



Adult Stem Cell Niches: Cellular and Molecular Components

Amélie Rezza^{*,†}, Rachel Sennett^{*,†}, Michael Rendl^{*,†,‡,§,1}

^{*}Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, USA

[†]Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, USA

[‡]Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, USA

[§]Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, USA

¹Corresponding author: e-mail address: michael.rendl@mssm.edu

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Abstract

As stem cells (SCs) in adult organs continue to be identified and characterized, it becomes clear that their survival, quiescence, and activation depend on specific signals in their microenvironment, or niche. Although adult SCs of diverse tissues differ by their developmental origin, cycling activity, and regenerative capacity, there appear to be conserved similarities regarding the cellular and molecular components of the SC niche. Interestingly, many organs house both slow-cycling and fast-cycling SC populations, which rely on the coexistence of quiescent and inductive niches for proper regulation. In this review we present a general definition of adult SC niches in the most studied mammalian systems. We further focus on dissecting their cellular organization and on highlighting recently identified key molecular regulators. Finally, we detail the potential involvement of the SC niche in tissue degeneration, with a particular emphasis on aging and cancer.



1. INTRODUCTION

Stem cells (SCs) have been studied for decades and tremendous advances have been made regarding SC functions and potential applications in different biological and biomedical domains, such as organ physiology, cancer pathology, aging, and regenerative medicine. The concept of a specific microenvironment or “niche” that is required for SCs to function emerged more than three decades ago (Schofield, 1978). Numerous studies have since confirmed in multiple invertebrate and mammalian organ systems that adult SCs reside in specific local microenvironments providing structural support and molecular signals to regulate SC quiescence, self-renewal, and activation for tissue maintenance.

Early and ongoing studies utilized *Drosophila* and *Caenorhabditis Elegans* to investigate adult SC niches, as the identification of SC reservoirs and accompanying niche structures are relatively straightforward in these model organisms (Byrd & Kimble, 2009; de Cuevas & Matunis, 2011). In mammalian systems a great deal of work has explored three archetypal examples of adult SCs and their corresponding niches: the hematopoietic system in the bone marrow, the small intestine, and hair follicles (HFs) in the skin. Numerous studies have identified markers to distinguish and isolate distinct SC populations, in addition to exploring the cells and signals constituting the niche within these model systems. All three organs are characterized by a high cell turnover and, under homeostatic conditions, rely on the steady activity of fast-cycling SC populations that self-renew and differentiate into all the different lineages necessary to maintain these complex tissues (Barker et al., 2007; Greco et al., 2009; Sangiorgi & Capecchi, 2008; Takeda et al., 2011; Takizawa, Regoes, Boddupalli, Bonhoeffer, & Manz, 2011; Wilson et al., 2008; Zhang, Cheong, Ciapurin, McDermitt, & Tumber, 2009). Importantly, these rapidly renewing tissues also contain separate slow-cycling SCs that are activated to replenish the cycling “workhorse” SCs or to regenerate damaged tissue upon injury, and therefore can be considered “back up” SCs. It is therefore an appealing concept to consider the coexistence of quiescent and inductive niches in these organs.

In contrast, organs that undergo slow rates of cell turnover, such as the brain (Beckervordersandforth et al., 2010; Coskun et al., 2008; Lugert et al., 2010; Nam & Benezra, 2009), muscle (Kuang, Gillespie, & Rudnicki, 2008; Pannerec, Marazzi, & Sassoon, 2012; Relaix & Zammit,

2012), and liver (Furuyama et al., 2011; Tanaka & Miyajima, 2012) contain only slow-cycling reserve SCs that maintain the tissue and can be activated following injury. Although specific SC markers have been identified in these organs, the exact cell types and specific signals composing the niche are just beginning to be uncovered in these systems.

The different functions of distinct SC populations appear to depend largely on extrinsic influences. Sustained signals from the environment are important for maintaining the quiescence, self-renewal, and continued survival of “reserve” SCs, while frequently different signals from unique niches are generated to induce SC activation for tissue turnover and repair. *In vitro* maintenance and/or activation of SCs requires addition of specific factors to the culture medium (Blanpain, Lowry, Geoghegan, Polak, & Fuchs, 2004; Roobrouck, Vanuytsel, & Verfaillie, 2011; Sato et al., 2009), confirming the importance of external inputs in regulating SC function. Interestingly, there is also accumulating evidence that this “medium-dependent” function of SCs exists for embryonic SCs (Nichols & Smith, 2012; Roobrouck et al., 2011).

In this review, we describe structural and cellular features of major adult SC niches in the mammalian system, mostly from well-characterized mouse models and where applicable also from human tissues. We further comprehensively discuss the molecular components of the niche that regulate SCs, distinguishing quiescent and activating signals. Lastly, we highlight the influence of the niche in nonphysiological conditions, such as during aging and in cancer.



2. CELLULAR ORGANIZATION OF ADULT SC NICHES

Along with characterization of SC reservoirs in adult tissues comes the exploration of their microenvironment. In most systems, the cellular components of SC niche can be classified into four categories (Fig. 12.1): (1) the SCs and progeny themselves, as they provide autocrine and paracrine regulation, respectively, within their own lineage; (2) neighboring mesenchymal or stromal cells providing paracrine signals; (3) extracellular matrix (ECM) or cell–cell contacts involving adhesion molecules; and (4) external cues from distant sources within the tissue or outside the tissue, such as from blood vessels, neurons, or immune cells. The concomitant expression and/or secretion of specific factors by these components creates a discretely

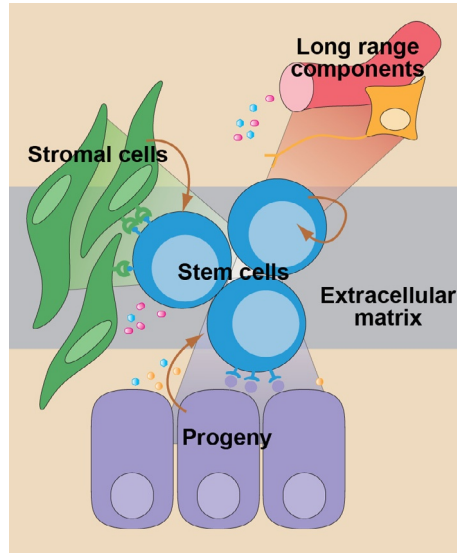


Figure 12.1 Common features of adult stem cell niches. Stem cells (SCs) reside in a specific microenvironment called the niche, composed of multiple components: SCs themselves (blue) and their progeny (purple), as well as close surrounding cells from the mesenchyme or stroma (green), more distant cells such as endothelial cells or neurons providing long-range signals (red and orange), and the extracellular matrix/basement membrane (ECM; gray). Each component expresses adhesion molecules, specific receptors, or secreted factors that signal to the SCs (gradients), creating a unique localized niche for SC maintenance and/or activation.

localized niche, allowing modulation of SC activity (Fig. 12.1). In this chapter we will detail the localization of fast- and slow-cycling SC reservoirs and point out conserved niche components within several adult tissues.

2.1. Autocrine/intrinsic SC regulation and feedback from direct progeny

Cellular contributions to the niche, especially in the form of secreted signals, are foremost and center in many adult SC systems. Important cellular input is generated by the SC pool itself and its direct progeny (blue and purple cells in Fig. 12.1; reviewed in Hsu & Fuchs, 2012). The role of SCs in adult hematopoiesis has been extensively studied, generating paradigms by which we consider the mechanisms behind SC function in other adult tissues. Hematopoietic stem cells (HSCs) proliferate and differentiate to give rise

to all blood cell lineages, and the behavior of distinct HSC subpopulations is influenced by niche stimuli present within the bone marrow (Fig. 12.2A). Traditionally, the localization of HSCs is thought to determine proliferative status, with quiescent SCs residing at the endosteum while activated SCs self-renew and differentiate in the perivascular space to maintain homeostasis, although more recent work has suggested the separation of these two niches is not as discrete as originally described (Lo Celso et al., 2009; Xie et al., 2009). A great deal of work has identified intrinsic regulation of HSC function and an important role of several stromal cell compartments within the HSC niche (discussed below), but recent studies are starting to highlight the important contributions of SC progeny as well. When macrophages are specifically targeted for ablation in mice, HSCs enter the peripheral bloodstream, suggesting that these myeloid progeny are important for maintaining HSC retention in the niche (Chow et al., 2011; Winkler et al., 2010). Further investigation revealed a circuitous mechanism, whereby macrophages secrete signals that act on other niche cells within the marrow to ultimately influence HSC localization. A separate study interrogated the role of lymphoid progeny in the niche by depleting regulatory T cells within the marrow of transplant recipient mice, before seeding donor HSCs. In the absence of Treg supportive stimuli, fewer transplanted HSCs survived (Fujisaki et al., 2011). Finally, neutrophil clearance from the bone marrow can also impact HSC activity (Casanova-Acebes et al., 2013).

