulate FGF signaling via direct interaction with FGF receptors, which raises the interesting possibility that it might also activate other signaling pathways (Williams et al., 2001). N-cadherin-mediated cell adhesion promotes HSC quiescence and thus long-term self-renewal, but it remains unclear whether its role in HSCs might also be due to its regulation of cell signaling (Hosokawa et al., 2010a). In the ventricular region of the developing brain N-cadherin is required for maintaining NSCs by preventing differentiation via regulation of Akt signaling (Zhang et al., 2010). Finally, in the *Drosophila* testis, BMP receptor complexes are localized to E-cadherin-rich AJs at the stem cell-niche junction, which might help restrict BMP signaling activity to the GSC niche (Michel et al., 2011). These lines of evidence support a role for cadherins in the regulation of cell signaling pathways important for stem cell self-renewal and proliferation (Fig. 3A).

Regulation of cell polarity and asymmetric cell division

Adult stem cells in the niche appear to be highly polarized, based on the localization of cell polarity markers and junctions. The best-studied cases are *Drosophila* ovarian and testicular stem cells (de Cuevas and Matunis, 2011; Xie, 2012). In these GSCs, AJs are always localized to the GSC-niche interface, which might help polarize GSCs toward the niche (Song et al., 2002; Yamashita et al., 2003). In the *Drosophila* testis, GSCs and CySCs precisely orient their spindle apparatus so that the self-renewing stem cell remains in contact with the niche and the differentiating daughter moves away from the niche, thereby balancing self-renewal and differentiation (Cheng et al., 2011; Yamashita et al., 2003). It has been shown that Adenomatous polyposis coli homolog 2 (APC2), which is localized to AJs at the stem cell-niche junction, is important for controlling spindle orientation in GSCs (Yamashita et al., 2003) (Fig. 3B). Furthermore, E-cadherin is required for polarizing GSCs toward the niche and properly orienting the GSC mitotic spindle (Inaba et al., 2010). Randomization of spindle orientation in the *Drosophila* testis leads to defective GSC self-renewal and proliferation (Cheng et al., 2008; Yamashita et al., 2003). In the *Drosophila* ovary, the spindle of the GSC is often oriented perpendicular to the niche (Deng and Lin, 1997). This spindle orientation is achieved by localization of the spectrosome, a germ cell-specific membrane skeletal organelle, to the GSC-niche junction, and absence of the spectrosome [as seen in GSCs of the *hu li tai shao* (*hts*) mutant ovary] results in the randomization of spindle orientation (Deng and Lin, 1997).

In contrast to *Drosophila* testicular GSCs, randomization of spindle orientation appears to have little effect on GSC maintenance and proliferation (Deng and Lin, 1997). Removal of E-cadherin often leads to spectrosome detachment from the GSC-niche junction, suggesting that AJs are required to anchor the spectrosome to the GSC-niche junction (Song et al., 2002). Consistent with the role of centrosomes in orienting the mitotic spindle, mislocalized centrosomes or a complete absence of centrosomes can also randomize the spindle orientation in *Drosophila* ovarian GSCs, but has no effect on either GSC maintenance or proliferation (Chen et al., 2010; Stevens et al., 2007). Therefore, regulation of spindle orientation is likely to be important for some stem cell types but not others.

Cadherin-mediated and integrin-mediated cell adhesion is also important for spindle orientation in skin and brain stem cells. In the mammalian skin, epidermal stem cells can orient their spindle either parallel or perpendicular to the basal layer, and this determines the mode (asymmetric or symmetric) of stem cell division. The removal of α-catenin, an obligate E-cadherin partner, from stem cells results in the randomization of spindle orientation (Lechler and Fuchs, 2005). In the developing ventricular region, NSCs can also divide asymmetrically or symmetrically with regard to partitioning of the apical membrane, and this is determined by spindle orientation and thus the cleavage plane (Kosodo et al., 2004). Transient antibody-mediated neutralization of integrin function increases the tendency of NSCs to divide symmetrically (Loulier et al., 2009). However, it remains to be determined whether N-cadherin and E-cadherin, or indeed other adhesion molecules, are required in NSCs for asymmetric cell division.

