Leading Edge
Review

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The Tortoise and the Hair: Slow-Cycling Cells in the Stem Cell Race

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Given the importance of stem cells to adult tissues, it has long been postulated that stem cells divide infrequently to preserve their long-term proliferation potential and to prevent the acquisition of errors during DNA replication. Yet, some stem cells must be able to continually churn out progeny in tissues that rapidly turn over or are subject to sudden injuries or growth spurts. This Review explores the challenges that mammalian stem cells face in balancing the competing demands of proliferation and differentiation in tissues.

Introduction

Adult stem cells have the capacity to self-renew and to regenerate tissue(s) long-term, in both homeostasis and wound repair. These remarkable fixtures of longevity place stem cells in an elite class of essential cells of living organisms. Given the importance of stem cells to body tissues, it has long been postulated that stem cells should be used sparingly and tucked safely away into resident niches, guarding them from harm's way.

Some tissues of the body, such as those in the brain and skeletal muscle, have very little turnover and are well protected, whereas others turnover constantly. Even though the intrinsic properties of stem cells are likely to be similar across tissues, each tissue has its own requisites for homeostasis and regeneration. We lose over 20 billion cells a day, requiring constant replenishment to stay alive. More than a billion of these lost cells come from our blood, necessitating a reservoir of constantly renewing hematopoietic stem cells (Orkin and Zon, 2008). The intestinal epithelium also undergoes constant turnover, taking only 3–5 days for undifferentiated cells at the bottom of the invaginating crypt to proliferate and differentiate into the enterocytes, goblet cells, or enteroendocrine cells of the adsorptive villus (Barker et al., 2008). Analogously, every 4 weeks, we have a brand new epidermis as cells in the basal layer terminally differentiate and are shed from the skin surface (Watt, 2002).

Some stem cells face even greater challenges. During pregnancy, the mammary epithelium undergoes a dramatic change as elaborate glands branch, differentiate, and produce milk. Hair follicles undergo cyclic bouts that entail not only periods of massive destruction and dormancy but also periods of active follicle regeneration and hair growth. Confounding the problem, the hair growth phase, which requires stem cells, is relatively uniform in length, but the resting phase increases with age, leading to extended periods where nothing appears to be happening (Blanpain and Fuchs, 2009). Finally, all of our tissues occasionally face traumatic injuries. Although this is commonplace for some tissues such as the skin epithelium, other tissues, such as the central nervous system, are not so well adjusted.

These sudden demands place a heavy burden on the nearby stem cell niches. All of these considerations mean that stem cells must be able to adjust swiftly in order to maintain a proper balance. When to cycle and how fast to cycle are features that vary considerably among stem cell populations. Moreover within a given tissue, more frequently cycling stem cells seem to function primarily in homeostasis while a reserve of more dormant master stem cells may be set aside for times of injury or unforeseen need. So when is "slow" slow and "fast" fast and what does this mean for maintaining stemness?

Below, I concentrate on three representative populations of adult mammalian stem cells—hematopoietic stem cells, hair follicle stem cells, and intestinal stem cells—and discuss the common themes that have emerged from studying their slow-cycling properties in normal homeostasis and in response to injury. The factors that enter into stem cell longevity are varied and complex and include not only the cellular interactions and stimuli that constitute the environment or "niche" in which stem cells reside but also intrinsic mechanisms governing such diverse processes as telomere length, cell survival, and asymmetric cell division. This Review highlights how the cycling kinetics of stem cells may enter into this medley.

Heterogeneity within the Hematopoietic Stem Cell Niche

The existence of stem cells within the bone marrow was demonstrated nearly 50 years ago by reconstitution of the hematopoietic system following irradiation (Till and McCulloch, 1961). These early serial transplantation studies revealed that less than 1% of bone marrow cells possess the capacity for long-term reconstitution. Detailed cell-cycle analyses have further revealed that most hematopoietic stem cells are quiescent and in the G0 phase of the cell cycle (Cheshier et al., 1999; Kiel et al., 2007; Passegue et al., 2005; Potten et al., 1978; Punzel and Ho, 2001; Spangrude and Johnson, 1990). Over the years, molecular markers have been identified to isolate and purify long-term hematopoietic stem cells (LT-HSCs) that exhibit special longevity (Christensen and Weissman, 2001; Muller-Sieburg

