Stem Cell Niche: Structure and Function

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Abstract

Adult tissue-specific stem cells have the capacity to self-renew and generate functional differentiated cells that replenish lost cells throughout an organism's lifetime. Studies on stem cells from diverse systems have shown that stem cell function is controlled by extracellular cues from the niche and by intrinsic genetic programs within the stem cell. Here, we review the remarkable progress recently made in research regarding the stem cell niche. We compare the differences and commonalities of different stem cell niches in *Drosophila* ovary/testis and *Caenorhabditis elegans* distal tip, as well as in mammalian bone marrow, skin/hair follicle, intestine, brain, and testis. On the basis of this comparison, we summarize the common features, structure, and functions of the stem cell niche and highlight important niche signals that are conserved from *Drosophila* to mammals. We hope this comparative summary defines the basic elements of the stem cell niche, providing guiding principles for identification of the niche in other systems and pointing to areas for future studies.

Contents

INTRODUCTION

Stem Cell Behavior is Regulated by Both Extrinsic Signals and Intrinsic Programs

Stem cells are a subset of cells that have the unique ability to replenish themselves through self-renewal and the potential to differentiate into different types of mature cells. These characteristics therefore play essential roles in organogenesis during embryonic development and tissue regeneration. There are two main types of stem cells: embryonic and adult. The pluripotent embryonic stem cell is derived from the inner cell mass of blastocysts and has the ability to give rise to all three embryonic germ layers—ectoderm, endoderm, and mesoderm (Chambers & Smith 2004, Thomson et al. 1998). As development proceeds, the need for organogenesis arises, and the embryo proper forms germ line stem cells (GSCs) for reproduction and somatic stem cells (SSCs) for organogenesis. Although diversified, GSCs and SSCs retain the feature of self-renewal. They either are progressively restricted in development, giving rise to multiple lineages (including tissue-specific cells), or are unipotent, giving rise to single lineage cells destined for certain tissues (Fuchs et al. 2004, Rossant 2004, Weissman 2000). After birth, adult stem cells, including both GSCs and SSCs, reside in a special microenvironment termed the "niche," which varies in nature and location depending on the tissue type. These adult stem cells are an essential component of tissue homeostasis; they support ongoing tissue regeneration, replacing cells lost due to natural cell death (apoptosis) or injury. To sustain this function throughout the organism's life span, a delicate balance

between self-renewal and differentiation must be maintained. The underlying mechanisms that control this delicate balance are fundamental to understanding stem cell regulation, the nature of cancer/tumor formation, and the therapeutic use of stem cells in human disease.

There are various intrinsic programs that control stem cell self-renewal and potency (Morrison et al. 1997). For example, HoxB4 is sufficient to induce and expand hematopoietic stem cells (HSCs) when introduced into embryonic stem cells (Kyba et al. 2002, Sauvageau et al. 1995). Bmi, a member of the polycomb family, is required for self-renewal of stem cells in the hematopoietic and neural systems (Lessard & Sauvageau 2003, Molofsky et al. 2003, Park et al. 2003). To ensure appropriate control of stem cell behavior, these intrinsic genetic programs must be subject to environmental regulation. This is supported by many studies, some of which are discussed later. Therefore, both environmental regulatory signals and intrinsic programs are required to maintain stem cell properties and to direct stem cell proliferation and differentiation.

The Hypothesis of and Evidence for the Stem Cell Niche

In 1978, Schofield proposed the "niche" hypothesis to describe the physiologically limited microenvironment that supports stem cells (Schofield 1978). The niche hypothesis has been supported by a variety of coculture experiments in vitro and by bone marrow transplantation, in which the niche is first "emptied" through irradiation or drug treatments (Brinster & Zimmermann 1994, Dexter et al. 1977, Moore et al. 1997, Rios & Williams 1990, Roecklein & Torok-Storb 1995, Sitnicka et al. 1996). However, these studies did not resolve the issue of the exact stem cell location and niche structure in vivo (Simmons et al. 2001, Verfaillie et al. 1999).

Although locating and further identifying stem cell niches in mammals has been difficult owing to their extremely complicated anatomic structures, studies regarding stem cells and their location/niche in other genetic model systems, including those of *Drosophila* and *Caenorhabditis elegans*, have been fruitful. In *Drosophila*, GSCs were located in the anterior region of ovary germarium on the basis of lineage tracing and laser ablation (Lin & Spradling 1993, Wieschaus & Szabad 1979). In 2000, the germarial tip adjacent to GSCs was defined as the niche supporting GSCs in the *Drosophila* ovary (Xie & Spradling 2000), whereas the hub, located at the tip of *Drosophila* testis, served this function in testis (Kiger et al. 2001, Tulina & Matunis 2001). In *C. elegans*, a distal tip cell (DTC) located at the tip of the germ line organization region was found to function as the niche in supporting GSCs (Crittenden et al. 2002).

In mammals, the epithelial stem cell location was successfully identified in the bulge area of hair follicles, and the intestinal stem cell location was identified near the crypt base. These were based on the adult stem cell's ability to retain the BrdU or ^{3}H-thymidine labels (Cotsarelis et al. 1990, Potten et al. 2002). Recently, there has been significant progress regarding stem cells and their surrounding microenvironments in a variety of mammalian models. In 2003, two independent, simultaneous studies using genetic mutant mouse models led to the identification of osteoblastic cells, primarily those lining the trabecular bone surface, as the key component of the HSC niche (Calvi et al. 2003, Zhang et al. 2003). In the neural system, the stem cell niche was found in endothelial cells located at the base of the subventricular zone (SVZ) and subgranular zone (SGZ) (Doetsch et al. 1999, Palmer et al. 1997, Shen et al. 2004).

Historically, "niche" is generally used to describe the stem cell location. In our view, however, "niche" is composed of the cellular components of the microenvironment surrounding stem cells as well as the signals emanating from the support cells. In this review, we summarize the research defining the stem cell niche in *Drosophila* and mammals;

compare the differences and commonalities of stem cell niches in these different systems; and use this information to define the basic features, structures, and functions of the stem cell niche.

STEM CELL NICHES IN *DROSOPHILA* OVARY AND TESTIS

Germ Line Stem Cell and Somatic Stem Cell Niches in the *Drosophila* Ovary

Two or three GSCs are located at the tip of the ovariole in the structure referred to as the germarium. These GSCs are surrounded by three types of somatic cells: terminal filament, cap cells, and inner germarial sheath (IGS) cells (**Figure 1**). The stem cells are easily identified by their direct contact with cap cells and the presence of a spectrosome (Lin 2002, Xie & Spradling 2001). Normally, a GSC divides to generate two daughter cells: one daughter that stays in association with cap cells and another daughter that moves away from the cap cells to form a cystoblast, which eventually becomes, through incomplete cytokinesis, an interconnected 16-cell cyst. Genetic and cell biological studies demonstrate that cap cells are the niche for GSCs (Xie & Spradling 2000). The anchorage of GSCs to cap cells through E-cadherin-mediated cell adhesion is essential for maintaining GSCs (Song & Xie 2002). Also, the number of GSCs correlates with the number of cap cells (Xie & Spradling 2000). Finally, cap cells express genes, such as *dpp*, *gbb*, *hh*, *piwi*, and *Yb*, that are known to be important for maintaining GSCs (Cox et al. 2000, King et al. 2001, Song et al. 2004, Xie & Spradling 1998, 2000) (**Figure 1**).

BMP-, Hh-, and Piwi-mediated signaling pathways play an important role in the control of ovarian GSC self-renewal (**Figure 1**). Two BMP-like genes, *dpp* and *gbb*, are expressed in niche cells, and GSCs mutant for *dpp*, *gbb*, and their downstream components are lost prematurely (Song et al. 2004, Xie & Spradling 1998, 2000). Dpp overexpression completely prevents GSC differentiation and thereby causes GSC-like tumor formation (Song et al. 2004, Xie & Spradling 1998). BMP signaling was recently shown to exert control of GSC self-renewal by repressing expression of *bam* (Chen & McKearin 2003, Song et al. 2004), which is necessary and sufficient for cystoblast differentiation (Ohlstein & McKearin 1997).

Piwi- and Yb-mediated signaling is also required for controlling ovarian GSC self-renewal (Cox et al. 2000, King et al. 2001, Lin & Spradling 1997). Interestingly, Yb regulates expression of *piwi* and *hh* in TF/cap cells; these genes in turn control GSC self-renewal (King et al. 2001). Yb-mediated signaling is also involved in repressing *bam* expression in GSCs (Chen & McKearin 2005, Szakmary et al. 2005). It would be interesting to know the relationship between BMP signaling and Piwi-mediated signaling in controlling GSC self-renewal. Zero population growth (a *Drosophila* homolog of mammalian innexin-4) is expressed in GSCs and is also required for GSC maintenance, although the underlying molecular mechanism for such maintenance is largely unknown (Gilboa et al. 2003, Tazuke et al. 2002).