Stem cell competition and quality control

Stem cell competition for niche occupancy is thought to serve as a quality control mechanism and is a niche feature that could potentially be exploited to enhance transplantation efficiency in future stem cell therapies. It is now clear that the anchorage strength between stem cells and their niche can affect the ability of stem cells to remain in the niche for long-term self-renewal. Indeed, in the *Drosophila* ovary, the GSC expressing high levels of E-cadherin gains a competitive advantage over the GSC expressing less E-cadherin for occupancy of the niche (Jin et al., 2008). Furthermore, as discussed below, many important GSC self-renewal factors are required to maintain E-cadherin expression, whereas key GSC differentiation factors negatively regulate E-cadherin expression at the translational level (Chen et al., 2010; Shen et al., 2009). If a GSC is experimentally induced to differentiate, downregulation of E-cadherin is observed in this GSC, which, as a result of the E-cadherin-mediated cell competition, is then pushed out of the niche by its non-differentiating stem cell neighbor. Because E-cadherin-mediated regulation of stem cell-niche adhesion is directly linked to the functions of intrinsic self-renewal and differentiation factors, it can function as a quality control mechanism to ensure that only undifferentiated and functional stem cells remain in the niche and that differentiating GSCs lose the competition for niche occupancy and are forced to depart (Xie, 2012) (Fig. 3C). Interestingly, N-cadherin overexpression in HSCs enhances their ability to home to the niche, whereas N-cadherin knockdown or overexpression of dominant-negative N-cadherin reduces their homing capacity, indicating that manipulation of N-cadherin expression in HSCs might influence their homing to the niche (Hosokawa et al., 2010a). It will be interesting to investigate whether N-cadherin performs a function in the HSC niche similar to that of E-cadherin in the *Drosophila* ovarian GSC niche.

In the *Drosophila* testis, CySCs and GSCs share a common niche, the hub, and require Janus kinase-Signal transducer and activator of transcription (JAK-STAT) signaling for their maintenance (de Cuevas and Matunis, 2011). JAK-STAT signaling is required in GSCs to maintain E-cadherin expression, niche anchorage and self-renewal, and in CySCs to control BMP expression (Leatherman and Dinardo, 2010). In the *Drosophila* testis, competition exists between GSCs and CySCs and among CySCs themselves for occupancy of the hub (Issigonis et al., 2009). Interestingly, the CySCs with higher JAK-STAT signaling activity, which can be achieved experimentally by removing the function of the JAK-STAT negative regulator SOCS36E, can outcompete normal CySCs and, surprisingly, can also push GSCs out of the niche. This JAK-STAT-regulated stem cell competition is dependent on the cell adhesion protein βPS integrin, but not E-

cadherin. Integrin-mediated cell competition is thus thought to play a crucial role in balancing two stem cell populations in the same niche, but it remains unclear whether it could also function as a quality control mechanism for CySCs, GSCs or both (Fig. 3C).

Stem cell aging

The adhesion strength between stem cells and their niche also determines their long-term self-renewal potential. An age-dependent decline in E-cadherin expression is observed in the GSCs of the *Drosophila* ovary and testis, and increased expression of E-cadherin can slow down age-dependent GSC loss in both systems (Boyle et al., 2007; Pan et al., 2007). Similarly, the adhesion strength between HSCs and their niche appears to decrease with age, as the ability to mobilize HSCs drastically increases in older mice (Xing et al., 2006). Although the adhesion molecules affected by age in this system have not yet been identified, this aging-dependent increase in mobilization, which correlates with elevated levels of GTP-bound Cdc42, is intrinsic to HSCs. Interestingly, Cdc42 has also been shown by an independent study to be required in HSCs to maintain β1 integrin and N-cadherin expression, thereby regulating HSC homing and niche retention (Yang et al., 2007). Furthermore, platelet selectin (P-selectin), a transmembrane adhesion protein, is essential for hematopoiesis and its deficiency can enhance HSC repopulation (Sullivan et al., 2011). Interestingly, P-selectin expression in HSCs increases with age, and aged mice deficient in P-selectin show increased levels of long-term HSCs in comparison to controls of the same age, suggesting that P-selectin might be required for removing aged HSCs from the niche (Sullivan et al., 2011). Therefore, these findings demonstrate that dynamic stem cell-niche interactions are important for removing aged GSCs from the niche (Fig. 3D).

