Stem cells and their niche: an inseparable relationship

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A recent Keystone symposium on ‘Stem Cell Interactions with their Microenvironmental Niche’ was organized by David T. Scadden and Allan C. Spradling. The meeting was held in conjunction with another Keystone symposium, ‘Stem Cells and Cancer’, at Keystone, Colorado. Among the work that was presented at this meeting, scientists presented data that advances our understanding of the contribution that the niche makes to stem cell maintenance. Novel types of stem cells and niches were also reported and new findings that clarify our understanding of the molecular mechanisms that regulate and maintain stem cells were presented.

Introduction

Stem cells have the remarkable ability to undergo both self-renewal and to give rise to progeny that can differentiate. This capacity, in adult stem cells, has recently been shown to be dependent on the microenvironment or niche in which the stem cell resides (Li and Xie, 2005) (Fig. 1). The idea that a niche could regulate such cell fate decisions was first proposed by Schofield (Schofield, 1978). The location and structure of the Drosophila and Caenorhabditis elegans germline stem cell (GSC) niches were among the first to be defined (Cox et al., 1998; Crittenden et al., 2002; Kiger et al., 2001; Tulina and Matunis, 2001; Xie and Spradling, 2000). Subsequently, adult stem cells and their niches have been identified in different types of mammalian tissues, including in the hematopoietic system (Arai et al., 2004; Calvi et al., 2003; Kiel et al., 2005; Zhang et al., 2003), in the epithelial system (Blanpain et al., 2004; Cotsarelis et al., 1990; Niemann et al., 2002), in the neural system (Doetsch et al., 1999; Palmer et al., 1997; Shen et al., 2004) and in the intestinal system (He et al., 2004; Potten et al., 1997). The last few years have heralded the discovery of new types of stem cells and their respective niches, in addition to the identification of the major signaling events that regulate stem cell self-renewal. In this meeting review, we summarize recent advances in the stem cell field and highlight the contribution that the niche makes to maintaining different stem cells.

Germline stem cells

The well-studied GSCs in C. elegans and Drosophila are maintained by distinct mechanisms. Judith Kimble (University of Wisconsin, Madison, WI, USA) summarized our current understanding of how GSC self-renewal is controlled in C. elegans. The single niche cell, the distal tip cell, expresses LAG-2/Delta (Dl), which activates GLP-1/Notch in neighboring GSCs to control their self-renewal via the activation of the Pumilio-like FBF genes, which repress meiosis-promoting genes (Crittenden et al., 2003). BrdU label retention has been widely used and accepted as a method for detecting stem cells in a variety of mammalian systems (Li and Xie, 2005). However, Kimble showed that no BrdU label retention was observed in the mitotic region where GSCs are localized, indicating that, in C. elegans, GSCs do not progress slowly through the cell cycle nor do they undergo immortal DNA-strand segregation (Crittenden et al., 2006). It has also recently been reported that Drosophila ovarian GSCs fail to retain BrdU labeling, indicating that GSCs in the Drosophila ovary also undergoing constant cycling (Song et al., 2007). These findings demonstrate that the use of BrdU long-term retention (LTR) to detect quiescent stem cells is an inappropriate strategy for localizing the position of stem cells in an organism or tissue in which stem cells undergo constant cycling, unless stem cells do indeed segregate chromosomes conservatively, as first proposed by Cairns (Cairns, 1975). Despite this, the label-retention stem cell-detection assay may still be effective in detecting the location of stem cells in certain mammalian tissues that have a much longer life span and that require the long-term maintenance of stem cells. Kimble further reported that the spindle orientation in dividing GSCs is irrelevant for GSC maintenance, leading her to propose that, in C. elegans, GSCs are maintained as a unique population. By contrast, the spindle orientation in male Drosophila GSCs is important for determining whether a GSC divides asymmetrically or symmetrically (Yamashita et al., 2003). Normally, one of the two centrosomes must be positioned apically near the hub (the niche) to ensure correct asymmetric division. Margaret Fuller (Stanford University, Stanford, CA, USA) used a genetic strategy to differentially label the mother centrosome from the daughter centrosome in GSCs, and discovered that the mother centrosome is invariably inherited by the GSC, whereas the duplicate daughter centrosome is passed on to the differentiated gonialblast (Yamashita et al., 2007). It is still unclear what the significance of such centrosomal inheritance is to stem cell regulation and what mechanisms underlie such centrosomal segregation.