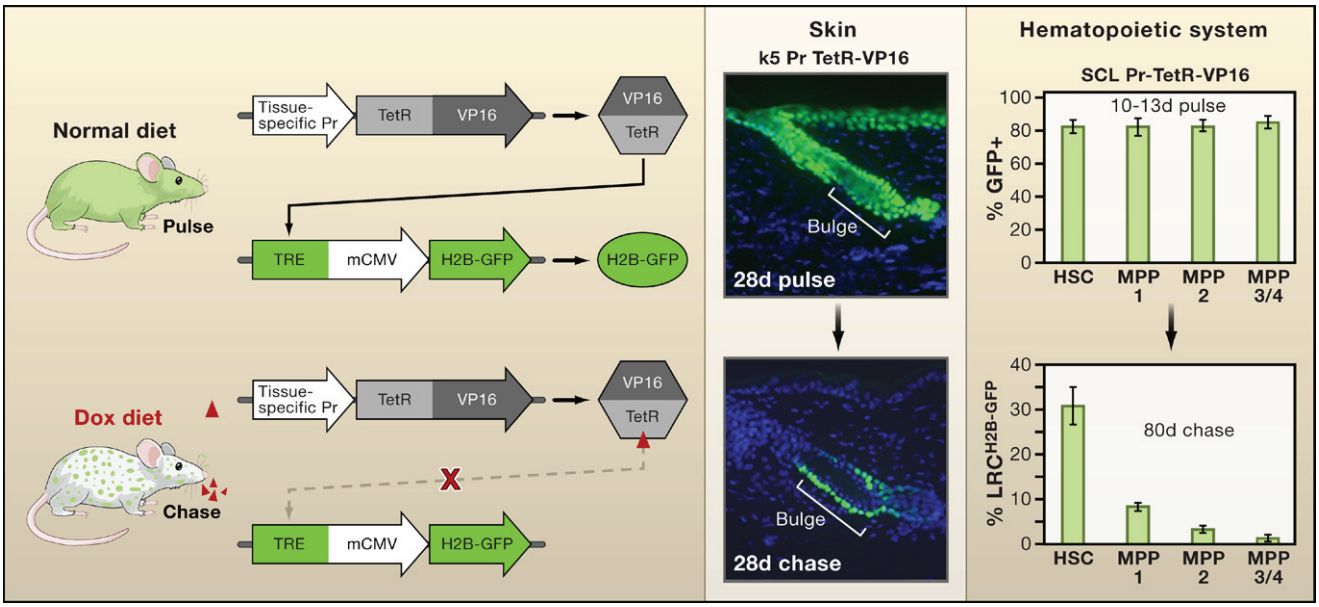

Figure 1. The Histone H2B-GFP Pulse-Chase System

The mating of two parent strains of transgenic mice is needed to make progeny in which expression of a transgene encoding histone H2B-green fluorescent protein (H2B-GFP) can be turned off when tetracycline is added to the animal's diet. The first parent strain harbors the H2B-GFP transgene under the control of a tetracycline (doxycycline; dox) regulatory element (TRE). The second expresses a transcription factor regulated by tetracycline (TetRVP16) that is under the control of a cell-type-specific promoter. In the first example shown, the *keratin 5* (k5) promoter drives expression of TetRVP16 leading to expression of H2B-GFP in the skin epithelium until 4 weeks of age, at which time tetracycline is administered for 4 weeks so that dividing cells dilute out the label and differentiating cells are sloughed from the skin (Tumbar et al., 2004). In the second example, TetRVP16 driven by the promoter of the *stem cell leukemia* (*SCL*) gene is used to express H2B-GFP in hematopoietic stem cells (HSCs) and different multipotent progenitors (MPPs) of the hematopoietic system for 10–13 days, after which tetracycline is administered for 80 days to identify the label-retaining cells (LRCs) (Wilson et al., 2008). Schematic adapted from Nowak (2009).

et al., 1986; Spangrude et al., 1988). This offers an ideal system for study, as evidenced by the fact that between 20% and 50% of purified ($Lin^-Sca1^+c\text{-}kit^+CD150^+48^-$) cells possess repopulation activity when serially transplanted in vivo (Challen et al., 2009; Christensen and Weissman, 2001; Foudi et al., 2009; Kiel et al., 2007; Spangrude et al., 1988; Wilson et al., 2008).

The steady-state pool of HSCs has been estimated at ~20,000–100,000. A subset of these are responsible for regenerating the shorter-lived and often rapidly dividing progeny, known as multipotent progenitors (MPPs), which produce nearly a billion circulating blood cells per day (Passegue et al., 2005; Wagers et al., 2002; and references therein). Serial transplantations of these HSCs in mice have been extended up to 5–7 rounds (Harrison and Astle, 1982; Harrison et al., 1978). It is not yet clear whether the inability to carry out serial transfer endlessly is a limitation of the assay or rather reflects a limited self-renewal capacity of these stem cells. However, such in vivo tests for longevity of HSCs are presently superior to those for other adult stem cells.

It has been estimated that two-thirds of the LT-HSCs are in G0 at any one time, a feature that correlates with their ability to function in hematopoietic reconstitution (Passegue et al., 2005). Proliferation kinetics suggest that cell divisions are less frequent in LT-HSCs than in short-tem HSCs and in more committed MPP cells of hematopoietic lineages. In BrdU pulse-chase experiments, ~5%–6% of HSCs still retain label even after 2 months (Cheshier et al., 1999; Foudi et al., 2009; Kiel et al., 2007; Morrison et al., 1997; Wilson et al., 2008). Triple label studies with nucleotide tracers demonstrate that these label-retaining cells (LRCs) do not arise from asymmetric segregation of a master template DNA strand while passing the newly synthesized strand to the committed daughter (Kiel et al., 2007). Such a feature would otherwise skew the use of nucleotide label as a means of monitoring slow-cycling properties of stem cells.

Although the kinetics of BrdU incorporation and retention have long argued for the existence of HSCs that are also LRCs (Cheshier et al., 1999; Kiel et al., 2007), researchers have wondered whether nucleotide label administered to adult mice might fail to mark highly quiescent stem cells if they divide only very rarely. Of further concern is whether BrdU as a mutagen might elicit some cell damage in LRCs. If so, this might prompt snoozing HSCs to awaken, proliferate, and enter a damage-repair mode, diluting nucleotide label in the process. BrdU toxicity also clouds the reliability of testing the stem cell functionality of LRCs.

Some of the possible caveats arising from long-term studies with BrdU have been circumvented by generating mice that harbor an histone 2B-green fluorescent protein (H2B-GFP) transgene driven by a tetracycline-regulatable enhancer element and its requisite transcription factor driven by a tissue-specific promoter (Figure 1) (Tumbar et al., 2004). This method was first devised and tested for the isolation and purification of LRCs of the hair follicle (Tumbar et al., 2004). By constitutively expressing H2B-GFP during embryogenesis, and then switching off expression postnatally in response to tetracycline, cells