Regulation of adhesion between stem cells and the niche

Stem cell-niche adhesion can be regulated by niche-derived and systemic signals

The studies discussed above demonstrate that stem cell-niche adhesion is modulated by E-cadherin expressed in both the stem cells and the niche cells. Thus, there are two different mechanisms by which E-cadherin-mediated stem cell-niche adhesion can be modulated: via the stem cells themselves or via the niche cells. In the *Drosophila* ovary, E-cadherin expression in niche cells has been shown to be regulated by the systemic signal insulin (Hsu and Drummond-Barbosa, 2011). In addition, it is known that BMP signaling directly represses the expression of *bag of marbles* (*bam*) and that Bam works with Benign gonial cell neoplasm (Bgcn) to directly repress E-cadherin translation (Shen et al., 2009). It is therefore likely that BMP signals from the niche contribute to the regulation of E-cadherin expression in GSCs, although this possibility remains to be directly demonstrated. The best example of regulation of stem cell-niche adhesion by niche signals is the control of E-cadherin expression in *Drosophila* testis GSCs by niche-activated JAK-STAT signaling (Leatherman and Dinardo, 2010); inactivation of JAK-STAT signaling in GSCs leads to a decrease in E-cadherin accumulation at the GSC-niche junction and consequently to the departure of GSCs from the niche.

Although less is known about how niche signals regulate stem cell-niche adhesion in mammalian systems, a recent study has shown that, in the mammalian subventricular zone, Vcam1-mediated cell adhesion between NSCs and their niche is regulated by the systemic factor interleukin 1β (IL1β), which is known to be involved in inflammation (Kokovay et al., 2012). Therefore, it appears that both niche-derived and systemic signals regulate stem cell-niche interactions (Fig. 4A).

Stem cell-niche adhesion is regulated by stem cell-intrinsic factors

Genetic studies have identified a number of stem cell-intrinsic factors that are important for regulating stem cell-niche adhesion. Notably, many of these factors act by modulating E-cadherin expression or function in stem cells (Fig. 4A), although some function by regulating integrin activity (Fig. 4B). In the *Drosophila* ovary, Lissencephaly-1 (Lis-1), which is encoded by the *Drosophila* homolog of the causative gene (*LIS1*, also known as *PAFAH1B1*) for the human disease lissencephaly, is required in GSCs to maintain E-cadherin accumulation at the GSC-niche junction via an unknown mechanism (Chen et al., 2010). In addition, Lis-1 is required for centrosome positioning in *Drosophila* ovarian and testicular GSCs as well as for spindle orientation in neuroblasts and mouse neural progenitor cells (Chen et al., 2010; Siller and Doe, 2008; Sitaram et al., 2012; Yingling et al., 2008). However, it remains unclear whether centrosome positioning and spindle orientation are connected with the adhesion role of Lis-1. Eukaryotic initiation factor 4A (eIF-4A) is also required for maintaining E-cadherin expression in GSCs. By contrast, the differentiation factors Bam and Bgcn can repress E-cadherin translation, probably through the *E-cadherin* 3′UTR (Jin et al., 2008; Shen et al., 2009), and eIF-4A functions through direct interaction with Bam, antagonizing Bam-mediated translational repression of E-cadherin (Shen et al., 2009). Additionally, Poly(ADP-ribose) glycohydrolase (Parg) and the heterogeneous nuclear ribonucleoprotein Hrp38 (also known as Hrb98DE) are required for GSC maintenance and act by sustaining E-cadherin expression through translational regulation (Ji and Tulin, 2012); Parg degrades poly(ADP-ribose), which binds to Hrp38 to prevent Hrp38 association with the 5′UTR of *E-cadherin*. In FSCs, Cyclin E is required for niche anchorage and it is proposed to act by maintaining E-cadherin expression; *Cyclin E* mutant FSCs are lost from the niche, but the forced expression of E-cadherin can restore niche retention, suggesting that the FSC cell cycle is coupled with niche adhesion (Wang and Kalderon, 2009). Interestingly, the FSC loss phenotype caused by many other mutations, such as *SCAR*, *smoothened*, *Coprox* or *Actin-related protein 2/3 complex subunit 1* (*ArpC1*), can be partially rescued by forced expression of E-cadherin, indicating that E-cadherin-mediated cell adhesion between FSCs and their niche might represent the focal point for FSC regulation (Wang et al., 2012).