Similarly, SCs and progeny in hair follicles (HFs) generate important niche signals. In adult skin, HFs cycle through periods of apoptotic destruction and regeneration (Fig. 12.2B) (Blanpain & Fuchs, 2009; Sennett & Rendl, 2012). Slow-cycling hair follicle stem cells (HFSCs) reside in a specialized “bulge” pocket, located near the top of the follicle that remains intact throughout the destruction phase of the hair cycle. During the subsequent regeneration phase, fast-cycling SC progeny in the HF germ multiply and reconstitute a new follicle, followed by proliferation and differentiation of direct SC progeny to continuously supply the growing HF until the next hair cycle. The distinct behavior of slow- and fast-cycling HFSCs is thought to depend on differential exposure to quiescent and activating niche signals. While housed in the relatively isolated bulge pocket, HFSCs are exposed only to quiescence-maintaining signals believed to be generated by the SCs themselves and possibly by nearby blood vessels and neurons (Blanpain et al., 2004; Greco et al., 2009; Tumber et al., 2004). After the first hair cycle round, differentiated SC progeny also provide important niche input to maintain quiescence among bulge HFSCs (Hsu,

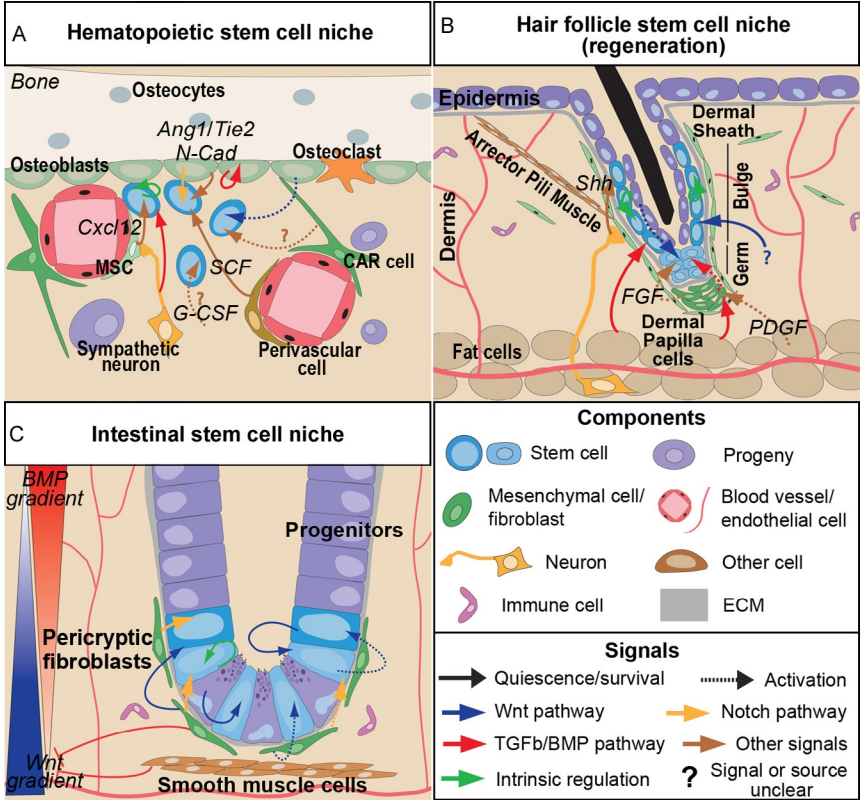


Figure 12.2 Cellular and molecular components of three fast-cycling adult stem cell niches: the hematopoietic, the hair follicle, and the intestinal systems. (A) The hematopoietic stem cell (HSC) niche includes osteoblasts (green), osteoclasts (orange), endothelial cells (red), perivascular cells (light brown), HSC progeny (purple), CXCL12-abundant reticular (CAR; dark green) cells, and mesenchymal stem cells (MSC; light green). Many signals regulate HSCs (blue): SCs autoregulate their cell cycle to prevent exhaustion (green arrow). TGFb from neural cells (red arrow), Notch signaling (yellow arrow), Ang1 from osteoblasts, perivascular SCF, and CXCL12 from MSC (brown arrows) are important for HSC maintenance. Activating signals are Wnts (still debated; dashed blue arrow), secreted factors from CAR cells and G-CSF (dashed brown arrows). (B) Hair follicle SCs (HFSCs) during regeneration reside in a niche composed of mesenchymal dermal papilla (DP) cells (dark green), fat cells (light brown), and dermis populated by blood vessels (red), immune cells (pink), loose fibroblasts and dermal sheath fibroblasts (light green), arrector pili muscle cells (dark brown), and neurons (yellow). Two populations of SCs are distinguishable during regeneration: bulge SCs (dark blue) and germ SCs (light blue). Maintenance signals to bulge SCs come from different niche components: HFSCs control quiescence through CDKi, Runx1/p21, Tbx1, NFATc1, Lhx2, and Sox9 (green arrow); subcutaneous fat cells express BMP ligands (red arrows); canonical Wnt signals from an unknown source (dark blue arrow); and nerve-secreted Shh (brown arrow). Activating signals include TGFb2

Pasolli, & Fuchs, 2011; Takeda et al., 2013). HFSCs can also act as niche for neighboring melanocyte SCs (Chang et al., 2013; Tanimura et al., 2011).

As opposed to HFs, constant SC activation is required for maintaining homeostasis in the intestine because of rapid cell turnover (Fig. 12.2C). Most studies have focused on the small intestine, where the epithelium is organized into crypts that house fast- and slow-cycling intestinal SCs (ISCs) together with transit-amplifying progeny. These crypts are continuous with villous extensions that consist of terminally differentiated absorptive and secreting cell progeny (Bjerknes & Cheng, 2006). Intriguingly, despite years of study, there is currently intense ongoing debate about the origins and relationship of the two ISC populations that are characterized by different marker expression and cell cycling activity (Rizk & Barker, 2012; Clevers, 2013). *Lgr5* + ISCs are interspersed with differentiated Paneth cell progeny at the crypt base, cycle quickly under homeostatic conditions, and have been shown through lineage tracing to self-renew and give rise to all cell types within a crypt/villus unit (Barker et al., 2007). Because of their localization they are also known as crypt basal cells, or CBCs. Separate studies have characterized *Bmi1* + SCs in the +4 position counting from the base, which similarly demonstrate both the ability to self-renew and give rise to all crypt cell lineages under homeostatic conditions (Sangiorgi & Capecchi, 2008). These +4 SCs were considered quiescent (Chwalinski, Potten, & Evans, 1988), although more recent studies contradict this hypothesis and suggest that both ISC populations are cycling under homeostatic conditions (Cambuli, Rezza, Nadjar, & Plateroti, 2013; Sangiorgi & Capecchi, 2008). These cells can repair the intestinal epithelium in the absence of *Lgr5* + cells, and even give rise to new *Lgr5* + cells after targeted ablation (Tian et al., 2011). However, complementary experiments revealed that CBCs are also capable of giving rise to +4 SCs (Takeda et al., 2011).

(dashed red arrow) and FGF7 (dashed brown arrow) expressed by DP cells, Wnt ligands expressed by epithelial cells (dashed dark blue arrow), and PDGF from the fat probably acting through DP cells (dashed brown arrow). (C) The intestinal SC niche is composed of ISC progeny Paneth cells (purple), pericryptic fibroblasts (green), and smooth muscle cells (brown). Blood vessels (red) and immune cells (pink) are present in the pericryptic mesenchyme. Two SC pools can be distinguished: *Lgr5* + Columnar Basal Cells (light blue) and +4 SCs (dark blue); and many signals regulate their behavior: *Ascl2* and *Lrig1* control ISC maintenance (green arrow), Wnt signaling regulates SC survival through Wnt ligands from Paneth cells and surrounding fibroblasts (plain and dashed blue arrows), Notch is involved in cell proliferation and differentiation (plain and dashed yellow arrows), BMP signaling is repressed by antagonists from pericryptic fibroblasts and smooth muscle cells (red blunt arrows).