Two or three SSCs located in the middle of the germarium are responsible for generating somatic follicle and stalk cells (**Figure 1**). The follicle cells encapsulate 16-cell cysts, whereas the stalk cells connect adjacent egg chambers. Although the ovarian SSCs lack a unique marker, they can be identified using lineage tracing (Margolis & Spradling 1995, Song & Xie 2002, 2003, Zhang & Kalderon 2001). SSCs have low levels of Fasciclin III (Fas 3) expression, whereas differentiated follicle cells have high levels of Fas 3 expression. Loss of adhesion between SSCs and IGS jeopardizes SSC self-renewal, suggesting that the proximal IGS cells are at least a part of the SSC niche, anchoring the SSCs (Song et al. 2002). Although cap cells are not physically associated with SSCs, they produce two

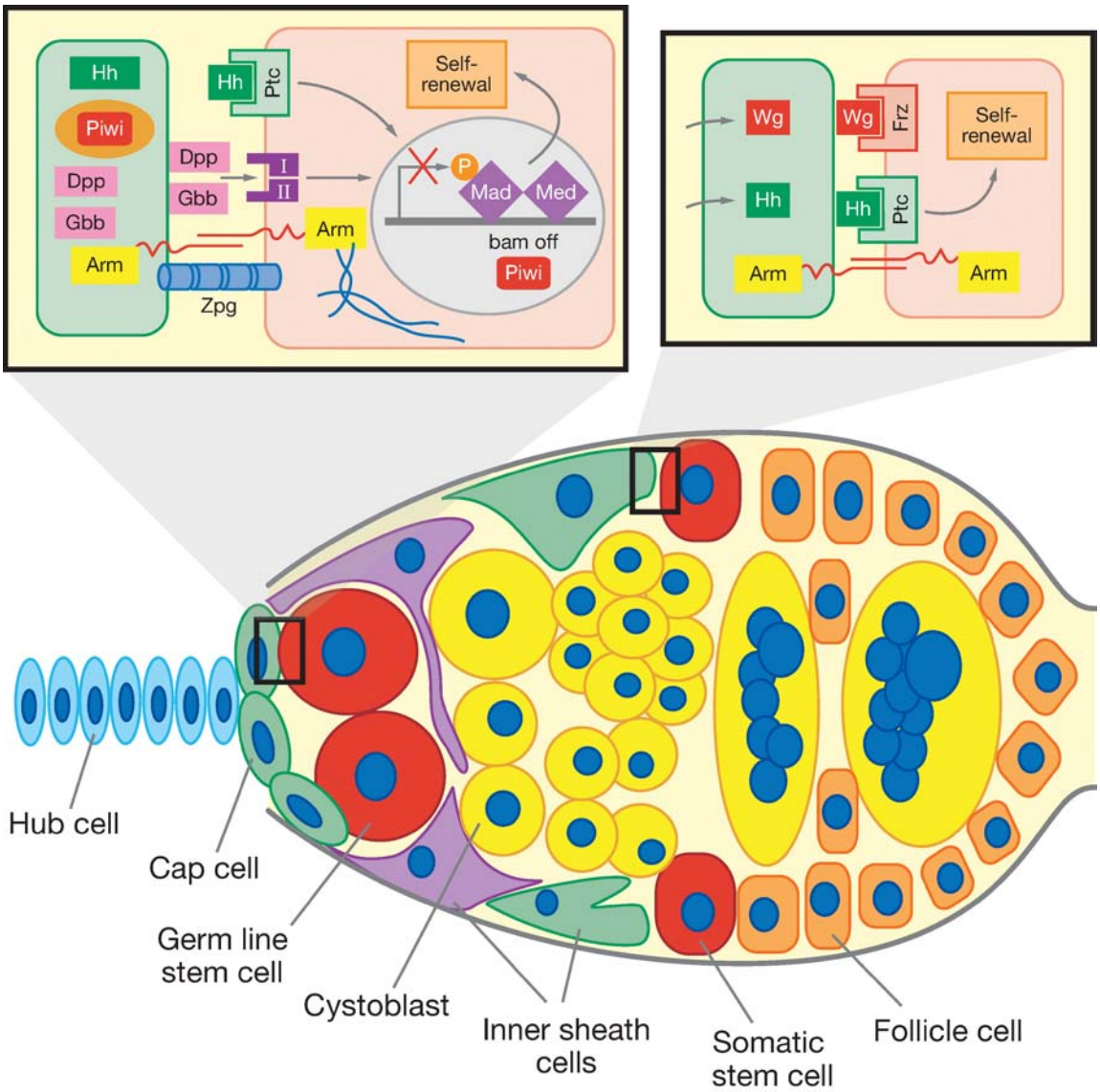


Figure 1

Drosophila germarium cross section showing the locations of germ line stem cells (GSCs), somatic stem cells (SSCs), and their niches. Two or three GSCs (*red* cells, *left*) are situated in their niche, composed of cap cells (*green* cells, *left*) and terminal filament cells (*light blue* cells, *left tip*), whereas their differentiated progeny, including cystoblasts and differentiated cysts (*yellow* cells, *middle*), are surrounded by inner sheath cells (*purple* cells and *green* cells, *bottom* and *top*). Two or three SSCs (*red* cells, *bottom* and *top*) directly contact the posterior group of inner sheath cells (*green* cells, *bottom* and *top*) forming their niche, whereas their differentiated progeny, also known as follicle progenitor cells (*orange* cells on *right*), further proliferate and generate differentiated follicle cells. Two inserts depict major signaling pathways controlling GSC (*top* and *left*) and SSC (*top* and *right*) self-renewal and proliferation; these inserts also depict niche cells (*green*) and stem cells (*pink*).

diffusible growth factors, Hh and Wg, that are required for controlling SSC maintenance and proliferation (Forbes et al. 1996, King et al. 2001, Song & Xie 2003). This supports the hypothesis that these cap cells are also a part of the SSC niche (Forbes et al. 1996; King et al. 2001, Song & Xie 2003, Zhang & Kalderon 2001).

The Germ Line Stem Cell Niche in the *Drosophila* Testis

In the apical tip of the *Drosophila* testis, two types of stem cells, GSCs and SSCs (the latter are also known as cyst progenitor cells), are responsible for producing differentiated germ cells and somatic cyst cells, respectively (Fuller 1993, Kiger et al. 2001) (**Figure 2**). Seven to nine GSCs, each containing a spectrosome, are attached to the hub (Hardy et al. 1979, Lindsley & Tokuyasu 1980, Yamashita et al. 2003). A male GSC divides asymmetrically, giving rise to one stem cell that remains in contact with the hub and one gonialblast that moves away from the hub and differentiates (Hardy et al. 1979, Lindsley & Tokuyasu 1980, Yamashita et al. 2003). As a GSC divides to produce a gonialblast, the neighboring SSCs also divide to generate two cyst cells, which envelop the gonialblast. This process leads to production of 64 sperm (Gonczy & DiNardo 1996, Hardy et al. 1979). The hub generates signals, including Unpaired (Upd) and BMP, to control GSC self-renewal (Kawase et al. 2004, Kiger et al. 2001, Shivdasani & Ingham 2003, Tulina & Matunis 2001) (**Figure 2**).

Upd from the hub activates the JAK-STAT pathway in GSCs and promotes their self-renewal (Kiger et al. 2001, Tulina & Matsunis 2001). Additionally, the activation of JAK-STAT signaling can reprogram mitotic germ cysts into GSCs (Brawley & Matunis 2004). As in the ovary, BMP signaling is required for controlling GSC self-renewal in the testis (Kawase et al. 2004, Schulz et al. 2004, Shivdasani & Ingham 2003). Hub cells and somatic cyst cells express *gbb* at high levels and *dpp* at much lower levels; consequently, BMP downstream components are essential for controlling testicular GSC self-renewal (Kawase et al. 2004). Because *dpp* overexpression fails to suppress completely spermatogonial cell differentiation, BMP signaling likely plays a permissive role in controlling male GSC self-renewal. BMP and JAK-STAT signaling pathways are required for controlling male GSC self-renewal; thus, they must somehow interact with each other. The integration between these two pathways in male GSCs is an important area in need of future exploration.

Gonialblast differentiation is tightly controlled by unknown signals from SSCs and somatic cyst cells (Kiger et al. 2001, Tran et al. 2000). In somatic cells mutant for *Egfr* and *raf*, GSC- and gonialblast-like single germ cells are greatly increased in number and remain active longer than do wild type cells.

One mechanism ensuring that only one of the two stem cell daughters self-renews is control of the spindle orientation of the stem cell so as to place one self-renewing daughter in the niche and the other daughter destined to differentiate outside the niche (**Figure 2**). Cnn and APC1, centrosomal components in GSCs, control orientation of the spindle perpendicular to the hub. Mutation in these components leads to an increase in GSC number and subsequent crowding in the niche. APC2, which is concentrated at the junction between GSCs and hub cells, also controls correct GSC spindle orientation (Yamashita et al. 2003).