In the Drosophila ovary and testes, committed germ cell progenitors can revert back to stem cells (Brawley and Matunis, 2004; Kai and Spradling, 2004). Erika Matunis (Johns Hopkins, Baltimore, MD, USA) reported that the reversion of differentiated germ cells back into GSCs is dependent upon the niche signal. Genetic screens conducted in her laboratory have identified mutants that can increase the efficiency of reversion from committed germ cell progenitors back into GSCs. Further molecular characterization of these mutants could provide novel insight into how committed germ cell progenitors are reprogrammed. Intriguingly, Niels Geijsen (Harvard Medical School, Boston, MA, USA) reported that mouse embryonic stem cells (ESCs) can be propagated under human ESC culturing conditions. These alternatively cultured mouse ESCs have a morphology that resembles that of human ESCs, in addition to their identification in the mitotic region where GSCs are localized, indicating that, in Drosophila, GSCs do not progress slowly through the cell cycle nor do they undergo immortal DNA-strand segregation (Crittenden et al., 2006). It has also recently been reported that Drosophila ovarian GSCs fail to retain BrdU labeling, indicating that GSCs in the Drosophila ovary also undergoing constant cycling (Song et al., 2007). These findings demonstrate that the use of BrdU long-term retention (LTR) to detect quiescent stem cells is an inappropriate strategy for localizing the position of stem cells in an organism or tissue in which stem cells undergo constant cycling, unless stem cells do indeed segregate chromosomes conservatively, as first proposed by Cairns (Cairns, 1975). Despite this, the label-retention stem cell-detection assay may still be effective in detecting the location of stem cells in certain mammalian tissues that have a much longer life span and that require the long-term maintenance of stem cells. Kimble further reported that the spindle orientation in dividing GSCs is irrelevant for GSC maintenance, leading her to propose that, in C. elegans, GSCs are maintained as a unique population. By contrast, the spindle orientation in male Drosophila GSCs is important for determining whether a GSC divides asymmetrically or symmetrically (Yamashita et al., 2003). Normally, one of the two centrosomes must be positioned apically near the hub (the niche) to ensure correct asymmetric division. Margaret Fuller (Stanford University, Stanford, CA, USA) used a genetic strategy to differentially label the mother centrosome from the daughter centrosome in GSCs, and discovered that the mother centrosome is invariably inherited by the GSC, whereas the duplicate daughter centrosome is passed on to the differentiated gonialblast (Yamashita et al., 2007). It is still unclear what the significance of such centrosomal inheritance is to stem cell regulation and what mechanisms underlie such centrosomal segregation.
addition to reporting that *scrawny* (*scny*), a possible *Drosophila* ortholog of the yeast histone H2B deubiquitylating enzyme UBP10, is essential for controlling GSC self-renewal. The link between epigenetic control and dedifferentiation is fascinating; however, future work in this area should provide a clearer picture about the contribution that epigenetic regulation makes to stem cell maintenance.

The importance of small RNAs in germ cell development has been recognized only recently (Jin and Xie, 2006). Haifan Lin (Yale University, New Haven, CT, USA) reported that *mili* (also known as *Piwi2* – Mouse Genome Informatics), a mouse homolog of the *Drosophila piwi*, is required for GSC maintenance in the mouse testis. Interestingly, however, *mili* appears to function cell-autonomously, in contrast to the function of MO, USA) reported that 75% of the long-term mouse HSCs that were isolated using Flk2-LSK markers also express N-cadherin, as shown by antibody staining and real-time PCR. It was further shown that an anti-N-cadherin-neutralizing antibody is able to compromise HSC engraftment. Toshio Suda (Keio University, Tokyo, Japan) confirmed that quiescent mouse HSCs in the osteoblastic niche express N-cadherin, whereas mobilized HSCs express less N-cadherin. Suda then showed that ectopic expression of a dominant-negative N-cadherin in HSCs reduces their attachment to the osteoblastic niche. Moreover, the expression of a mutant N-cadherin that lacks a β-catenin-binding site in HSCs results in the increased nuclear localization of β-catenin and, thus, proliferation, but reduced long-term HSC reconstitution. These observations reveal the important role that N-cadherin plays in HSC anchorage and suggests a role in the maintenance of a quiescent state. Further to this, Suda reported that the anti-oxidant treatment of HSCs prevents increased levels of intracellular reactive oxygen species (ROS) and prolongs the lifespan of HSCs, raising the possibility that ROS involvement in niche regulation is mediated via the downregulation of N-cadherin.