Even more recently, a study demonstrated the existence of label-retaining progenitors that only contribute to crypt repair after injury (Buczacki et al., 2013). These appear to exist as a subpopulation of Lgr5+ cells, but their relation to the +4 SC pool is unclear. Attempts to link the activity and origins of these distinct SC populations have generated controversy within the field, and explorations of important niche influences have been similarly ambiguous. Although SC progeny Paneth cells were highlighted as an important constituent of the niche (Sato et al., 2011), the physiological relevance of this finding has since been challenged (Kim, Escudero, & Shivdasani, 2012), and SCs themselves, their epithelial progeny and surrounding pericryptal fibroblasts are thought to secrete important factors for SC maintenance (Fig. 12.2C) (Farin, Van Es, & Clevers, 2012; Sakamori et al., 2012).

Quiescent niche signals are thought to prevail in adult muscle tissue, which has little proclivity for homeostatic regeneration and limited capacity for wound repair. While satellite SCs were identified within adult muscle many years ago (Mauro, 1961), the muscle fiber progeny was only recently recognized as an important source of niche signaling (Fig. 12.3A; Ratajczak et al., 2003; Sherwood et al., 2004; Tatsumi et al., 2006; Wozniak & Anderson, 2007). Satellite SCs reside along the edge of these fibers, nestled underneath an encompassing basement membrane, and their nuclei comprise only a small percent of those within and along the muscle fiber (Wagers & Conboy, 2005). Recently, studies found that satellite SCs are heterogeneous: some proliferate more frequently, presumably in order to achieve normal tissue homeostasis, whereas others cycle less frequently and are considered reserve quiescent SCs (Ono, Boldrin, Knopp, Morgan, & Zammit, 2010; Ono et al., 2012; Relaix & Zammit, 2012). The influence of differential niche stimuli on these two populations has not yet been precisely described.

Likewise, adult neurogenesis by SC activation is a young field currently under great investigation. Based on label incorporating studies it was first determined that two regions of the adult brain undergo active proliferation to generate new cells: the subventricular zone (SVZ) bordering the lateral ventricles, and the subgranular zone (SGZ) of the dentate gyrus within the hippocampus (Ming & Song, 2011). In both regions, glial fibrillary acidic protein-expressing radial-glia like cells are the putative SCs that proliferate and can generate differentiated neurons, but are largely quiescent until stimulated following tissue injury. Some evidence exists for a relatively more “active” SC population that exists in the SGZ, characterized by expression of Sox2 (Suh et al., 2007). Based on lineage tracing experiments,

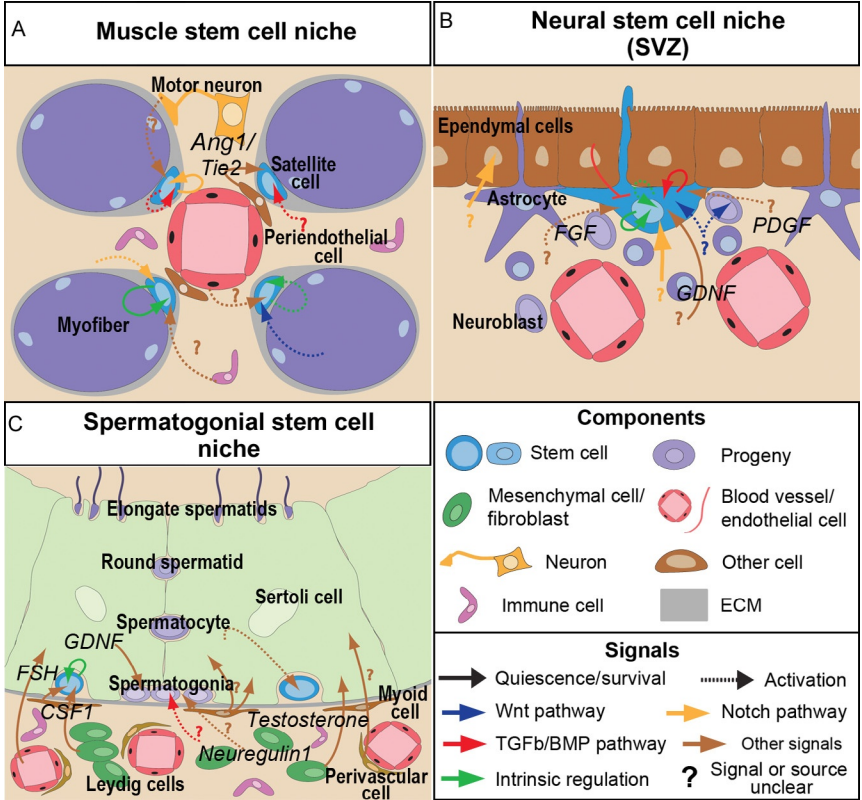


Figure 12.3 Components of the adult muscle, brain, and germ stem cell niches. (A) In the muscle, satellite SCs (blue) are present along muscle fiber progeny (purple), beneath the basal lamina (grey). The SC niche also includes blood vessels (red), periendothelial cells (brown), neurons (yellow), and immune cells (pink). Satellite SCs autoregulate their quiescence through Notch signaling (yellow arrow) and the miRNA machinery (green arrow). Periendothelial cells secrete Ang1 to regulate SC maintenance (brown arrow). SCs control their activation through the regulation of S1P (dashed green arrow). TGFb signaling also influences the regenerative capacity of satellite SCs (dashed red arrows). Myofibers secrete Wnt ligands that promote activation and regeneration (dashed blue arrow). Neural, endothelial, and immune cells secrete factors to impact SC behavior, but precise mechanisms are unclear (dashed brown arrows). (B) The neural SC niche in the SVZ is composed of ependymal cells (brown) and astrocyte progeny (purple) that both directly interact with SCs. Also present in the close environment are other NSC progeny (round purple cells) and blood vessels (red). NSCs autoregulate their own proliferation and quiescence (green arrow). The BMP pathway is precisely regulated with ligands from NSCs (red arrow) and antagonists from ependymal cells (red blunt arrow). Notch (yellow arrows) and GDNF (brown arrow) signaling are critical for ependymal and SCs maintenance as well, but the ligand source is unknown. The miRNA machinery regulates NSC activation (dashed green arrow). The Wnt pathway (dashed blue arrow) also

(Continued)

Sox2-expressing cells generate small clusters of Sox2-expressing and more differentiated progeny cells under homeostatic conditions, but the physiological implications of this observation are not yet clear. The niche system within the SVZ has been best studied and incorporates vascular cells, ependymal cells, astrocytes, and even other differentiated SC progeny (Fig. 12.3B). The ependymal cells of the SVZ line the lateral ventricle to shield neighboring SCs, and use beating cilia to generate gradients of morphogens that specify unique cell fates during differentiation (Sawamoto et al., 2006). Recent papers explored the largely ignored question of niche cell establishment and maintenance using neural SC niche cells (NSC) as a model system, and intriguingly found plasticity between ependymal cells and astrocytes (Carlen et al., 2009; Nomura, Goritz, Catchpole, Henkemeyer, & Frisen, 2010).