THE GERM LINE STEM CELL NICHE IN *C. ELEGANS*

In the *C. elegans* hermaphrodite gonad, only the 225 germ cells closest to the distal tip cell (DTC) are mitotic; those further proximal are arrested in meiotic pachytene (Crittenden et al. 1994) (**Figure 3**). Specific stem cells within the mitotic region have not been

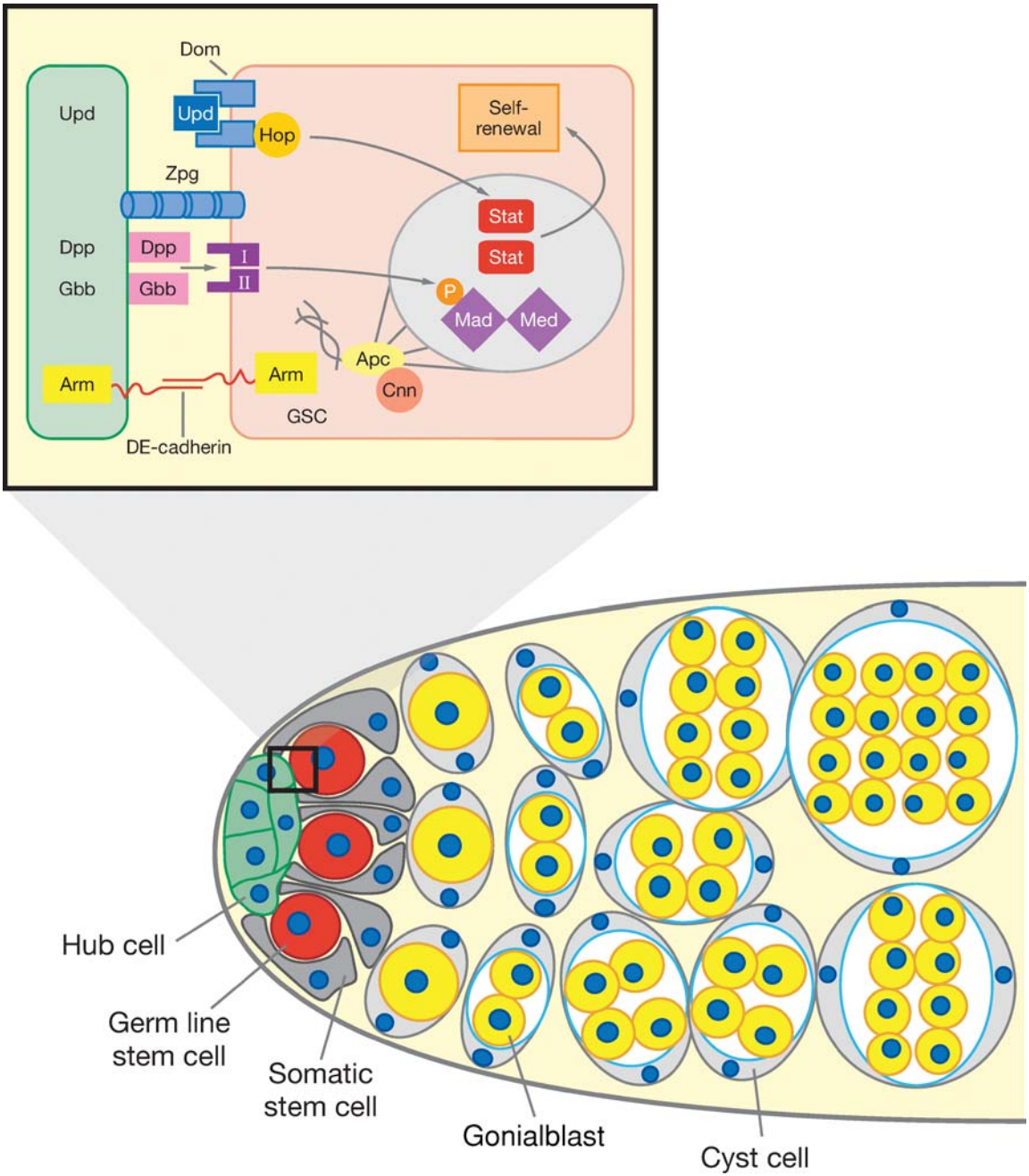


Figure 2

Cross section of the apical tip of the *Drosophila* testis, showing the locations of germ line stem cells (GSCs), somatic stem cells (SSCs), and their niches. Hub cells (*green*) at the apical tip of the testis form niches for both GSCs (*red*) and SSCs (*gray*, *left*), which generate, respectively, spermatogonial cells (*yellow*) and somatic cyst cells (*light gray*) encapsulating differentiated spermatogonial cells. The insert on top describes major signaling pathways involved in communication between GSCs and the niche cells for controlling self-renewal and proliferation.

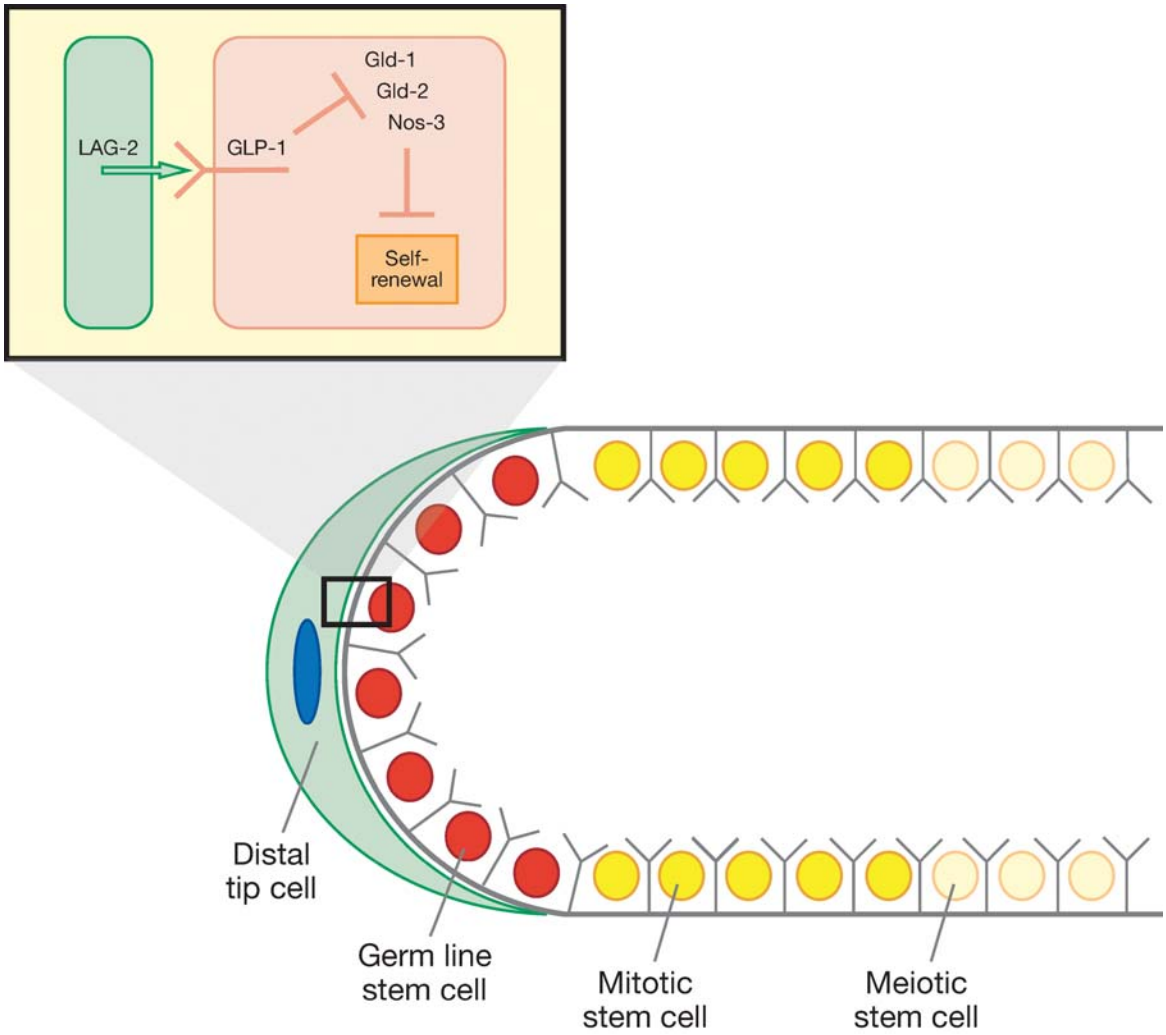


Figure 3

Cross section of the *C. elegans* hermaphrodite gonad. The putative germ line stem cells (GSCs) (*red*) are directly associated with their distal tip cell (DTC) niche cell (*green*), whereas their differentiated progeny (*light yellow*) move away from the DTC, progressing from the mitotic phase to the meiotic phase. The GLP-1 (Notch-like) signaling pathway is involved in communication between the DTC and GSCs and represses functions of differentiation-promoting gene products, such as Gld-1, Gld-2, and Nos-3, which regulate entry into meiosis (*insert*).

identified. The somatic DTC is required for maintaining these cells in mitosis (Kimble & White 1981). Although mitotic and meiotic germ cells in the tube share a central core of cytoplasm, only those mitotic germ cells located at the most distal tip (i.e., GSCs) adjacent to the DTC behave like stem cells, capable of self-renewing and generating differentiated gametes. The proximal mitotic neighbors behave more like transient amplifying cell populations, described in other systems. As germ cells move further away from the DTC, they terminate their mitotic activities and commit meiosis. Only those germ cells

that physically interact with the DTC maintain their GSC identity; thus, the signal from the DTC either must be short ranging or mediated by a direct cell-cell interaction.