Slam* (also known as Slamfl and CD150 – Mouse Genome Informatics) HSCs are predominantly localized to the vascular niche (Kiel et al., 2005). Interestingly, Sean Morrison (University of Michigan, Ann Arbor, MI, USA) reported that HSCs do not express N-cadherin. He further reported that HSCs cannot be reliably identified based on BrdU label retention. biglycan mutant mice, he showed, display a severe osteoblast deficiency, while possessing a normal number of HSCs. These findings indicate that a quantitative reduction in osteoblasts does not necessarily lead to an equivalent reduction in HSCs, implying that the majority of HSCs are not acutely dependent upon contact with osteoblasts. However, whether the activity of HSCs, in *biglycan* mutant mice, is altered is not known. Reconciling these observations with previous studies showing that HSCs (revealed by BrdU LTR) are predominantly
localized in close contact with osteoblasts, could it be that these Slam+ HSCs represent a distinct population of HSCs from those that express N-cadherin in the osteoblastic niche? This awaits future verification. Kateri Moore (Princeton University, NJ, USA) reported that green fluorescent protein (GFP)-labeled mouse long-term retaining cells (LRCs) are frequently detected near the osteoblastic-niche surface and can also be seen in close proximity to blood vessels. Furthermore, these GFP-labeled LRCs are predominantly in the G0 phase of the cell cycle and are able to efficiently form stem cell colonies in long-term culture.

Molecular and genetic screens provide powerful approaches for the identification of novel factors involved in developmental regulation, including the regulation of stem cells. David Scadden (Harvard Stem Cell Institute, Cambridge, MA, USA) has used molecular screens to identify genes, such as Gsα and P2Y14 (also known as P2yr14 – Mouse Genome Informatics; a nucleotide receptor), that are expressed in purified HSCs. The conditional knockout of Gsα in mice inhibits HSC niche engagement by interfering with HSC migration. P2Y14-knockout mutant mice display normal HSCs under normal conditions, but show defects in HSC expansion in response to bone marrow injury. Leonard Zon (Harvard Medical School, Boston, MA, USA) reported the identification of small molecules that affect the number of HSCs in zebrafish. Results from a chemical genetic screen designed to isolate chemicals that modify HSCs revealed several chemicals that disrupt the prostaglandin-synthesis pathway. It was shown that Prostaglandin E2 (PGE2) promotes HSC formation in zebrafish. Furthermore, Zon showed that PGE2 exposure in mice can enhance hematopoietic recovery following radiation therapy and can also promote a dose-dependent increase in the formation of hematopoietic colonies from murine ESCs in vitro. This work represents a prime example of the transfer of findings from studies in model organisms to possible clinical applications.

**Stem cells in the epithelia**

The skin is a unique system in which to study the developmental regulation of epithelial stem cells because these stem cells reside in well-defined regions (Fuchs et al., 2004). Elaine Fuchs (Rockefeller University, NY, USA) reported that Wnt signaling activates mouse hair follicle stem cells (HFSCs) via the regulation of interactions between β-catenin and Tcf3 or Lef1. Tcf3 activity alone (without β-catenin) is important for maintaining stem cells in an undifferentiated state, which it does via the transcriptional repression of lineage-specific transcription factors, whereas the β-catenin-Tcf3
complex activates stem cell proliferation and the β-catenin-Lef1 complex governs differentiation. Fuchs also discussed the importance of α-catenin in maintaining the appropriate spindle orientation and, thus, asymmetric division in epidermal precursor cells. Additionally, she reported that α-catenin has a further role in the repression of the Ras-MAPK pathway activity in the proliferative compartment of the skin. Fiona Watt (Cancer Research UK, London, UK) also reported that high, intermediate and low Wnt signaling promotes the formation of hair cells, sebaceous glands and interfollicular epidermal cells, respectively, in the mouse. She further reported that the expression of an N-terminal truncation of Lef1 under the control of the K14 promoter induces the formation of squamous sebaceous cell tumors by blocking p53 induction.