2.2. Input from neighboring mesenchymal or stromal cells

In numerous tissues, SCs and their progeny are closely associated with mesenchymal or stromal cell neighbors, which have been shown to play a central role in SC regulation (green cells in Fig. 12.1). Stromal cells in the bone marrow are the best studied component of the HSC niche (green cells in Fig. 12.2A). Cells of the osteoblastic lineage have classically been considered the dominant cell type of the quiescent niche, crucial for maintaining and sequestering HSCs through a combination of secreted cytokines, morphogens, and cell–cell adhesion molecules. *In vivo* experiments that depleted osteoblasts caused a corresponding drop in HSC production, suggesting these niche cells are important for maintaining SC numbers, and experimental conditions that expanded osteoblast numbers also increased the size of the HSC pool (Calvi et al., 2003; Kiel, Radice, & Morrison, 2007; Visnjic et al., 2004; Wilson & Trumpp, 2006; Zhang et al., 2003; Zhu et al., 2007). Many *in vitro* experiments also demonstrated the utility of coculturing osteoblasts

Figure 12.3—Cont'd activates proliferation. FGF and PDGF signals from unknown sources promote NSC proliferation (dashed brown arrows). (C) The germ SC niche in the testis is composed of Sertoli cells (light green), myoid cells (dark brown), and Leydig cells (green). Blood vessels (red) surrounded by perivascular cells (brown) and immune cells (pink) are present in the interstitial tissue. SSC maintenance and activation is dependent on Sertoli cell maintenance, which is regulated by testosterone from Leydig cells, FSH from the pituitary gland, and other signals from perivascular cells (brown arrows). SSC survival and maintenance depends on integrin beta1 expression in SSCs (green arrow), GDNF from Sertoli cells (brown arrows), or CSF1 from Leydig and myoid cells (brown arrows). SSCs can be activated by a wide range of signals such as BMP (dashed red arrow).

with HSCs to improve survival and sustain functionality (Chitteti et al., 2010; Taichman, Reilly, & Emerson, 1996). Other important stromal cells contribute to the bone marrow niche as well; most notably, endothelial cells and CXCL12-abundant reticular (CAR) cells release soluble factors and establish cell–cell contacts to regulate HSC activation and dispersion. Experiments targeting CAR cells for ablation resulted in decreased numbers of activated HSCs with increased expression of progeny genes, suggesting CXCL12-producing cells mediate the survival, retention, and differentiation of primed HSCs in the marrow (Omatsu et al., 2010). Other recent work has provided substantial evidence implicating nestin+ mesenchymal stem cells (MSCs) as potent regulators of HSC activity as well (Mendez-Ferrer et al., 2010). Importantly, in very recent studies, expression of the essential niche signal CXCL12 was disrupted within several niche cell types, that is, osteoblasts, osteoprecursors, endothelial cells, MSCs, and other specific stromal subpopulations to determine differential cellular contributions to HSC maintenance (Ding & Morrison, 2013; Greenbaum et al., 2013). Remarkably, significant reductions in HSC numbers were only observed when CXCL12 was ablated from MSCs, indicating a subset of CXCL12-producing perivascular stromal cells is most instrumental in maintaining HSC survival and retention in the bone marrow, together with some input from endothelial cells. Meanwhile, osteolineage niche cells appear to support more differentiated HSC progeny through CXCL12 production.

Neighboring mesenchymal niche cells are thought to play a central role in activating HFSCs in the transition between the destruction and regeneration phases of the hair cycle. After a period of rest, when HFSCs are first induced to regrow a new follicle, activating signals are thought to emanate from the dermal papilla (DP) (dark green cells in Fig. 12.2B). In the mature follicle the DP exists as a pocket of mesenchymal cells normally localized to the lower bulb where it can regulate hair growth (Clavel et al., 2012), but right before regeneration this compartment approaches the bulge as most of the follicle base undergoes apoptosis. After exposure to stimuli provided by the activating DP niche (Greco et al., 2009; Oshimori & Fuchs, 2012), few fast-cycling HF germ SCs generate transit-amplifying progeny that subsequently multiply and differentiate in order to regenerate a new follicle (Greco et al., 2009; Lee & Tumber, 2012; Sennett & Rendl, 2012). Recent studies found that the DP is absolutely essential for new HF regeneration (Rompolas et al., 2012) and that the number of cells in the DP determines successful activation of SC during the hair cycle. Below a certain threshold, the DP lose its ability to signal to SCs (Chi, Wu, & Morgan, 2013).

However, a complete understanding of the signals involved in this process remains unknown.

In the intestine, mesenchymal cells neighboring the crypt base have also been implicated as part of the niche (green cells in Fig. 12.2C), although the precise identity of important cells and their corresponding signaling modes are not yet clear (reviewed in Smith, Davies, Silk, & Wong, 2012). In humans, it was shown that pericryptic fibroblasts and underlying smooth muscle cells secrete factors influencing crypt cells (Kosinski et al., 2007). Although the ability of Lgr5+ SCs to form organoids in culture without the influence of neighboring mesenchymal cells challenges this theory (Sato et al., 2009), necessary survival factors of the *in vitro* system are known to be expressed by surrounding cells *in vivo* (Farin et al., 2012; Kosinski et al., 2007).

In the testes, SCs are supported by neighboring cells from another lineage. Testes consist of interstitial tissue and seminiferous tubules in which postmitotic Sertoli cells associate with differentiated germ cells to form the seminiferous epithelium. The basal compartment of the epithelium contains spermatogonial SCs (SSCs) and spermatogonia, whereas more differentiated germ cells are present in the adluminal compartment (reviewed in Oatley & Brinster, 2012). The seminiferous epithelium is bound by a basement membrane that separates it from the interstitial tissue, populated by myoid cells, Leydig cells, blood vessels, and different immune cells (Fig. 12.3C). Sertoli cells in the seminiferous tubules are the most important cellular component of the immediate SSC niche. These cells form tight junctions with each other, creating the blood–testes barrier and physically constraining signals that emanate from the underlying interstitial tissue to the domain of undifferentiated SSCs associated with the basement membrane. More differentiated progeny still associate with the Sertoli cells, but outside of this tight junction barrier. Increasing the number of Sertoli cells in the seminiferous epithelium creates more niches for SSC seeding, and inhibiting the production of supportive signals from these cells depletes SSC numbers (Meng et al., 2000; Oatley, Racicot, & Oatley, 2011).

2.3. SC regulation by ECM and adhesion molecules

The influence of cellular components within the SC niche can extend beyond the production of secreted morphogens and chemokine gradients to include direct cell–cell contact (reviewed in Chen, Lewallen, & Xie, 2013). Because adhesion-mediated interactions are generally static, this kind

of niche input typically influences quiescence, maintenance, or retention of SCs no matter the system. Additionally, SCs interact with the ECM (gray area in Fig. 12.1) within the niche as it can act as a reservoir of secreted signals or potentially, as demonstrated largely by *in vitro* work, modulate SC activity in its own right (Engler, Sen, Sweeney, & Discher, 2006; Gilbert et al., 2010).

In the HF, different adhesion molecules were shown to have an important role in epidermal and HF development and maintenance. Complete ablation of integrin beta1 in epithelial cells results in severe skin blistering and impaired HF formation (Raghavan, Bauer, Mundschau, Li, & Fuchs, 2000). Similarly, ablation of the linker protein alpha-catenin impairs hair development and causes major defects in the epidermis (Vasioukhin, Bauer, Degenstein, Wise, & Fuchs, 2001). Also, E-cadherin ablation in adult epidermis leads to a severe differentiation defect, and to decreased proliferation of HF progenitors (Young et al., 2003), suggesting that E-cadherin is important for HF maintenance. However, the exact function of these proteins specifically in adult HFSCs remains unknown.

In the brain, astrocytes are present in both the SVZ and SGZ, producing morphogens and providing direct cell–cell contacts with NSCs to regulate proliferation, differentiation, migration, and synapse formation (Barkho et al., 2006). Vascular cells similarly disperse throughout neural tissue and can act via direct contact to influence progenitor cell fate choices by bringing blood-derived signals into close proximity with target cells, and can additionally provide paths that progenitors migrate along to reach different zones of the brain (Kojima et al., 2010). More specifically, targeted ablation of E-cadherin within all central nervous system (CNS) tissues revealed its functional role in mediating NSC self-renewal *in vivo*, while parallel studies confirmed E-cadherin expression was needed for NSCs to form colonies in culture (Karpowicz et al., 2009).

Both cadherins and integrins are expressed in a polarized orientation by muscle SCs, but the functional role of their localization is not yet clear (Kuang et al., 2008). In the muscle, where SCs are entirely surrounded by matrix, the ECM was shown to localize numerous factors that activate satellite cells, such as HGF, FGF, or IGF, especially in cases of injury (Yin, Price, & Rudnicki, 2013). It was also recently shown that Collagen VI regulates satellite cell self-renewal (Urciuolo et al., 2013).

Reports of direct cell–cell adhesions that regulate HSC activity have been conflicting. Although N-cadherin expression was first appreciated in HSCs and the osteoblastic niche a decade ago (Zhang et al., 2003),

subsequent studies have both confirmed and negated its role in mediating HSC maintenance (Bromberg et al., 2012; Haug et al., 2008; Hosokawa et al., 2010; Kiel, Acar, Radice, & Morrison, 2009). Separate functional investigations reveal integrins are also expressed by HSC and niche cells and have a role in mediating SC homing to the bone marrow, especially in transplantation experiments (Potocnik, Brakebusch, & Fassler, 2000; Qian, Tryggvason, Jacobsen, & Eklom, 2006), and *in vitro* studies have hinted at a role in long-term maintenance via interactions with osteoblasts (Schreiber et al., 2009).