Signaling from the DTC to control GSC self-renewal is through a Notch-like cascade. The mitotic germ cells express the Notch-type receptor, GLP-1, which is activated by the Delta-like signal from DTC, LAG-2 (Crittenden et al. 1994, Henderson et al. 1994). Constitutive GLP-1 activity downregulates the meiosis-promoting genes *gld-1*, *gld-2*, and *nos-3* and thereby causes expansion of germ cell numbers (Berry et al. 1997). Because individual stem cells have not been identified in the mitotic region, it remains unclear whether they are maintained through a population mechanism or an asymmetric division mechanism.

KNOWN STEM CELL NICHES IN MAMMALIAN SYSTEMS

The stem cell and the niche hypothesis, first developed in the hematopoietic system in mammals, has provided the conceptual background for stem cell studies in *Drosophila* and *C. elegans* (Schofield 1978, Weissman 1994). Conversely, studies in *Drosophila* on the molecular pathways controlling the stem cell niche have provided important insight into identification of the stem cell niche in mammalian systems (Lin 2002, Spradling et al. 2001). In this section, we describe and compare the location and physical organization (if known) of adult stem cells in bone marrow, skin/hair follicle, intestine, neuron, and testis.

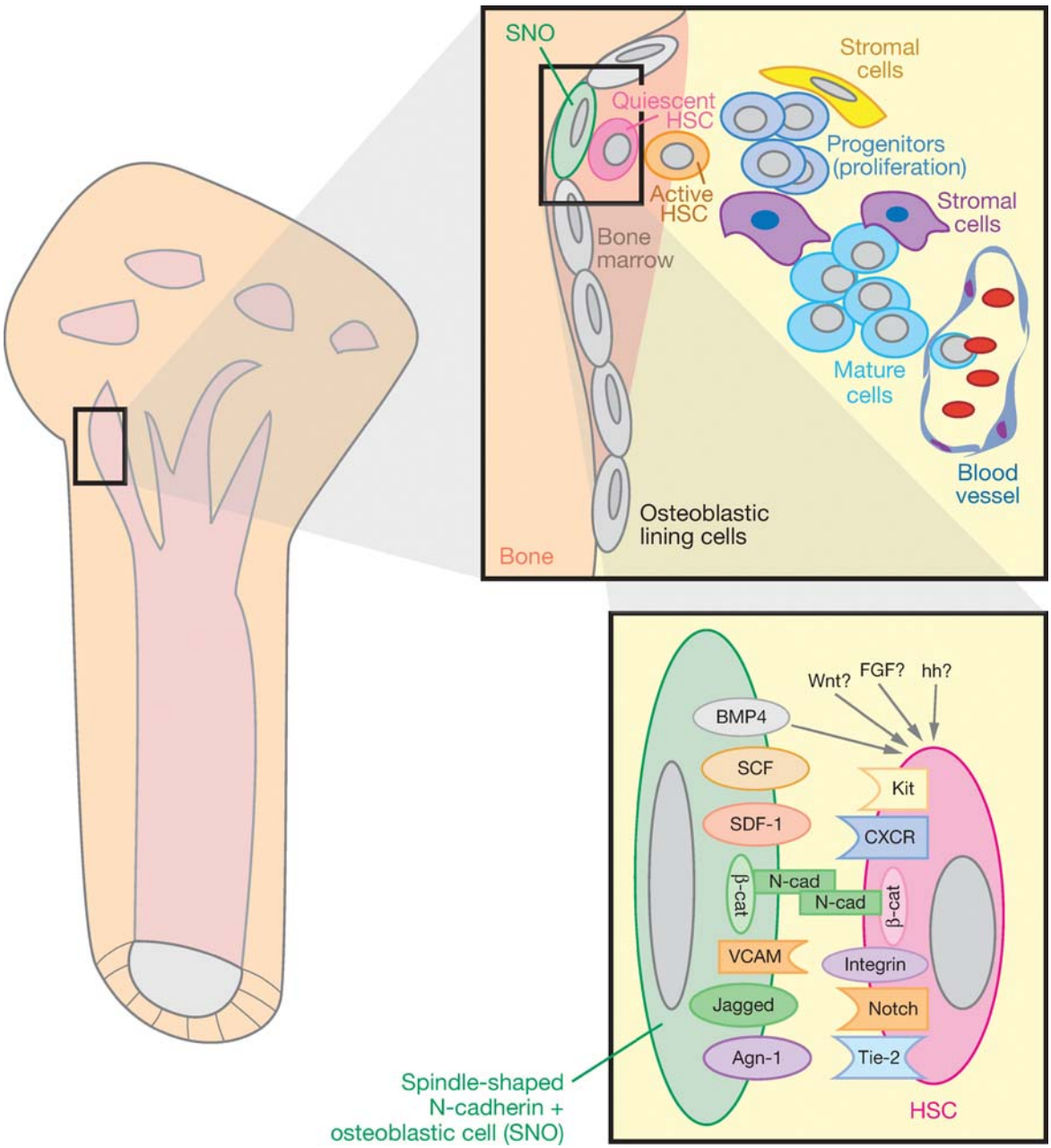
The Hematopoietic Stem Cell Niche

Bone marrow serves as the pioneer system for studying stem cells; the concept and basic features of stem cells were defined from studying hematopoietic stem cells (HSCs) (Orkin 2000, Till & McCulloch 1961, Weissman et al. 2001). However, the way in which HSCs interact with their local environment to promote stem cell maintenance has not been clear. Most studies of HSCs have examined their behavior in cell populations obtained from their natural niche in the bone marrow. Thus far, however, only limited culture systems exist that allow sustained maintenance and expansion of HSCs in vitro, attesting to the importance of as-yet poorly defined interactions in the bone marrow niche. Two independent studies recently 1) identified a subset of osteoblastic cells (N-cadherin$^+$CD45$^-$) to which HSCs physically attach in the bone marrow, 2) identified an N-cadherin/β-catenin adherens complex between HSCs and osteoblastic cells, 3) showed that Jagged1, generated from osteoblasts, influences HSCs by signaling through the Notch receptor, and 4) demonstrated that the number of N-cadherin$^+$ osteoblastic lining cells controls the number of HSCs (Calvi et al. 2003, Zhang et al. 2003). Homing studies to trace the location of GFP-labeled HSCs after transplantation also pointed to the endosteal surface as a possible stem cell niche (Nilsson et al. 2001). In vitro coculture of HSCs with osteoblasts can expand the HSC population (Taichman & Emerson 1998), and depletion of osteoblasts leads to loss of hematopoietic tissue (Visnjic et al. 2004). In addition, N-cadherin is a key target of Angiopoietin-1 (Ang-1)/Tie-2 signaling that maintains HSC quiescence (Arai et al. 2004) (**Figure 4**).

A primary function of the niche is to anchor stem cells. In addition to N-cadherin, other types of adhesion molecules, including integrin, play an important role in the microenvironment/stem cell interaction (Simmons et al. 1997). Stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 are involved in homing of HSCs (Lapidot & Kollet 2002) (**Figure 4**).

Although the analysis of the signals generated by the niche has just begun, gene expression profiling studies of HSCs have revealed which signals HSCs potentially receive from the niche. The components of evolutionally conserved and developmentally regulated pathways are prominent in stem cells

and are indeed involved in the regulation of stem cell self-renewal or maintenance. These components include the Shh, Wnt, Notch, and TGF-β/BMP pathways (Akashi et al. 2003, Gomes et al. 2002, Ivanova et al. 2002, Park et al. 2002, Ramalho-Santos et al. 2002). For example, the Wnt/β-catenin pathway is important for self-renewal of HSCs (Reya et al. 2003). The Notch pathway is required for maintaining HSCs in the undifferentiated state (Calvi et al. 2003, Duncan et al. 2005, Li et al. 1998, Varnum-Finney et al. 2000).



The BMP signal plays a role in control of HSC number (Zhang et al. 2003). The Shh signal mediated by the BMP pathway is able to maintain stem cells in vitro (Bhatia et al. 1999). (**Figure 4**).

The Epithelial Stem Cell Niche in Skin

Skin, with its appendix hair follicle structure, has well-organized architecture (**Figure 5**) and provides an excellent system for studying the molecular mechanisms that regulate stem cell self-renewal, proliferation, migration, and lineage commitment (Fuchs & Segre 2000). Each hair follicle is composed of a permanent portion, which includes sebaceous glands and the underlying bulge area, and a dynamic renewing portion, which undergoes cycles of anagen phase (a period of active growth), catagen phase (apoptosis-driven retraction), and telogen phase (a short period of rest) (Hardy 1992). The bulge area functions as a niche, where epithelial stem cells (Niemann & Watt 2002) are located and maintained (Cotsarelis et al. 1990, Sun et al. 1991). Epithelial stem cells are multipotent, giving rise to daughter cells that either migrate upward to serve as epidermal progenitors for generating epidermal cells during wound repair or migrate downward to convert to hair-matrix progenitors, which further give rise to the hair shaft (Niemann & Watt 2002, Oshima et al. 2001, Taylor et al. 2000).