The intestinal crypt is another attractive system in which to study epithelial stem cells. Li reported that Wnt signaling positively controls, and bone morphogenetic protein (BMP) signaling negatively controls, intestinal stem cell (ISC) activation and proliferation, respectively. However, both are required for lineage specification. The phosphoinositide-3 kinase (PI3K)-Akt pathway, which is normally suppressed by PTEN, can cooperate with Wnt signaling to control the entry of ISCs into the mitotic cycle. In Drosophila, ISCs have been shown to exist in the posterior midgut and to be regulated by N signaling (Michielli and Perrimon, 2006; Ohlstein and Spradling, 2006). Spradling reported that the expression of DL, an N ligand, in Drosophila ISCs activates N signaling in neighboring ISC daughter cells. Tumor formation occurs if N signaling in these cells is prevented, indicating a crucial role for N signaling in ISC differentiation. No specific niche cells have yet been identified for these ISCs, suggesting that they have a non-cell based niche (see Fig. 1). Spradling further described the first detailed cellular characterization of the follicle stem cell (FSC) niche. FSCs in the Drosophila ovary are responsible for the continuous production of the epithelial somatic follicle cells that surround the germline cysts (Margolis and Spradling, 1995). He showed that FSC daughter cells frequently migrate across the germarium where they sometimes displace resident FSCs in a nearby niche. Sergei Sokol (Mount Sinai School of Medicine, NY, USA) used the epithelium of Xenopus embryos as a model in which to show that the cell polarity factors pARK and Par-1 are involved in cell fate determination via the regulation of DI expression and, thus, N signaling. It would be interesting to know whether these polarity genes also affect N signaling in epithelial stem cell lineages.

The cornea is thought to be maintained by a population of cornea stem cells in the limbus, the corneal-scleral junction (Li et al., 2007). Yann Barrandon (Ecole Polytechnique Fédérale de Lausanne, Switzerland) reported the surprising finding that, in fact, corneal stem cells are scattered throughout the cornea, which he demonstrated via a combination of laser ablation and cornea transplantation experiments. He further reported that the mTOR pathway is involved in the control of skin epithelial stem cell responses to environmental change via the binding of the transcriptional activators TORC1 and TORC2 to torrid sequences in the temperature-responsive target genes.

**Neural stem cells**

The subventricular zone (SVZ) and the subgranular zone (SGZ) are the primary and well-recognized germinial regions where neural stem cells (NSCs) reside in the adult brain (Li and Xie, 2005). Charles ffrench-Constant (University of Cambridge, UK) reported that tenasin C is expressed highly in the SVZ in mice, and is responsible for switching NSC responsiveness from fibroblast growth factor (FGF) to epidermal growth factor (EGF) by regulating the expression of the EGF receptor. Tenasin C-deficient mice also have altered numbers of CNS stem cells, indicating that tenasin C contributes to the stem cell niche function within the SVZ. He further reported that β1 integrin is highly expressed in NSCs, making it a reliable NSC marker. The ablation of β1 integrin does not affect NSC self-renewal in vitro, suggesting that it may have other roles in stem cell maintenance within the SVZ. Chay T. Kuo (University of California, San Francisco, CA, USA) revealed that the removal of Numb and numb-like function from mouse postnatal SVZ progenitors and ependymal cells resulted in severe damage to lateral ventricle integrity in the mammalian brain. Surprisingly, this ventricular damage was eventually repaired; SVZ reconstitution and ventricular wall remodeling were mediated by progenitors that escaped Numb deletion. These findings highlight the existence of a self-repair mechanism in the mammalian brain.

Wnt, N and Hedgehog (Hh) pathways have all been shown to be required for the self-renewal or the differentiation of stem cell progeny in a variety of systems, including NSCs (Li and Xie, 2005). Roel Nusse (Stanford University, Stanford, CA, USA) shared his insights into how Wnt signaling maintains an undifferentiated state in progenitor cells. In the SVZ of the developing brain, a Wnt reporter, Axin-lacZ, is expressed in radial glial cells, the putative progenitor cells. Radial glial cells, isolated by fluorescence activated cell sorting (FACS), from mouse embryonic brains are able to form stem cell-like colonies in the presence of Wnt3a. These cultured progenitor cells can differentiate into neurons, glial cells and oligodendrocytes after the removal of Wnt3a or with the addition of dickkopf, a Wnt inhibitor. Thus, Wnt3a functions as a self-renewing factor for neuronal progenitor cells, but it is not a mitogen. Andreas Androussis-Theotokis (NINDS, National Institutes of Health, Bethesda, MD, USA) reported that N signaling and ciliary neurotrophic factor (CNTF) can maintain NSC self-renewal in mice by regulating the phosphorylation of Stat3, indicating that different signaling pathways may converge on Stat3 to control NSC survival.