Cell adhesion is also believed to influence germ SSC activity, especially with the confirmed expression of several integrins on SSCs (Shinohara, Avarbock, & Brinster, 1999). Undifferentiated SSCs are closely associated with both the basement membrane lining the seminiferous epithelium and next to somatic Sertoli cells within the tubules. Specific studies have shown that SSC expression of integrin beta1 is important for sustaining SC activity and crucial for directing SC homing to the appropriate niche during transplantation studies (Kanatsu-Shinohara et al., 2008). SSCs also directly interact with neighboring Sertoli cells, potentially through cadherin-mediated interactions (Tokuda, Kadokawa, Kurahashi, & Marunouchi, 2007).

2.4. Long-range contributors and macroenvironment

Although the original concept of the SC niche embodied only neighboring elements capable of influencing SC self-renewal and differentiation, we now know that SCs also receive signals from cells more distant in the tissue or even outside the tissue (red and orange cells in Fig. 12.1).

The activation and quiescence of SCs within the HF is known to be influenced by signals that originate globally from the dermis, which helps to coordinate hair cycling between many follicles throughout the skin (Plikus et al., 2011). A recent study further elucidated a role for nascent and mature adipocytes in signaling to the bulge compartment to initiate hair cycling, possibly via signaling through the DP compartment (Festa et al., 2011). Another study also showed the importance of neural input in regulating HFSCs capacity to act as epidermal SCs (Brownell, Guevara, Bai, Loomis, & Joyner, 2011).

Long-range neural input is indisputably important for regulating HSC release into the peripheral bloodstream within the hematopoietic system.

G-CSF stimulation drives SCs out of the bone marrow by affecting norepinephrine signaling, which in turn downregulates osteoblast production of CXCL12, and ultimately allows HSCs to escape into the circulation (Katayama et al., 2006). Remarkably, even light stimuli can influence the sympathetic nervous system with downstream effects on HSC mobilization, which occurs in a circadian rhythm pattern during normal homeostasis (Mendez-Ferrer, Lucas, Battista, & Frenette, 2008). Sympathetic tone has similarly been shown to influence the activity of other components of the cellular niche including nestin + MSCs, promoting the egress of HSCs from the bone marrow through additional avenues (Mendez-Ferrer et al., 2010). In the case of the hematopoietic system, far-reaching neural input acts on multiple targets to achieve SC mobilization into the circulation.

Within the muscle, satellite SCs are consistently localized near vasculature, ensuring ready access to systemic signals that can regulate SC activity (Christov et al., 2007; Fukada et al., 2007). Signals from the interstitial space outside the muscle basal lamina, such as from immune cells or neurons, can also reach underlying satellite cells and influence SC behavior (Girgenrath et al., 2006). Additional evidence that systemic factors can modulate satellite cell potency comes from experiments characterizing the effects of parabiosis or calorie restriction on the ability of muscle SCs to self-renew and repair degenerating tissue (Cerletti, Jang, Finley, Haigis, & Wagers, 2012; Conboy et al., 2005).

Humoral signals also appear to be crucial for maintenance and maturation of germ SCs, as SSCs preferentially localize near vessels in the interstitium and migrate away as they differentiate (Chiarini-Garcia, Hornick, Griswold, & Russell, 2001; Chiarini-Garcia, Raymer, & Russell, 2003; Yoshida, Sukeno, & Nabeshima, 2007). Signals that reach the SSCs through the interstitial vasculature are prevented from spreading throughout the circulatory system by Sertoli cell tight junctions. Interstitial Leydig cells produce cytokines to support SSC self-renewal, and also secrete testosterone, which is similarly important to ensure proper functioning of Sertoli cells (Davidoff et al., 2004; Oatley, Oatley, Avarbock, Tobias, & Brinster, 2009). Signals from interstitial myoid cells have also been implicated in supporting SSC self-renewal (Nurmio et al., 2012; Qian et al., 2013), while perivascular cells are thought to secrete supporting signals for Sertoli cells (Verhoeven & Cailleau, 1988). Important roles for systemic factors have been demonstrated, but more in the context of supporting the cellular components of the SSC niche (Oatley & Brinster, 2012).



3. MOLECULAR REGULATORS OF ADULT SC NICHES

Adult SC niches can be populated by many different cell types and structural components, each providing molecular and/or physical support to regulate SCs, as described in the previous section. In this part, we discuss signals that have been identified in different adult SC niches. These signals can be organized into two main categories: (1) quiescent or survival signals and (2) activating signals. Quiescent signals are emitted by niches maintaining dormant SCs, whereas activating signals are present after injury or in rapidly renewing tissues during homeostasis.

3.1. Signals maintaining quiescence, survival, and self-renewal

In all organs that contain SCs specific signals promote the survival of these cells. In slowly renewing tissues that contain slow-cycling SC populations, niche signals also allow maintenance of quiescence. Some molecular regulation comes from within the SCs themselves, but most originates from different components of the niche.

3.1.1 *Cell autonomous regulation of SC quiescence and survival*

The main intrinsic process by which SCs balance quiescence and self-renewal is through regulation of cell cycle entry. This cell-autonomous mechanism regulates HSC quiescence by preventing HSC exhaustion (green arrow in Fig. 12.2A) (Pietras, Warr, & Passegue, 2011). Several studies have highlighted the critical role of the Rb protein family in controlling HSC cell cycle entry, thereby maintaining HSC self-renewal capacity (Viatour et al., 2008). Concomitant ablation of Rb, p130 and p107 activated HSCs proliferation, limiting their reconstitution capacity in long-term transplantation assays. This phenotype was not observed in single deleted mice (Cobrinik et al., 1996; LeCouter et al., 1998; Walkley & Orkin, 2006), suggesting an important functional redundancy within the Rb protein family. Similarly, mice deficient for a single D-cyclin or associated kinase have only minimal hematopoietic phenotypes, whereas concomitant deletion of several D-cyclins or Cyclin-dependent kinases (CDKs) results in embryonic lethality with severe hematopoietic defects (Kozar et al., 2004; Malumbres et al., 2004). The potential importance of D-cyclins/CDKs in adult HSCs is still unknown. The Ink4 protein family of D-cyclin–Cdk4/6 antagonists also regulates HSC cell cycle entry by balancing quiescence and proliferation (reviewed in Pietras et al., 2011). CIP/KIP family

proteins p21, p27, and p57, but also master transcriptional regulator p53, are key controls of HSC quiescence as well. Additionally, suppression of PI3K signaling by tumor suppressor PTEN is critical to control D-cyclins for HSC quiescence, illustrating again the central role of cell cycle regulation in the maintenance of HSCs. Similarly in HF, HFSCs autonomously regulate quiescence through CDKi (green arrow in Fig. 12.2B). The Runx1/p21 complex controls HFSC quiescence during the hair cycle (Lee et al., 2013). During the destruction/catagen phase, downregulation of Runx1 results in p21 upregulation maintaining HFSCs in a quiescent state. Upon regeneration (anagen phase), Runx1 represses p21 expression and then promotes HFSC self-renewal. Cell cycle inhibitors are also crucial for maintenance of adult NSC quiescence (green arrow in Fig. 12.3B). P21 represses NSC proliferation (Kippin, Martens, & van der Kooy, 2005) specifically during development (Molofsky, He, Bydon, Morrison, & Pandal, 2005). P73 also inhibits premature senescence of NSC (Taloz et al., 2010).