During the early anagen phase, the dermal papilla region may provide the dynamic signals that activate stem cells; however, the cellular components of the niche in the bulge are yet to be defined other than as stem cells per se. The dermal sheath derived from mesenchymal cells adjacent to the epithelial stem cells in the bulge area most likely provides the niche function. The recent identification of markers for epithelial stem cells, such as CD34, will be helpful in further identifying the adjacent niche cells and the related niche structures, including adhesion molecules (i.e., $\alpha 6$ integrin) (Blanpain et al. 2004).

Recent studies showed that label-retaining cells can regenerate the entire HF structure in transplantation experiments, thus demonstrating that these cells are bona fide epidermal stem cells (Blanpain et al. 2004, Braun et al. 2003). Molecular analysis of epithelial stem cells has revealed the following features: 1) the expression of adhesion molecules known to be involved in stem cell-niche interaction, 2) the presence of growth inhibition factors such as TGFβ/BMP molecules and cell cycle inhibitors, and 3) the components of Wnt signaling pathways, including receptors and inhibitors such as Dkk, sFRP, and WIF. Taken together, these molecular features indicate that the epithelial stem cell niche is a growth- and differentiation-restricted environment (Tumbar et al. 2004). This conclusion is, in general, consistent with the many previous studies that have used genetic

Figure 4

Illustration of the hematopoietic stem cell (HSC) niche. The HSC niche is located primarily on the surface of trabecular bone, where a small subset of spindle-shaped N-cadherin-positive osteoblastic cells (indicated as SNO cells) are the key component of the HSC niche. N-cadherin and β-catenin form an adherens complex at the interface between stem cells and niche cells, assisting stem cells in attaching to the niche. Multiple growth factors and cytokines are involved in stem-niche interaction. These include SCF/Kit, Jagged/Notch, SDF-1/CXCR4, and Ang1/Tie2. BMP4 is expressed in osteoblastic cells, but the type of receptor expressed in HSCs is unknown. The Wnt signal is important for stem cell self-renewal, but the Wnts present in the niche are unknown. The same is true for FGF and hedgehog. In vitro data suggest they affect HSC behavior; however, whether they are present as niche signals is unknown. Different types of stromal cells (illustrated as different colors and shapes) may regulate stem cell activation, proliferation, and differentiation by secreting different microenvironmental signals. Finally, maturated blood cells migrate and infiltrate into blood vessel.

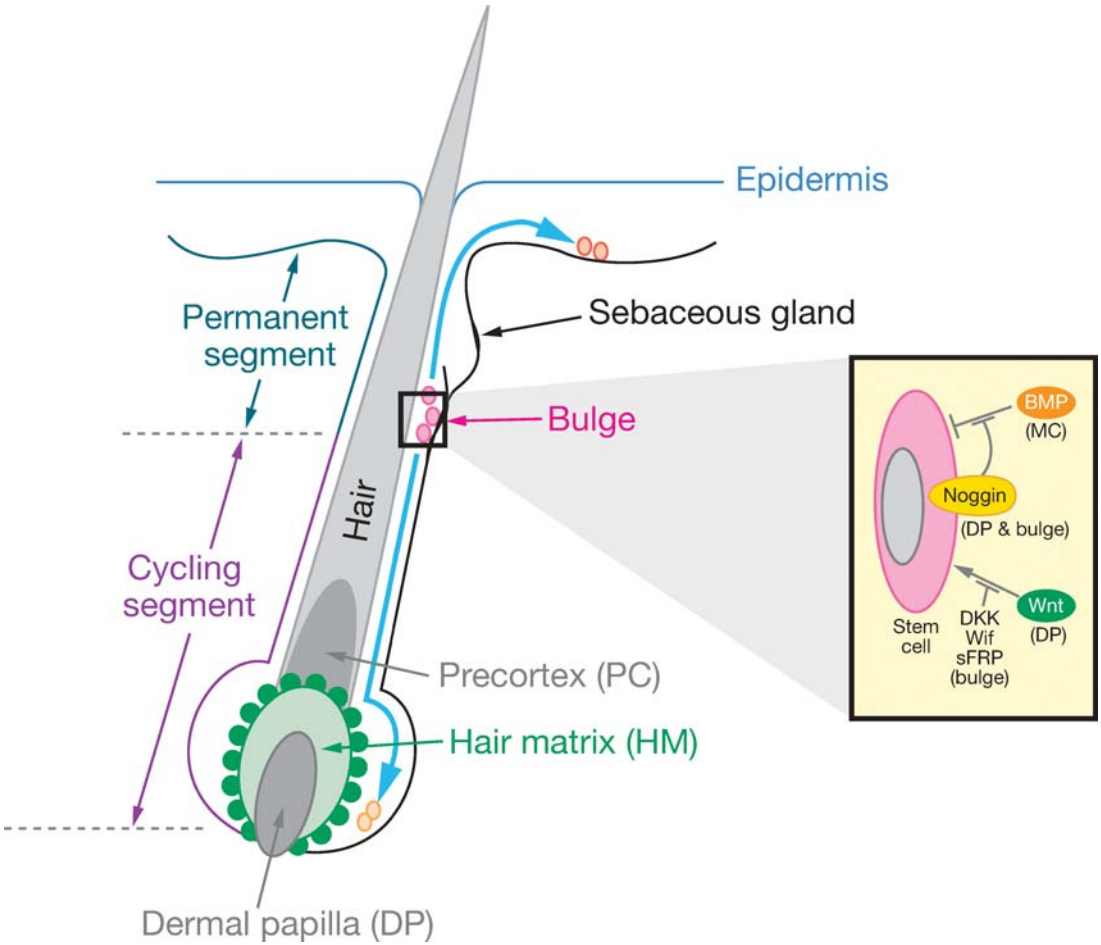


Figure 5

Illustration of the epidermal stem cells. Stem cells are located in the bulge region of the hair follicle beneath the sebaceous gland. Upon activation, stem cells undergo division; the daughter cells retained in the bulge remain as stem cells while other daughter cells migrate down to become hair-matrix progenitors responsible for hair regeneration. In neonatal mice or in damaged skin, stem cells can also migrate upward and convert to epidermal progenitors that replenish lost or damaged epidermis. The bulge area is an environment that restricts cell growth and differentiation by expressing Wnt inhibitors, including DKK, Wif, and sFRP as well as BMPs. During the early anagen phase, Wnts from dermal papilla (DP) and Noggin, which is derived from both DP and bulge (J. Zhang & L. Li, unpublished data), coordinate to overcome the restriction signals imposed by both BMPs and Wnt inhibitors; this leads to stem cell activation and subsequent hair regeneration. The FGF and Notch pathways are also involved in DP function for hair-matrix cell proliferation and lineage fate determination, but their influence on stem cells is not clear.

targeting and transgenic models to reveal that signaling molecules, including Wnts, Notch, and BMPs, have important roles in the regulation of HF development and regeneration (Fuchs et al. 2001, Jones et al. 1995, Lavker et al. 1993, Watt 2001).

Among these various signaling molecules, two family members are prominent, reflecting their important roles in controlling stem cell behavior. One is the Wnt signaling pathway which, through regulating β-catenin activity, controls stem cell activation, fate

determination (by favoring HF over epidermal cell lineages), and differentiation (Gat et al. 1998, Huelsken et al. 2001, Merrill et al. 2001, Niemann et al. 2002). The second controlling pathway is the BMP signaling pathway (Hogan 1996). Although it is also required for HF differentiation at a later stage, BMP signaling, as opposed to Wnt signaling, restricts the activation of stem cells and favors epidermal cell fate (Botchkarev 2001, 2003, Kulessa et al. 2000). These observations also support the theory that coordination between Wnt and Noggin (through temporarily overriding the BMP restriction on stem cells) is required to initiate each hair growth cycle (Jamora et al. 2003 and J. Zhang & L. Li, unpublished data).

The Intestinal Stem Cell Niche

The intestinal architecture is composed of a sequential array of zones (or compartments) along the villus-crypt axis (**Figure 6**). Intestinal regeneration begins with intestinal stem cells (ISCs), which give rise to four different types of epithelial lineages: columnar enterocytes, mucin-producing goblet cells, Paneth cells, and enteroendocrine cells (Bjerknes & Cheng 1999, Hermiston & Gordon 1995, Winton 2000). ISCs are generally proposed to be located at the fourth or fifth position from the crypt bottom, above the Paneth cells (Booth & Potten 2000, He et al. 2004, Sancho et al. 2004), as evidenced either through a DNA-labeling retaining assay (Booth & Potten 2000, He et al. 2004, Potten et al. 1997, 2002) or through regeneration dynamics using chimeric mouse lines (Winton 2000, Bjerknes & Cheng 1999). A number of molecules—Telomerase, Tcf4, EphB3, P-PTEN, P-Akt, 14-3-3ζ, Noggin, and Musashi-1—are expressed in the proposed ISC position near the crypt base (Batlle et al. 2002, Booth & Potten 2000, He et al. 2004, Korinek et al. 1998, Nishimura et al. 2003). However, a combination of these markers and the cell position is required to locate ISCs more accurately.