Endocytosis is important for regulating E-cadherin membrane targeting and adhesion in *Drosophila* epithelial cells (Classen et al., 2005; Leibfried et al., 2008; Shaye et al., 2008). Rab11, a key regulator of recycling endocytosis, has been suggested to be required for targeting E-cadherin to the apical side of GSCs, thereby maintaining E-cadherin accumulation at the stem cell-niche junction (Bogard et al., 2007). However, this finding requires further verification, as an independent study did not observe any changes in E-cadherin localization to the stem cell-niche junction in *Rab11*-deficient ovaries (Lighthouse et al., 2008). In the *Drosophila* testis, Rap-GEF/Rap signaling controls the accumulation of E-cadherin at the GSC-niche junction, thereby anchoring GSCs to the niche (Wang et al., 2006). Recently, the transmembrane receptor tyrosine phosphatase Leukocyte-antigen-related-like (Lar) has also been shown to be required in GSCs for maintaining E-cadherin accumulation between GSCs and their

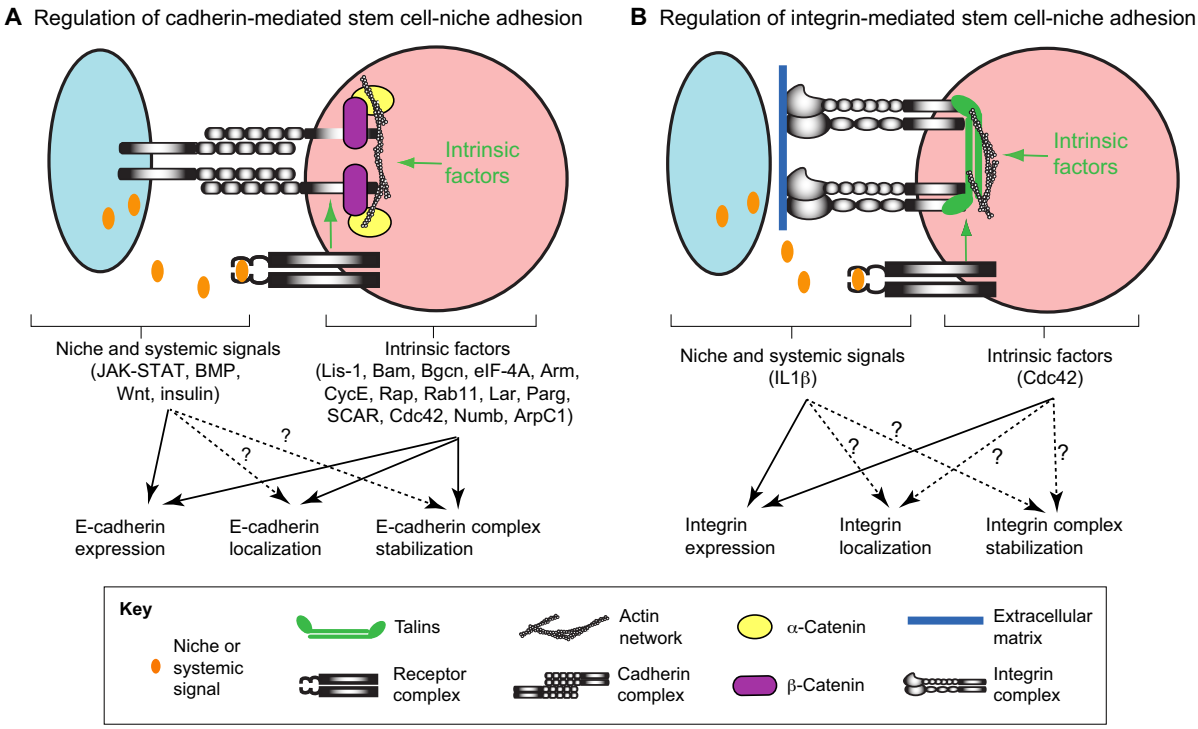

Fig. 4. Cadherin- and integrin-mediated stem cell-niche interactions are subject to regulation by niche-derived and stem cell-intrinsic factors. (**A**) Cadherin-mediated stem cell-niche adhesion is regulated by niche, systemic and intrinsic factors. Most of our current understanding comes from *Drosophila* stem cell systems. (**B**) Integrin-mediated stem cell-niche interaction can also be regulated by intrinsic, niche and systemic factors. Dashed lines indicate potential connections, which require further experimental verification.

niche in the *Drosophila* testis (Srinivasan et al., 2012). It is likely that on-going genetic studies in the *Drosophila* stem cell systems will identify more regulators of stem cell-niche adhesion.

Relatively few intrinsic factors are known to regulate stem cell-niche interactions in mammalian systems. In the mammalian hematopoietic system, Cdc42 regulates HSC-niche interaction by controlling the expression of β1 integrin and N-cadherin (Yang et al., 2007). In the developing ventricular region of the mouse brain, NSC-expressed Numb and numb-like, which interact with both N-cadherin and E-cadherin, are required to maintain AJs and the tissue integrity of the NSC niche (Rasin et al., 2007). Future genetic studies in mice are needed to identify additional intrinsic regulators of stem cell-niche adhesion.