Other transcription factors have similarly been implicated in SC-autonomous survival. In HF, HFSCs deficient for Tbx1 were unable to replenish their niche, suggesting a key role in long-term SC maintenance (Chen et al., 2012). NFATc1 intrinsically maintains HFSC quiescence as well (Horsley, Aliprantis, Polak, Glimcher, & Fuchs, 2008). Interestingly, both studies mechanistically link SC survival to bone morphogenetic protein (BMP) signaling. Lhx2 is also involved in HF formation and HFSC maintenance (Rhee, Polak, & Fuchs, 2006), whereas Sox9 is dispensable for hair induction but critical for the formation of the HFSC compartment (Nowak, Polak, Pasolli, & Fuchs, 2008; Vidal et al., 2005). In the muscle, the Transforming Growth Factor beta (TGFb) signaling factor Myostatin is expressed by satellite SCs to maintain their own quiescence (green arrow in Fig. 12.3A) (McCroskery, Thomas, Maxwell, Sharma, & Kambadur, 2003). Genetic ablation of the Notch signaling effector Rbpj also causes satellite SCs to lose quiescence in resting muscle (Mourikis et al., 2012). In this case, the source of the Notch ligand Delta is still unclear, although satellite SCs upregulate its expression after injury (Conboy, Conboy, Smythe, & Rando, 2003). In the intestine, the Wnt target gene Ascl2 is important for CBC survival/maintenance (green arrow in Fig. 12.2C), although the status of Ascl2 in the +4 ISCs is not yet characterized (van der Flier et al., 2009). The pan-ErbB negative regulator Lrig1 marks a quiescent ISC population in the colon and maintains intestinal homeostasis likely by regulating quiescence. Upon deletion of Lrig1, this subpopulation of SCs starts to proliferate and eventually forms tumors (Powell et al., 2012).

In the CNS, the nuclear orphan receptor Tlx (Sun, Yu, Evans, & Shi, 2007), the transcription factor Sox2 (Favaro et al., 2009; Hu et al., 2010), and the longevity-associated factor Foxo3 (Renault et al., 2009) have all been identified as regulators of NSC quiescence. Interestingly, a recent study suggested that GSK3, a downstream effector of numerous signaling pathways, may be a central regulator of NSC homeostasis (Kim et al., 2009). Recently, even components of the miRNA pathway have been shown to regulate SC quiescence. miRNA-489 suppresses satellite cell expression of a protein promoting progenitor expansion in muscle (Cheung et al., 2012). SCs in the skin similarly express miRNA-125b to preferentially self-renew instead of differentiating (Zhang, Stokes, Polak, & Fuchs, 2011). Finally, it is worth noting that some of the autocrine regulators mentioned earlier may nevertheless be dependent on external input, such as from the basement membrane or secreted ligands.

3.1.2 Extrinsic signals that regulate SC quiescence and self-renewal

As described in the first section, many different cell types can constitute the niche and signal to SCs as extrinsic regulators. The TGF β superfamily encompasses multiple signaling pathways known to contribute to SC quiescence. In the bone marrow niche, TGF β maintains HSC hibernation (Yamazaki et al., 2011, 2009). This process involves neighboring glial cells which activate latent TGF β (red arrow in Fig. 12.2A) (Yamazaki et al., 2011). In the intestinal epithelium, it was shown that quiescence of ISC can be promoted by oral administration of TGF β 1 (Puolakkainen et al., 1994), although data demonstrating that this process happens endogenously is lacking.

The BMP family, part of the TGF β superfamily, was shown to be important for SC quiescence as well. Inactivation of BMPRIa in epithelial cells causes HFSC activation and premature anagen (Kobielak, Stokes, de la Cruz, Polak, & Fuchs, 2007). BMP also seems important for maintenance of HFSC niche integrity as DP cells deficient for BMPRIa lose hair inducing capacity (Rendl, Polak, & Fuchs, 2008). Moreover, the resting phase of the hair cycle is characterized by a high concentration of BMP ligands in the dermis, generated from subcutaneous fat, which instructs HFSCs to stay quiescent (red arrow in Fig. 12.2B; Plikus et al., 2008). Outside of the skin, the BMP pathway is also involved in preventing proliferation in the intestine. BMP4 is expressed in the intravillus mesenchyme to activate the BMP pathway in differentiated epithelial cells in the villi (Haramis et al., 2004). Another study showed that inactivation of BMPRIa in the intestinal epithelium leads to an expansion of the stem compartment through activation of

the canonical Wnt pathway (He et al., 2004). Interestingly, similar observations were made in the colon (Singbrant et al., 2010). Also, it was shown that human pericryptic fibroblasts and smooth muscle cells express BMP antagonists that repress the BMP pathway in ISCs (Kosinski et al., 2007). Taken together these data suggest that a gradient of BMP ligands and antagonists exists along the crypt–villus axis, repressing the BMP pathway in ISC to limit proliferation and thus maintain quiescence (red gradient and blunt arrows in Fig. 12.2C). Also, Wnt5a regulated TGF β has been shown to have a crucial role in *de novo* crypt formation after injury (Miyoshi, Ajima, Luo, Yamaguchi, & Stappenbeck, 2012). Interestingly, a separate study reported that intestinal mesenchymal cells express Wnt5a (Farin et al., 2012), but its link to TGF β and wound repair was not mentioned. Similarly in the hippocampus, the BMP pathway, through BMPRIa, contributes to adult NSCs quiescence maintenance (Mira et al., 2010). A specific balance of BMP signaling occurs in the SVZ to maintain NSC quiescence, involving the production of BMP ligands by NSCs and expression of the BMP antagonist Noggin by ependymal niche cells (red arrow and blunt arrows in Fig. 12.3B) (Lim et al., 2000). Finally, the BMP pathway regulates the niche size for HSCs in the bone marrow through its action on osteoblasts (red arrow in Fig. 12.2A) (Zhang et al., 2003).

Another signal pathway commonly involved in SC homeostasis is canonical Wnt signaling. For years, the Wnt/ β -catenin pathway has been associated with intestinal tumorigenesis and specifically the proliferation of intestinal progenitors (Reya & Clevers, 2005). Several studies have implicated this pathway in regulating ISC maintenance, as ablation of Tcf4 (Korinek et al., 1998; van Es et al., 2012) and β -catenin (Fevr, Robine, Louvard, & Huelsken, 2007) leads to reduced ISC survival. Interestingly, a recent study demonstrated numerous and redundant sources of Wnt ligands in the ISC microenvironment (Farin et al., 2012). Indeed, abrogated Wnt3 secretion by Paneth cells is compensated by mesenchymal cell production of Wnt ligands (blue arrows in Fig. 12.2C). Similarly, canonical Wnt signaling is necessary to maintain HFSCs (Huelsenken, Vogel, Erdmann, Cotsarelis, & Birchmeier, 2001). Ablating β -catenin in epithelial cells results in loss of SC markers and active proliferation (Lowry et al., 2005). Interestingly, the source of essential Wnt ligands remains unclear and likely does not involve epithelial cells, as the ablation of Wntless (Wls), an essential factor in Wnt secretion, in these cells does not alter HFSC maintenance. (blue arrow in Fig. 12.2B) (Myung, Takeo, Ito, & Atit, 2013). On the other hand, the role of Wnt signaling in the hematopoietic system is

under ongoing debate, as several studies reported Wnt ligands maintain HSC self-renewal *in vitro* (Reya et al., 2003; Willert et al., 2003), but *in vivo* data contradict this conclusion (Cobas et al., 2004; Kirstetter, Anderson, Porse, Jacobsen, & Nerlov, 2006; Reya et al., 2003).

The Notch pathway, known to be important for cell fate decisions, has also been identified as a key regulator in SC biology. Notch1 signaling is particularly crucial for the generation of definitive HSCs (Kumano et al., 2003). In adults, Notch is thought to inhibit cytokine-induced differentiation through regulation of GATA2 and Hes1, thus maintaining HSCs (Kumano et al., 2001; Kunisato et al., 2003). Interestingly, the Notch ligand Jagged1 is highly expressed in osteoblasts (yellow arrows in Fig. 12.2A) (Calvi et al., 2003). Notch also controls cell proliferation in the intestine in a Wnt-dependent manner (Fre et al., 2009; Riccio et al., 2008; Rodilla et al., 2009), although its specific mode of action on SCs is unclear. Regardless, several Notch ligands are expressed by surrounding intestinal mesenchymal cells (yellow arrows in Fig. 12.2C) (Fre et al., 2005). In the brain, several studies have identified Notch signaling in maintenance of adult NSCs. In the hippocampus, Notch1 ablation increases proliferation and leads to loss of NSCs (Breunig, Silbereis, Vaccarino, Sestan, & Rakic, 2007). A similar observation was made in the whole brain upon deletion of the downstream transcription factor Rbpjk (Imayoshi, Sakamoto, Yamaguchi, Mori, & Kageyama, 2010). Ependymal cells, which give rise to neuroblasts and astrocytes after a stroke, maintain quiescence through Notch signaling (Carlen et al., 2009), and Notch and PEDF signaling cooperate to regulate self-renewal in the brain (Andreu-Agullo, Morante-Redolat, Delgado, & Farinas, 2009). Although these studies do not identify Notch ligand-expressing cells, these should be in close distance to the NSCs (yellow arrows in Fig. 12.3B).