During postnatal intestinal regeneration, mesenchymal cells subjacent to epithelial cells play a role in directing epithelial cell proliferation, differentiation, and apoptosis. BMP4, expressing in the ISC-adjacent mesenchymal cells, is one of the putative niche signals (He et al. 2004). However, the type of mesenchymal cells that expresses BMP4 adjacent to the ISCs is yet to be identified. Endothelial cells composed of vascular vessels have also been proposed to provide ISCs with survival signals such as FGF (Paris et al. 2001). Myofibroblasts that are distributed to the surrounding epithelial cells are proposed to be the candidate "niche" supporting ISCs and influencing other epithelial cells (Mills & Gordon 2001).

We have just begun to understand which niche signals regulate self-renewal and maintain the balance between self-renewal and differentiation of ISCs. An increasing number of molecules, including Wnt, BMP, FGF, Notch, and the underlying signal pathways, may play roles in this regard (Brittan & Wright 2002, Roberts et al. 1995, Sancho et al. 2004). Gene expression profiling revealed that Myc-related pathways and the PI3K/Akt pathway are predominantly present in these stem/progenitors (Mills et al. 2002, Stappenbeck et al. 2003). Inappropriate activation of the Wnt/β-catenin, which targets on Myc, results in the development of tumors as a consequence of an overproduction of stem cells (Clevers 2004). In addition, mutations in BMPRIA and its signaling mediator SMAD4 have been found in Juvenile polyposis syndrome (Howe et al. 1998a,b). Recent studies using gene targeting demonstrated that BMP signaling has a role in suppression of Wnt signaling and thereby maintains a balanced control of stem cell activation and self-renewal (Haramis et al. 2004, He et al. 2004). Mechanistically, inhibition of Wnt signaling by the BMP signal involves both the PTEN/PI-3k/Akt pathway and Smad-mediated transcriptional control (Haramis et al. 2004, He et al. 2004)

In summary, Wnt signaling plays a positive role in promoting ISC activation/self-renewal

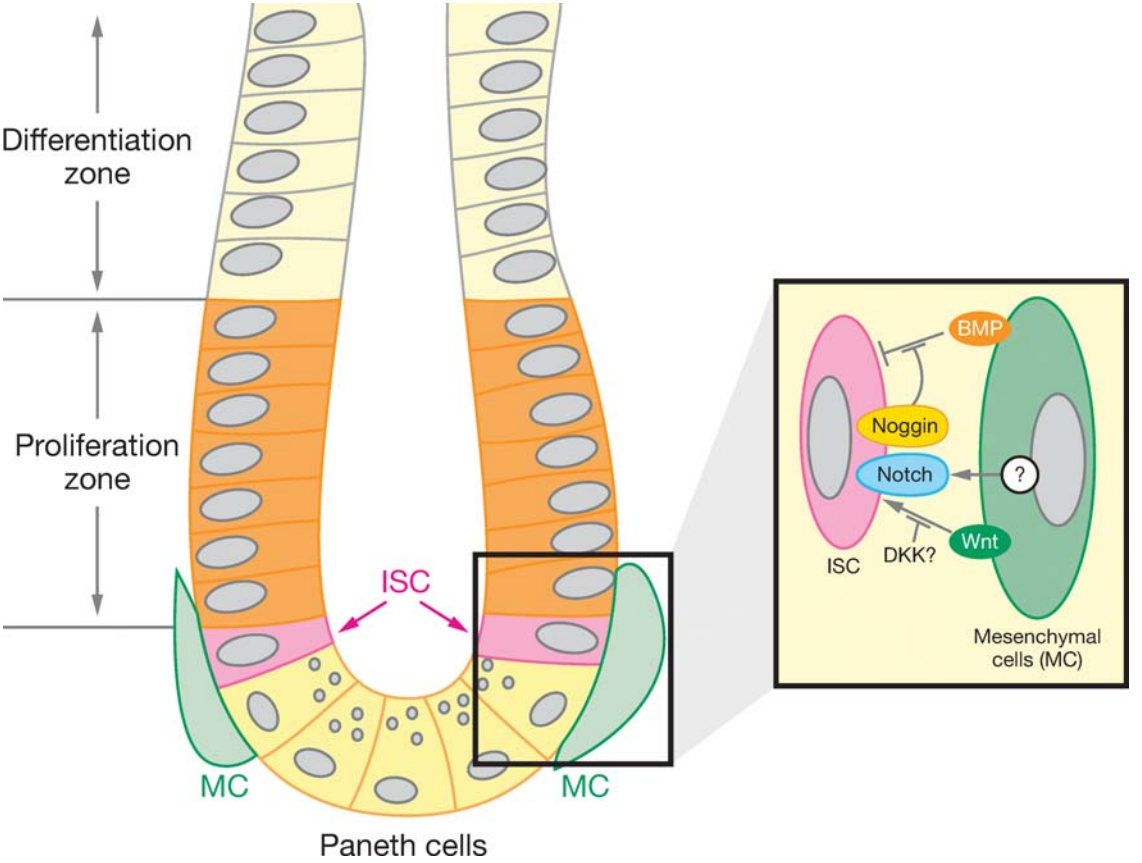


Figure 6

Illustration of the intestinal stem cell (ISC) niche. ISCs (*pink*) are located at the fourth or fifth position above the Paneth cells, as measured from the crypt base. Mesenchymal cells (*green*) adjacent to the ISCs function as the niche. BMP4 expressed from the niche influences the ISCs through its receptor Bmpr1a. Wnt signaling is present throughout the crypt, as revealed by phosphorylated coreceptor LRP6 (He et al. 2004). However, which Wnt receptor is expressed in stem cells is not yet well defined. Whether Wnt inhibitors, such as Dkk, are expressed in the ISC niche also is still unknown. The Notch pathway is known to affect stem/progenitor lineage fate. The expression patterns of the Notch receptor and ligand need to be determined. Noggin expression can be detected in stem cells, but its expression is transient. Noggin is proposed to be a molecular switch coordinating with Wnt signaling to fully activate stem cells by overriding BMP restriction signaling (He et al. 2004).

and crypt cell fate (van de Wetering et al. 2002); in contrast, BMP signaling restricts ISC activation/self-renewal and crypt cell fate (Haramis et al. 2004, He et al. 2004). Importantly, in intestine as well as in HF, overriding the restriction of BMP activity by Noggin as well as by active Wnt signaling is required to fully activate stem cells and support ongoing regeneration (He et al. 2004, Jamora et al. 2003).

The Neural Stem Cell Niche

In the 1990s, studies from a number of research groups led to the identification of neural stem cells (NSCs) (Alvarez-Buylla et al. 1990, Johe et al. 1996, Lois & Alvarez-Buylla 1993, Reynolds & Weiss 1992). NSCs can be isolated from various regions in the adult brain and peripheral nervous system. However, the subventricular zone (SVZ) and the

subgranular zone (SGZ) of the hippocampus region are the primary and well-characterized germinal regions in which NSCs reside and support neurogenesis in the adult brain (Doetsch 1999, 2003, Lois & Alvarez-Buylla 1993, Palmer et al. 1997, Temple 2001).

There are four types of cells in the SVZ (**Figure 7**). A layer of ependymal cells lining the lateral ventricle (LV) region separates the SVZ from the LV. SVZ astrocytes are located adjacent to the ependymal cells, with a single cilium structure extending through the boundary of ependymal cells to contact the LV region and to form a glial tunnel that embraces a group of neuroblasts. Immature cells derived from SVZ astrocytes are precursors for neuroblasts. A specialized basal lamina extending from the blood vessels to the ependymal cells contacts all cell types in the SVZ. The SVZ astrocytes, which express astrocyte marker glial fibrillary acidic protein (GFAP), have stem cell features: They undergo self-renewal and give rise to transient amplifying precursor C cells, which further give rise to neuroblasts. Neuroblasts differentiate into neurons that migrate toward the olfactory bulb and other regions. In addition to producing neurons, SVZ astrocytes can also generate oligodendrocytes (Doetsch 2003, Mirescu & Gould 2003, Temple 2001). In the hippocampus, the SGZ is a germinal layer between the hilus and the dentate gyrus, and is responsible for generating dentate gyrus neurons (Palmer et al. 1997). In the SGZ, neurogenesis occurs locally in direct contact with blood vessels. SGZ astrocytes also express GFAP and function as stem cells, undergoing self-renewal and generating daughter cells that further produce granule neurons (**Figure 7**) (Doetsch 2003, Temple 2001).