Conclusions

It has been nearly a decade since it was shown that GSCs and FSCs are anchored in the niche via E-cadherin-mediated cell adhesion (Song and Xie, 2002; Song et al., 2002). Rapid progress has since been made toward understanding how different adhesion molecules can mediate stem cell-niche interaction as well as play other important roles in stem cell regulation. Based on findings from various stem cell systems (Fig. 1), the cadherin and integrin families of adhesion molecules have emerged as common mediators of stem cell-niche anchorage, although other classes of adhesion molecules are also involved (Fig. 2). Thus, depending on cell type, stem cells can use cadherins, integrins, or varying combinations of these and other adhesion molecules for their physical interaction with the niche. Such cadherin-mediated cell-cell interactions and integrin-mediated cell-ECM interactions can tether stem cells to their niche, allowing continuous exposure to niche-secreted short-range signals and cell contact-dependent signals, thereby maintaining long-term self-renewal. Stem cell-niche adhesion might also help transplanted stem cells home to suitable niches and establish stable stem cell-niche interactions.

In addition to niche anchorage, adhesion-mediated signaling events can help maintain stem cell self-renewal, proliferation and survival (Fig. 3). The most conceivable way for stem cell-niche adhesion to regulate stem cell function is by directly activating, facilitating or potentiating the intracellular signaling events that are important for stem cell self-renewal, proliferation and survival. One means by which stem cell-niche adhesion can regulate stem cell self-renewal is to control asymmetric versus symmetric cell division, and hence cell fate, through regulation of spindle and cleavage orientation (Fig. 3B). The modulation of stem cell-niche adhesion is thus an effective strategy for terminating or maintaining a stem cell's 'right' to self-renew, and stem cell competition for niche occupancy can represent an efficient quality control mechanism for eliminating differentiating or dysfunctional stem cells (Fig. 3C). Finally, because the adhesion strength between stem cells and the niche is affected by aging, manipulation of adhesion molecule expression might represent an effective way to fight against stem cell aging and, consequently, tissue and organismal aging (Fig. 3D).

Because it plays such an important role, stem cell-niche adhesion is tightly regulated by numerous factors, which can be niche-derived, stem cell-intrinsic or systemic (Fig. 4). In the *Drosophila* male and female germline systems, both niche signaling and stem cell-intrinsic factors are involved in the regulation of E-cadherin expression, membrane targeting and function. Although some progress has been made in understanding the regulation of stem

cell-niche adhesion in mammalian stem cell systems, adhesion in these systems remains poorly characterized relative to *Drosophila* stem cell systems. In the future, the systematic stem cell- or niche-specific knockout of adhesion molecules will elucidate their requirement in stem cell anchorage, proliferation and survival.

Despite the progress discussed above, many crucial questions concerning the regulation and function of stem cell-niche adhesion remain to be answered. The first and most important step is to define all of the adhesion molecules that are required for a given stem cell type to interact with its niche. Although this is a daunting task, it has become easier with more powerful tools for purifying stem cells and for identifying surface molecules that could potentially mediate interaction with the niche. Identifying such molecules will lay a solid foundation for determining their role in niche anchorage and in the regulation of stem cell behavior. The identified surface receptor molecules can then be studied effectively in different stem cell systems using gene knockout, knockdown and blocking antibodies to assess their roles in stem cell anchorage, self-renewal, proliferation and survival. However, the most challenging task in establishing the role of adhesion molecules in stem cell biology is to distinguish their adhesion-based roles from their other roles in stem cell maintenance *in vivo*. To directly decipher the roles of adhesion molecules in niche anchorage, live imaging techniques are likely to prove effective in gaining more insight into the dynamic physical interactions between stem cells and their niche. It is thus important to develop suitable *in vitro* binding assays using stem cells and niche cells for assaying the role of surface receptors in mediating stem cell-niche interaction. Additionally, it will be crucial to investigate whether the adhesion molecules that are important for niche anchorage also regulate stem cell function by directly and/or indirectly participating in intracellular signaling in stem cells. The last important question to address is how adhesion molecules are regulated at both the expression and functional levels by intrinsic self-renewal factors and niche signaling.

The answers to these questions will provide a better understanding of how adhesion molecules regulate stem cell biology, including niche anchorage, self-renewal, proliferation/quiescence and survival. New knowledge regarding stem cell adhesion will help the fight against cancer stem cells and age-dependent stem cell loss, and will also provide the foundation for improving the homing efficiency of transplanted stem cells for future stem cell therapy.

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