Several other signaling pathways are involved in regulating SC quiescence. Receptor tyrosine kinase Tie2 signaling, initiated by its ligand Angiopoietin1, promotes HSC quiescence in the bone marrow (Arai et al., 2004), and satellite SC quiescence in the muscle (Abou-Khalil et al., 2009). Osteoblasts produce Angiopoietin1 in the hematopoietic system (brown arrow in Fig. 12.2A) (Arai, Ohneda, Miyamoto, Zhang, & Suda, 2002), and periendothelial cells in the muscle (brown arrow in Fig. 12.3A) (Abou-Khalil et al., 2009). The growth factor GDNF plays an essential role in SC quiescence in the germline and the brain. GDNF-deficient mice have seminiferous tubules that lack germ cells, most likely due to the inability of Sertoli cells to sustain undifferentiated SSCs

(Kubota, Avarbock, & Brinster, 2004; Meng et al., 2000). GDNF is expressed by Sertoli cells upon activation by FSH (brown arrow in Fig. 12.3C) (Tadokoro, Yomogida, Ohta, Tohda, & Nishimune, 2002), and its receptor, GFRalpha1, is expressed by spermatogonia (brown arrow in Fig. 12.3C) (Grisanti et al., 2009). Interestingly, the effect of GDNF signaling on SSC maintenance is promoted *in vitro* by FGF2, EGF (Kanatsu-Shinohara et al., 2005), and IGF (Kubota et al., 2004). In the brain, GDNF is widely expressed and important for neuronal precursor survival (brown arrow in Fig. 12.3B) (Arenas, Trupp, Akerud, & Ibanez, 1995). A number of additional secreted factors are important for maintaining SCs. Stem cell factor (SCF), also known as KITL, is an important promoter of HSC maintenance and is expressed by perivascular cells in the bone marrow (Fig. 12.2A) (Ding, Saunders, Enikolopov, & Morrison, 2012). Interactions between HSCs and osteoblasts involving the adhesion proteins *N*-cadherin and integrins also support HSCs (Schreiber et al., 2009; Zhang et al., 2003). Two recent studies demonstrated a central role of CXCL12 from MSCs in HSC self-renewal and maintenance (brown arrow in Fig. 12.2A) (Ding & Morrison, 2013; Greenbaum et al., 2013). Interestingly, long-range CXCL12 signaling is also important for homing SSC precursors in the embryo, and *in vitro* studies suggest an importance for maintaining adult SSCs as well (Ara et al., 2003; Kanatsu-Shinohara et al., 2012). In the adult testes, CSF1 is expressed in Leydig cell clusters and peritubular myoid cells as a component of the SSC niche that controls self-renewal of the germline (brown arrows in Fig. 12.3C) (Oatley et al., 2009). Finally, in the HF, nerve-secreted Shh is important for the maintenance of bulge HFSCs (brown arrow in Fig. 12.2B) (Brownell et al., 2011).

3.2. SC activating signals for tissue regeneration and repair

As described in the previous section, SC quiescence depends on cell-autonomous factors as well as external inputs in both fast and slow-cycling tissues. Interestingly, only few intrinsic activating SC signals have been described. In the muscle, sphingolipid signaling, through its soluble form S1P, acts in an autocrine/paracrine manner to promote satellite cell proliferation and muscle regeneration (Nagata, Partridge, Matsuda, & Zammit, 2006; Sassoli et al., 2011). In the brain, the miRNA machinery was recently implicated in SC regulation (Szulwach et al., 2010).

In general, SC activation to regenerate tissues during homeostasis or after injury depends almost exclusively on external signals. While TGF β signaling

maintains quiescence in several organs, it is also important for SC activation. In the HF, TGF β 2 expressed by DP cells transiently activates Smad2/3 and antagonizes refractory BMP signals to allow HF regeneration (dashed red arrow in Fig. 12.2B) (Oshimori & Fuchs, 2012). In the muscle, TGF β is upregulated in satellite cells of aged mice, impairing the regeneration capacity of aging muscle (Carlson, Hsu, & Conboy, 2008). Interestingly, high TGF β levels were also detected in the ECM, but its source remains unclear (dashed red arrow in Fig. 12.3A). In the germline, BMP4 seems to regulate SSC differentiation by acting on spermatogonia, as exposure to BMP4 decreases SSC numbers *in vitro* (dashed red arrow in Fig. 12.3C) (Nagano, Ryu, Brinster, Avarbock, & Brinster, 2003; Pellegrini, Grimaldi, Rossi, Geremia, & Dolci, 2003), but this effect has not been confirmed *in vivo*.

Similarly, Wnt/ β -catenin signaling activates SCs in different organs, in addition to promoting survival, self-renewal, and maintenance as previously described. Canonical Wnt signaling activates HSC proliferation in the bone marrow (Reya et al., 2003; Willert et al., 2003) via ligands most likely expressed by osteoblasts (dashed blue arrow in Fig. 12.2A). In the SVZ of the brain, this pathway promotes progenitor cell proliferation. Pharmacological inhibition of GSK3 stabilizes β -catenin in SVZ cells and activates their proliferation (Adachi et al., 2007), although the specific source of Wnt ligands in homeostatic conditions remains unclear (dashed blue arrows in Fig. 12.3B). In the hippocampus, Wnt pathway activation is dispensable for maintenance of the SC compartment, but important for the survival of neural progenitors and neuronal maturation (Kuwabara et al., 2009). Here again, the source of Wnt ligands in the hippocampus was not explored. In addition, the *in vitro* sphere formation capacity of NSCs is Wnt dependent, as well as NSC activation after injury (Wang et al., 2011). The central role of Wnt/ β -catenin signaling in intestinal homeostasis was specifically linked to its ability, when overactivated, to promote SC proliferation and adenoma formation. Interestingly, the putative ISC marker Musashi1 is a potent activator of Wnt and Notch pathways, leading to tumor formation (Rezza et al., 2010). More strikingly, specific activation of the Wnt pathway in ISC through stabilization of β -catenin (Sangiorgi & Capecchi, 2008) or Adenomatous Polyposis Coli (APC) deletion (Barker et al., 2009) activates SC proliferation to eventually form tumors. *In vivo*, different redundant sources of Wnt ligands have been identified (dashed blue arrows in Fig. 12.2C) (Farin et al., 2012). In HFs, the canonical Wnt pathway is also important for HFSC activation. Sustained β -catenin stabilization in epithelial cells leads to proliferation and precocious

activation of HFSC (Lowry et al., 2005). Interestingly, ablation of Wls specifically in epithelial cells results in decreased proliferation and defective regeneration (Myung et al., 2013), suggesting that Wnt ligands involved in HFSC activation are secreted by epithelial cells (dashed blue arrow in Fig. 12.2B). Two studies have highlighted a role of Wnt signaling in myogenic differentiation and muscle regeneration, as it is required for proper activation and differentiation of satellite cells and hence normal regeneration in an *in vitro* model of cultured myofibers (Brack, Conboy, Conboy, Shen, & Rando, 2008; Otto et al., 2008). Moreover, Wnt ligands are expressed in muscle fibers and canonical Wnt signaling is activated during muscle regeneration after injury (dashed blue arrow in Fig. 12.3A) (Otto et al., 2008).

The Notch pathway is also important for SC regulation and cell fate decisions in different organs. In the intestine as in the muscle, it is necessary for proper differentiation of certain cell types (dashed yellow arrows in Figs. 12.2C and 12.3A) (Conboy & Rando, 2002; Fre et al., 2005; van Es et al., 2005). In one study, Notch signaling promoted satellite cell proliferation during muscle regeneration and was shown to be impaired in aged muscle (Conboy et al., 2003). Other important pathways in SC activation include FGF and PDGF signaling. In HFs, FGF7 secreted by DP cells is upregulated during the transition from telogen to anagen, when HFSCs are activated (dashed brown arrow in Fig. 12.2B). Subcutaneous injection of FGF7 induces HFSC activation and precocious follicle regeneration (Greco et al., 2009). In the brain, FGF2 associated with EGF promotes NSC proliferation *in vitro*, although its role *in vivo* is unclear (dashed brown arrow in Fig. 12.3B) (Gritti et al., 1999; Kuhn, Winkler, Kempermann, Thal, & Gage, 1997). PDGF signaling promotes HFSC activation as well, as PDGF-A ligands generated by subcutaneous fat is required for hair regeneration in the hair cycle, most likely through activation of the PDGFR pathway in DP cells (dashed brown arrow in Fig. 12.2B) (Festa et al., 2011). In the brain, this pathway activates adult NSCs (dashed brown arrow in Fig. 12.3B). In the SVZ, PDGF signaling is necessary for oligodendrogenesis, although it is dispensable for neurogenesis in general. Additionally, PDGF can be a potent mitogen of NSCs, leading to tumor-like hyperplasia (Jackson et al., 2006; Lachapelle, Avellana-Adalid, Nait-Oumesmar, & Baron-Van Evercooren, 2002).