In both the SVZ and SGZ structures, endothelial cells that form blood vessels and the specialized basal lamina are an essential component of the NSC niche: These endothelial cells provide attachment for SVZ and SGZ astrocytes and generate a variety of signals that control stem cell self-renewal and lineage commitment (Doetsch 2003, Shen et al. 2004). Signals generated from the niche include BMPs and their antagonist Noggin, FGFs, IGF, VEGF, TGFα, and BDNF. The BMP signal favors astrocyte lineage fate by inhibiting neuronal fate. In contrast, Noggin functions to inhibit BMP signaling and thereby favors neurogenesis (Temple 2001). An adherens junction composed of cadherins and β-catenin also plays a role in maintenance of stem cells. Interestingly, overexpression of β-catenin leads to expansion of the NSC population; this presumably reflects activation of Wnt signaling (Chenn & Walsh 2002). This phenotype is very similar to overexpression of IGF in transgenic mice, in which an increased brain size is also observed (Aberg et al. 2003). Both EGF and bFGF are able to expand NSCs in an in vitro culture system. In addition, signaling pathways, including Notch and PTEN/PI3K, are also involved in NSC regulation (Doetsch 2003, Temple 2001).

The Germ Line Stem Cell Niche in Mice

Stem cell transplantation capability, simple anatomy, and genetics make the mouse testis an attractive model for studying GSCs and their niche. The GSCs in mice are single cells that are located in the periphery of seminiferous tubules and that have the ability to self-renew and generate a large number of differentiated gametes (Brinster 2002) (**Figure 8**). GSCs in the mouse testis each divide asymmetrically to generate a GSC and a differentiated daughter, which forms an interconnected A_{pair} spermatogonial cell. The A_{pair} spermatogonial cell then divides synchronously to form a chain of interconnected spermatogonial cells. Stem cells, spermatogonia, spermatocytes, spermatids, and sperm cells can be distinguished by their spatial relation to differentiating sperm cells. GSCs are very rare and can be isolated using fluorescence-activated cell sorting (FACS) as a population of α_v-integrin$^{-/dim}$ α_6-integrin^{+} Thy-1$^{lo/+}$ C-kit^{-} cells (Kubota et al. 2003). Sertoli cells,

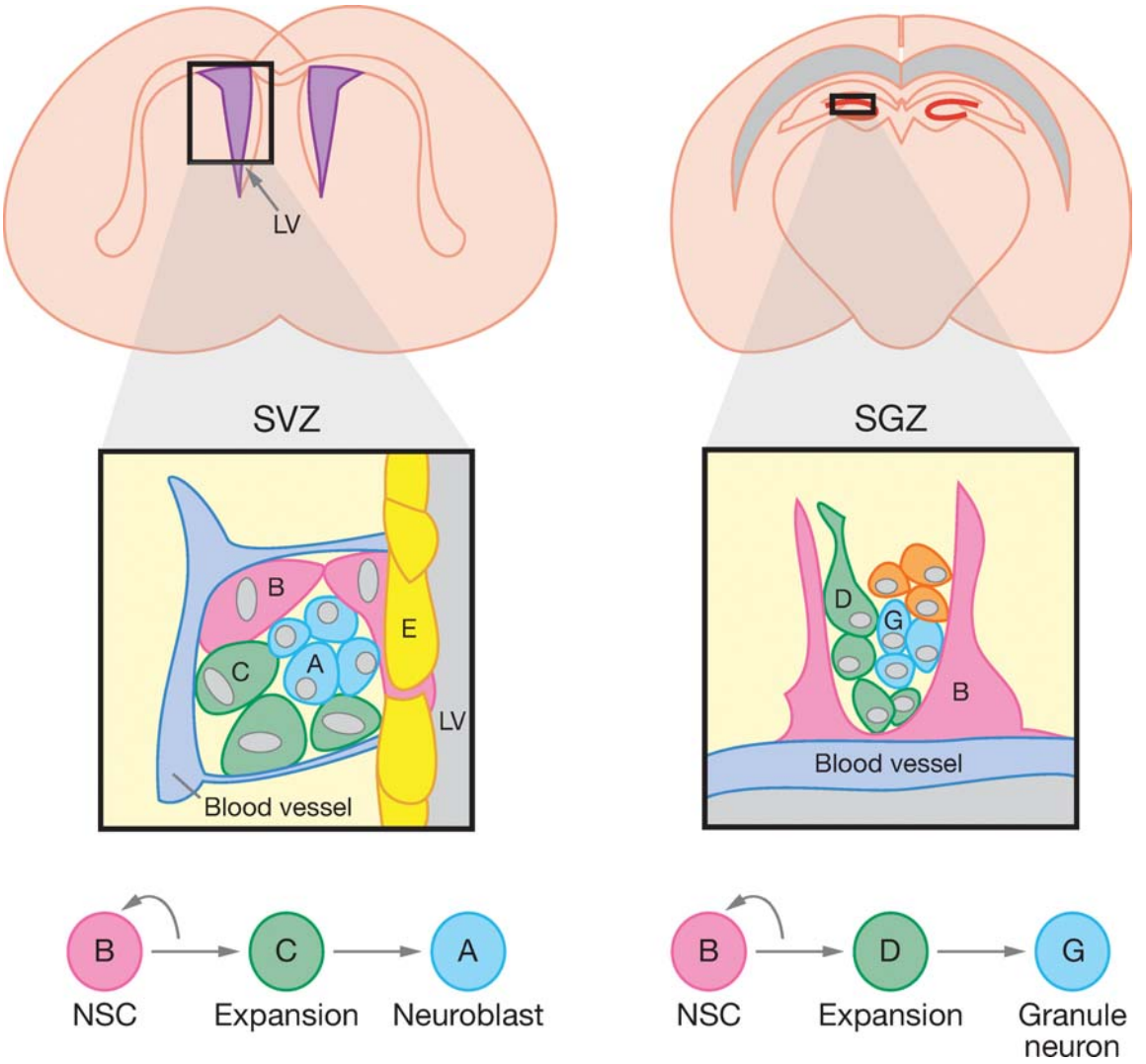


Figure 7

Illustration of the neural stem cell (NSC) niche. The subventricular zone (SVZ) and the subgranular zone (SGZ) are two well-characterized germinal regions in which NSCs (*pink*) are located. In the SVZ, astrocytes (*B*) lining the ependymal cells (*E*) function as NSCs; they give rise to transient amplifying cells (*C*) (*green*), which further produce neuroblast cells (*A*) (*blue*). Endothelial cells in the blood vessel/laminar maintain contact with astrocytes, which regulate NSC self-renewal and proliferation by generating different types of signals. In the SGZ, astrocytes (*B*) directly attach to the blood vessel and receive signals from the endothelial cells that direct NSCs to undergo self-renewal, proliferation (*D*), and differentiation (*G*). The figure is adapted and modified with permission from Doetsch 2003.

the somatic cells of the seminiferous tubules that physically interact with the stem cells, likely constitute functional niches for the stem cells by providing growth factors that promote stem cell self-renewal and/or proliferation.

Several studies support the idea that Sertoli cells regulate the maintenance of the stem cell pool (although little is known about the underlying molecular mechanisms). First, studies in which male GSCs and Sertoli cells