**In vitro stem cell niches**

As discussed earlier, the extracellular matrix (ECM) is an important aspect of the stem cell niche; it plays an essential role in anchoring stem cells to the niche and potentially modulates their function. Donald Ingber (Harvard Medical School, Boston, MA, USA) reported findings that show that the physical status of the microenvironment is as potent a regulator of stem cell and tissue development as molecular signaling is. He showed that mechanical forces that are applied to integrins, and the changes in ECM mechanics that alter the cytoskeleton and thereby simultaneously activate multiple signaling pathways, drive cell fate switching in vitro. He also described the induction of neutrophil differentiation in human promyelocytic precursor cells by specific hormones and showed that non-specific stimuli result in a common phase-transition-like switching among hundreds of genes distributed across the genome-wide gene regulatory network. Helen Blau (Stanford University, Stanford, CA, USA) reported the development of in vitro-engineered niches that have been used in her laboratory to culture muscle stem cells, pancreatic progenitor cells and HSCs. These engineered niches are constructed by fabricating hydrogel microwell arrays for single stem cells. It appears that engineered niches, for monitoring the fate of single cells via time-lapse microscopy, in conjunction with genetic fate mapping, are suitable for screening factors that are required for stem cell self-renewal and the differentiation of stem cell progeny on a large scale.
Cancer stem cell microenvironment

Normal stem cells depend on a niche to provide the necessary signals for self-renewal. Likewise, cancer cells also require a special microenvironment to maintain cancer stem cells and to support cancer cell growth. The BMP and Wnt pathways represent a ‘Yin-Yang’ type of controlled balance between self-renewal and differentiation (Li and Xie, 2005). The deregulation of these signals can lead to the uncontrolled self-renewal and proliferation of stem cells, risking tumorigenesis. Tannishtha Reya (Duke University, Durham, NC, USA) showed that β-catenin is required for long-term HSC maintenance in vivo, and that the conditional deletion of β-catenin in mice also impairs Bcr–Abl-induced chronic myeloid leukemia (CML) development. Consistently, Irving Weissman (Stanford University, Stanford, CA, USA) and Catherine Jamieson (University of California at San Diego, CA, USA) reported that mutations/splice abnormalities in GSK3β (GSK3B), a major β-catenin inhibitor, were frequently found in myeloid blast-crisis leukemia stem cells from human patients with CML. Phil Beachy (Stanford University, Stanford, CA, USA) revealed that the Sonic hedgehog (Shh) pathway is also involved in stem cell regulation, and that the abnormal activation of Shh signaling is very often associated with various cancers, including multiple myeloma. Changes in niche signaling, including the inactivation of proliferation inhibitory pathways, can also impact stem cell self-renewal and tumorigenesis (He et al., 2004). Patrick Brown (Stanford University, Stanford, CA, USA) reported the identification of a dominant BMP inhibitory factor, gremlin, which is upregulated in basal cell carcinoma tumor stromal cells. Gremlin enhances the proliferation of tumor cells, consistent with the role of BMP in suppressing stem/progenitor cell proliferation. The notion that an altered microenvironment might contribute to pre-cancerous conditions was discussed by Louise Purton (Massachusetts General Hospital, Boston, MA, USA). She reported that myeloid proliferative disorder (MPD) can be caused by a retinoic acid receptor γ (RARG)-deficient microenvironment. Carl Walkley (Harvard Medical School, Boston, MA, USA) and Stuart Orkin (Harvard Medical School, Boston, MA, USA) then showed that MPD is also seen in a retinoblastoma 1 (Rb1)-deficient mouse model, which has a substantial reduction in trabecular bone volume.

Conclusion

The meeting revealed several interesting aspects of the relationship between stem cells and their niche (Fig. 1). First, we have gained a deeper understanding of how the niche and the signals that emanate from the niche, such as Wnt, BMP, N and Shh, control self-renewal. Second, new stem cell types, such as ISC s in the Drosophila gut and cornea stem cells in mice, and their niches have been identified. Third, the existence of a non-cellular niche has been revealed, such as the Drosophila ISC niche. Fourth, the contribution that the tumor microenvironment makes to the initiation of tumorigenesis has been uncovered. And last, but certainly not least, the creation of stem cell niches in vitro not only helps our understanding of stem cell regulation but will also support future tissue-engineering efforts. We anxiously anticipate further progress in these areas over the next few years.

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References