A number of other secreted factors have been linked to SC activation in distinct model systems. G-CSF is an activator of HSCs and is now therapeutically utilized to harvest cells from the bone marrow for transplants (dashed brown arrow in Fig. 12.2A) (Katayama et al., 2006). For germ SCs,

Neuregulin1 has a potent differentiation effect on spermatogonia *in vitro* (Hamra, Chapman, Nguyen, & Garbers, 2007), although this regulation was not yet confirmed *in vivo* (dashed brown arrow in Fig. 12.3C). In the muscle, several long-range signals have been suggested to activate satellite cells, but precise factors and mechanisms are unclear. Endothelial cells express growth factors, such as VEGF, that promote satellite cell proliferation, while neurons secrete neurotrophins, NGF and BDNF, which are thought to influence satellite cell behavior (reviewed in Yin et al., 2013). Similarly, immune cells seem to promote satellite cell proliferation by secreting a range of diffusible factors such as MCP-1 (dashed brown arrows in Fig. 12.3A) (Chazaud et al., 2003; Yin et al., 2013). Androgens are also suggested to affect satellite cells, which express the androgen receptor, but direct evidence of this regulation is lacking (Yin et al., 2013).



4. SC NICHE DYSFUNCTION IN AGING AND CANCER

In addition to studying normal adult SC and niche interactions, it is important to consider the implications of a malfunctioning SC environment during aging and in disease. Aging tissues typically display a diminished capacity for repair, resulting in progressively decreasing integrity. It seems logical that declining SC function could contribute to global tissue aging, but only few studies have explored the role of the aging niche in this context. Some of the most convincing evidence comes from parabiotic studies, in which aged and young mice are joined together with a shared blood circulation (Conboy et al., 2005; Mayack, Shadrach, Kim, & Wagers, 2010). Long-range signaling factors present in the young serum revitalized aged SCs in the older mice, leading to increased long-term HSC numbers and differentiation capacity in the bone marrow, enhancing proliferation of muscle satellite SCs for tissue repair, and even promoting regeneration of aged hepatocytes.

A recent study directly examined signaling changes in aged myofibers, with interesting results on satellite SC maintenance (Chakkalakal, Jones, Basson, & Brack, 2012). With age, myofibers emit increasing levels of FGF2 that negatively affect satellite SC quiescence. While additional FGF ligands are being produced in the niche, aging satellite SCs start to produce fewer FGF pathway inhibitors, resulting in increased proliferation and depletion of reserve SCs. In this case the myofiber niche is important for keeping SCs in a quiescent state through the limited release of soluble factors, a protective mechanism that starts to fail in aging tissue. Convincing

studies have similarly demonstrated a central role for the niche in contributing to declining germ SC function and decreased fertility with age. Testes generally atrophy in aged mice; overall weight of testes and number of SSCs decreases, and these SCs have diminished functionality in serial transplantation assays (Zhang, Ebata, Robaire, & Nagano, 2006). However, young SSCs can repopulate old testes stroma, and SSC transplantation into a young environment can rejuvenate their activity, suggesting that both germ SCs and niche undergo decline during aging, but still maintain the potential to produce functional gonads. In another study serial transplantation of adult SSCs through young testes stroma maintains self-renewal and sperm-generating capacity for up to 3 years (Ryu, Orwig, Oatley, Avarbock, & Brinster, 2006), highlighting the powerful role of a young niche in supporting a SC lineage even longer than the lifespan of the animal.

An understanding of HFSC and niche cell maintenance during iterative hair cycles and aging is generally lacking. In mice, the first hair cycle takes only a few days to complete while subsequent cycles are increasingly lengthy (Sennett & Rendl, 2012). In humans, aging follicles similarly decline in productivity as cycling turnover slows, and shorter, smaller HFs become suspended in the resting telogen phase (Kligman, 1988; Trueb, 2006). Very little is known about the cellular dynamics behind these morphological changes, although correlative data has suggested a link between DP niche integrity and continued HF cycling (Chi et al., 2013), and when DP cells are physically ablated in mice, HF regeneration fails (Rompolas et al., 2012). It follows that loss of the mesenchymal compartment and/or activating niche signals during successive hair cycles might contribute to a decline in HF productivity, but the relevance to physiological aging has yet to be explored. Alternatively, an increase in quiescence-promoting signals from the aging skin macroenvironment could negatively impact follicle productivity (Chen et al., 2012).

As SC proliferation and differentiation into the appropriate progeny can be influenced by stimuli in the immediate environment, it also implies that dysregulation within the niche could promote tumorigenesis. The concept of cancer stem cells (CSCs) is still evolving, and so too is the distinct idea of a cancer SC niche, in which normal SCs are prompted to proliferate excessively or a specific lineage overexpands because of aberrant signals from the environment. Evidence for aberrant niche signaling driving tumorigenesis has been described in only a few cancer models. Studies of stromal cells derived from human basal cell carcinomas found increased expression of BMP antagonist Gremlin1 compared to normal fibroblasts, and subsequent

work in culture suggested sustained repression of BMP signaling is permissive for CSC expansion (Sneddon et al., 2006). *In vitro* work found enhanced Wnt signaling within colorectal CSCs, which could be enforced by secreted signals generated by myofibroblasts that normally reside in the crypt microenvironment (Vermeulen et al., 2010). Experimentally targeting niche cells for gene deletion in the bone marrow can induce myelodysplasia in mice, presenting the provocative idea that dysregulation in the niche can drive cancer development (Raaijmakers et al., 2010). Also, studies of glioblastoma have found evidence supporting the existence of CSCs that depend on interactions with nearby perivascular and immune niche cells to specifically promote cancer cell survival and proliferation (Charles et al., 2010; Filatova, Acker, & Garvalov, 2013; Heddleston, Li, McLendon, Hjelmeland, & Rich, 2009; Zhu et al., 2011). As powerful signals generated by glioblastoma cells can dramatically alter the gene expression and migration of surrounding vasculature cells, and cancer cells even transdifferentiate into tumor endothelial cells (Wang et al., 2010) it seems that CSCs can mold their own defective niche. Similarly, in the case of cutaneous squamous cell carcinomas, CSCs use secreted signals to simultaneously promote self-renewal and reshape the vasculature in their microenvironment (Beck et al., 2011).



5. CONCLUDING REMARKS

Accumulating data implicates the microenvironment of SCs in governing their behavior and capacity for tissue regeneration. Adult SC niches are composed of cells from different developmental origins, including the SCs themselves and their progeny, mesenchymal neighbors, and other more distant cells. The ECM, basement membrane, and other adhesion molecules are also key players in many adult SC niches (Fig. 12.1). From the molecular data collected in different model systems, three main signaling pathways have been identified as executors of quiescence, survival/maintenance, and/or activation of SCs: the TGF β superfamily signals, the Wnt pathway, and Notch signaling, although many other tissue specific regulators have also been described (Figs. 12.2 and 12.3).

By understanding how SC niches function in physiological conditions and disease we can create better systems to study and manipulate SCs *in vitro*, which will ultimately be crucial for future clinical applications. A small number of niche-centric therapeutic approaches are already being applied in clinic, the most notable example being advances in bone marrow transplantation (Thomas, Stein, Gentile, & Shah, 2010). Recent

experimental evidence suggests that agents directly targeting the niche could be useful in the future to enhance transplanted HSC viability (Naveiras et al., 2009). In the future, quiescence-promoting proteins or small molecule treatments could be applied to counteract cancer-driving signals from a diseased niche, or activating signals could be exogenously supplied to jump start tissue regeneration.

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