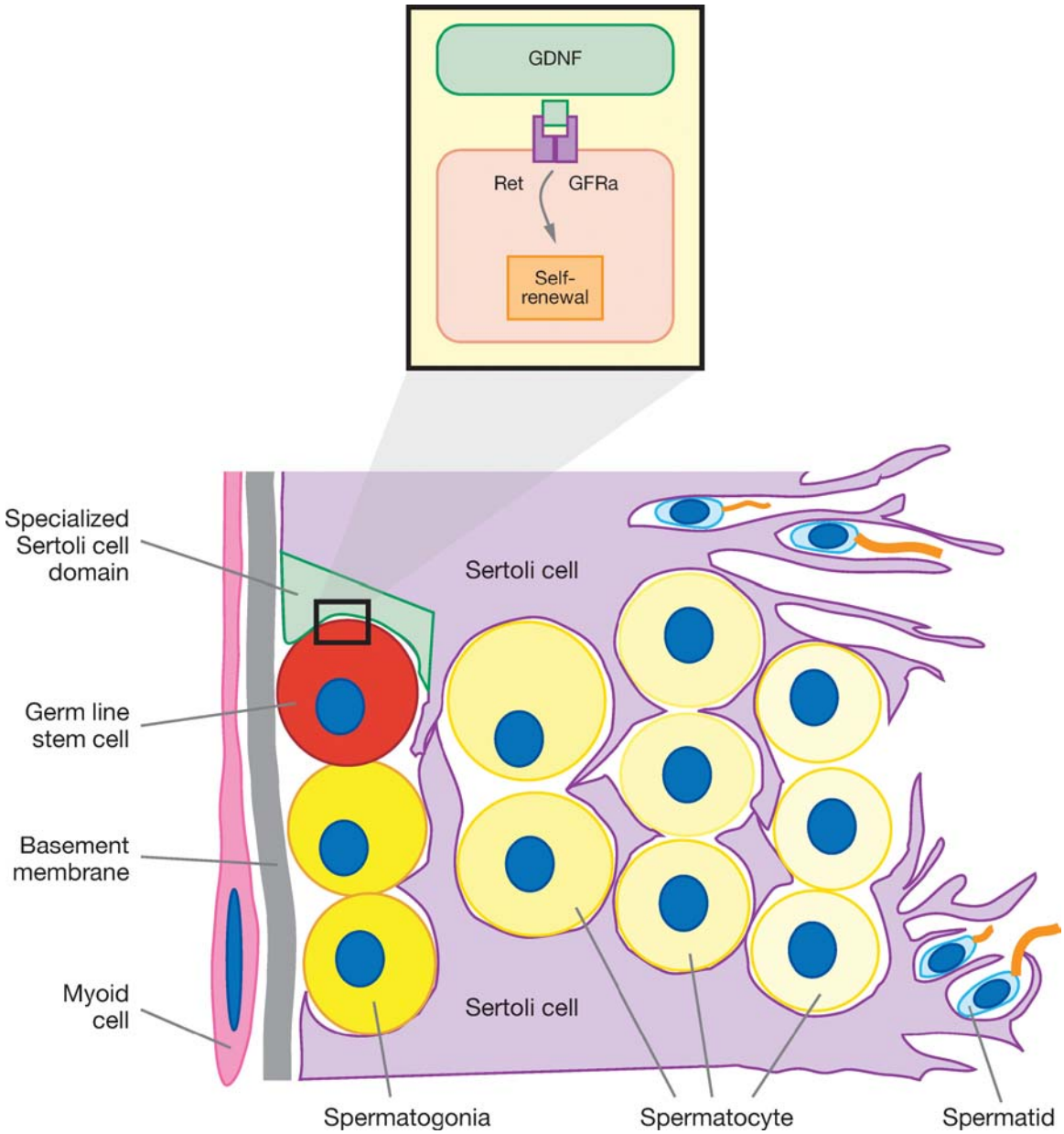


Figure 8

Cross section of a small section of mouse testis. A germ line stem cell (GSC) (*red*) directly contacts a Sertoli cell's (*purple*) basement membrane (*gray*) secreted by myoid cells (*pink*), and specialized region (*green*), which together may form a putative GSC niche. Myoid cells (*pink*) may also participate in niche function, as they are close to GSCs. The differentiated spermatogonial cells (*yellow*) are germ-line cysts that move through different domains formed by Sertoli cells toward the lumen, where mature sperm are released. The GDNF pathway, depicted in the insert (*top*), is a known major pathway for controlling GSC self-renewal in the mouse testis.

are transplanted into infertile mice show that Sertoli cells indeed can support GSC maintenance and spermatogenesis (Ogawa et al. 2000, Shinohara et al. 2000, 2003). Second, GDNF, a member of the TGF-β superfamily produced by Sertoli cells, can control GSC self-renewal and maintain GSCs in vitro (Kanatsu-Shinohara et al. 2004, Kubota et al. 2004). Therefore, Sertoli cells contribute to the function of the GSC niche. Future study is needed to define the physical structure of the GSC niche and its associated signals in the mouse testis.

CONCLUSION AND PROSPECTIVE

Common Features, Structures, and Functions of the Stem Cell Niche

After comparison of the stem cell niches in the ovary and testis of *Drosophila* and in *C. elegans*, as well as in mammalian bone marrow, hair follicle, intestine, brain, and testis, the common features, structures, and functions of the stem cell niche are summarized as follows:

1. The stem cell niche is composed of a group of cells in a special tissue location for the maintenance of stem cells. The niche's overall structure is variable, and different cell types can provide the niche environment. For example, N-cadherin-positive osteoblastic lining cells in the trabecular bone form the niche for HSCs, whereas endothelial cells form the NSC cell niche.
2. The niche functions as a physical anchor for stem cells. E-cadherin-mediated cell adhesion is required for anchoring GSCs and SSCs in *Drosophila*, and N-cadherin may be important for anchoring HSC in the bone marrow niche. Other adhesion molecules, such as integrins, may help anchor stem cells to extracellular matrixes.
3. The niche generates extrinsic factors that control stem cell fate and number. Many signal molecules have been shown to be involved in regulation of stem cell behavior, including hh, Wnts, BMPs, FGFs, Notch, SCF, Ang-1, and LIF or Upd through the JAK-Stat pathway. Among these, the BMP and Wnt signal pathways have emerged as common pathways for controlling stem cell self-renewal and lineage fate from *Drosophila* to mammals. Several pathways can be utilized to control self-renewal of one stem cell type, whereas one growth factor can regulate several different stem cell types. The presence of signaling components of multiple conserved developmental regulatory pathways in stem cells supports the ideas that stem cells retain the ability to respond to these embryonic regulatory signals and that orchestration of these signals is essential for proper regulation of stem cell self-renewal and lineage commitment.
4. In invertebrates and mammals, the stem cell niche exhibits an asymmetric structure. Upon division, one daughter cell is maintained in the niche as a stem cell (self-renewal); the other daughter cell leaves the niche to proliferate and differentiate, eventually becoming a functionally mature cell.

FUTURE DIRECTIONS

Recent studies regarding the stem cell niche in different organisms, including various mammalian organ systems, have resulted in significant progress; fundamental principles about the niche have been established. We hope that the knowledge gained from these studies discussed above will provide guidelines for defining the stem cell niche in other systems. Using a combination of genetic, molecular, and cell biological approaches, several important signaling pathways from the various niches have been identified for

their ability to maintain and regulate self-renewal of stem cells. In general, multiple conserved developmental regulatory signals coexist; therefore, orchestration of these signals is essential for proper regulation of stem cell self-renewal and lineage commitment. Further studies of the cross-talk between these signal pathways and the relationship between these pathways and the intrinsic factors required for self-renewal and maintenance of stem cells will provide further insight into the molecular mechanisms governing stem cell self-renewal and differentiation.

Cellular and Molecular Components of the Stem Cell Niche

In uncovering other molecular components of the stem cell niche, genetic screening in *Drosophila* will continue to be an efficient method of identification of novel factors. In mammals, systematic analysis of gene expression in the niche cells (Hackney et al. 2002) will be as important and fruitful as analysis of gene expression in stem cells. For example, systematic analysis of the N-cadherin-positive osteoblastic lining cells, using gene array to compare to other types of marrow stromal cells, including N-cadherin-negative osteoblastic cells, is required to uncover any unique genes predominantly expressed in the HSC niche cells. Furthermore, comparisons of niche- and stem cell–specific gene profiles in different systems will provide important insight into the critical niche signals and intrinsic factors that potentially influence stem cell behavior. Thus, conserved signal molecules and intrinsic factors important for stem cell self-renewal and maintenance and specific factors unique to each stem cell niche can be identified.

Asymmetric Versus Symmetric Stem Cell Division

The stem cell niche exhibits structural asymmetry, and asymmetric division of stem cells is one of the proposed mechanisms controlling the balance between self-renewal and differentiation. This has been well illustrated in the *Drosophila* system. Whether this mechanism is preserved in the mammalian system needs to be determined. The centrosome-associated proteins APC1 and centrosomin are important in controlling spindle orientation during stem cell division in *Drosophila* (Yamashita et al. 2003). It is important to investigate whether control of spindle orientation is essential for asymmetric division of stem cells in other systems as well.

Stem Cell Maintenance and Reversion from Committed Daughter Cells

As described above, asymmetric stem cell division leads to the retention of one daughter cell in the niche (stem cell) and to the other daughter cell leaving the niche to become committed, an irreversible process in the normal physiological condition. Whether the committed daughter cell can revert to a stem cell if restored to the niche is an interesting and important question. Two recent studies in *Drosophila* provide solid evidence indicating that this may be possible (Brawley & Matunis 2004, Kai & Spradling 2004). It remains to be seen whether this is a general feature for different types of stem cells in invertebrates and mammals.

Normal Stem Cells and Cancer Stem Cells: Niche-Dependent or Niche-Independent

The concept of cancer stem cells has changed the perspective on cancer, in which stem cells and their underlying self-renewal is key. In adults, the niche prevents tumorigenesis by controlling stem cells in the arrested state and maintaining the balance between self-renewal and differentiation. In this context, any mutation that leads stem cells to escape from the niche control may result in tumorigenesis. It is therefore reasonable to hypothesize that one of the differences between normal stem cells

and cancer stem cells is that cancer stem cells may no longer be dependent on niche signaling. This hypothesis needs to be tested.

CLOSING REMARKS

Stem cell behavior is regulated by coordination of environmental signals and intrinsic programs. Environmental signals are provided by the niche, which is composed of specialized cell populations located in unique topological relationships with the stem cells in different adult tissues. In this review, we compare the differences and commonalities of the niches in a variety of stem cell systems across different species and provide evidence demonstrating the impact of the niche on the homeostatic regulation of stem cells. Dissection of the niche's cellular and molecular components has revealed the basic features and functions of the stem cell niche and will provide important insights for identification of the stem cell niche in different systems. We believe that the ability to reconstitute the stem cell niche in vitro will open a new avenue for maintenance and expansion of adult stem cells. Uncovering the important signals generated by the niche will shed light on the mechanisms that regulate stem cell self-renewal and maintenance of stem cell multipotentiality. Finally, understanding the interaction between stem cells and their natural partners will substantially benefit therapeutic approaches to human degenerative diseases.

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