Asymmetric Cell Division in Normal and Malignant Hematopoietic Precursor Cells

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Hematopoietic precursors have long been postulated to divide in an asymmetric manner. In this issue of *Cell Stem Cell*, Wu et al. (2007) provide evidence for the existence of asymmetric cell division and its possible molecular control in normal and transformed blood precursor cells.

Every second of our lives, millions of blood cells are destroyed and must be regenerated. This regeneration is accomplished by hematopoietic stem cells (HSCs), the only cells that throughout life are able to both produce the required new cells of all blood lineages and maintain their own numbers. Obviously, the regulation of HSC fate decisions between remaining in the stem cell state (self-renewal) or becoming a cell without stem cell potential (differentiation) must be tightly balanced for normal hematopoietic homeostasis. Despite decades of research, our understanding of the molecular mechanisms controlling self-renewal in HSCs is still very limited. In addition, we know even less about how the total HSC pool size is kept constant over time. With individual HSCs dispersed over the whole body, how are the self-renewal decisions coordinated between them? Many different models, involving both extrinsic and intrinsic signals, have been suggested. Such models are generally classed as "instructive," in that systemic feedback signals or local environmental cues may regulate HSC decision making, or "stochastic," in which any given HSC is equally likely to exit the stem cell state at a given time, resulting in a constant pool size determined by the overall probability of self-renewal within the population.

Another model that provides a conceptual mechanism for the required tight control of HSC numbers is asymmetric cell division (ACD). In ACD, distinct fates of the two generated daughter cells are prospectively determined by a mechanism that is linked to mitosis (Figure 1 and reviewed in Morrison and Kimble, 2006). If HSC divisions produce one differentiated and one HSC daughter, overall HSC numbers would remain constant even while differentiated progeny are produced. It is important to note that ACD can not be the exclusive mode of HSC division, because expansion of the HSC pool is necessary and possible, e.g., after its reduction by injury or irradiation. However, ACD could be a central homeostatic mechanism controlling HSC self-renewal, possibly modulated by extrinsic signals under regenerative stress conditions.

Although it has long been postulated that HSCs divide in an asymmetric manner, clear mechanistic evidence has been missing to date. Importantly, asymmetric fates of HSC progeny do not necessarily constitute evidence of ACD. That is, the fates of two identical daughter cells could be independently influenced after a symmetric division, without being linked to the mitosis event itself. Asymmetric fates of the progeny of mammalian hematopoietic precursor cells (HPCs) have been described previously in a number of elegant studies using paired daughter cell analyses and microscopic time-lapse imaging (Brummendorf et al., 1998; Ema et al., 2000; Punzel et al., 2003). It was also demonstrated that the frequency of these asymmetric fate decisions can be influenced by extrinsic signals provided by the type of stroma or cytokines used in culture (Ema et al., 2000; Punzel et al., 2003). Furthermore, the polarization of various molecules has recently been described in fixed mitotic human HPCs (Beckmann et al., 2007). However, because the asymmetric fates observed in the daughter cells could not be linked to a mitotic mechanism, the existence of ACD in blood cells remained uncertain. Compelling evidence for ACD in differentiated hematopoietic cells was provided this year by Chang et al., who demonstrated that several proteins, including Numb, are polarized in dividing T cells upon contact with an antigen presenting cell (Chang et al., 2007). The proteins are distributed asymmetrically between the resulting daughter cells, whose individual fates correlate with the type of "asymmetry proteins" they inherited during the ACD.

In this issue of *Cell Stem Cell*, Wu et al. (2007) developed a system to assay for ACD specifically in primitive hematopoietic precursors. To do so, the authors had to overcome a major obstacle facing hematopoiesis researchers, in that the field has historically lacked reliable markers for the prospective and noninvasive discrimination of differentiating and immature HPCs. Wu et al. approached this problem in an innovative way by relying on a substitute marker for the immature state of cultured HPCs. Population analyses indicated that, in HPCs from transgenic mice engineered to express eGFP under the control of activated Notch signaling, maintenance of eGFP expression in culture correlates with the maintenance of their immature

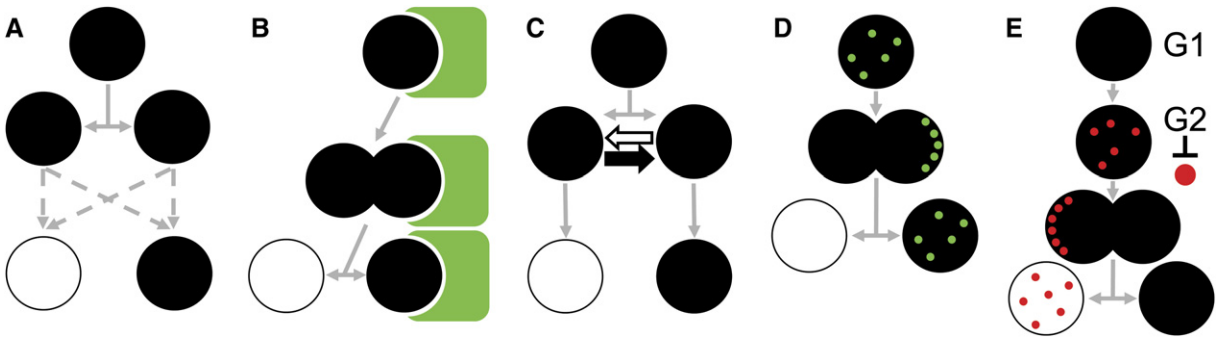

Figure 1. Some Possible Modes of Hematopoietic Precursor Divisions with Homeostatic Output

(A) Symmetric division: undifferentiated HPCs produce two undifferentiated daughters, and their later fate decisions are not linked to the mother's mitosis.

(B–E) Possible hypothetical mechanisms of asymmetric divisions in HPCs. (B) Orientation of the division plane leads to positioning of only one of the daughters close enough to localized extrinsic signals provided by a self-renewal (shown here in green) or differentiation niche. (C) Undifferentiated HPCs initially generate two identical undifferentiated daughters, which immediately after mitosis and, while still being in close spatial contact, engage in reciprocal feedback signaling, leading to the differentiation of only one of them. (D and E) Intrinsic cell fate determinants segregate asymmetrically between daughter cells, instructing either self-renewal (green dots in [D]) or differentiation (red dots in [E]) of the receiving daughter. (E) The activity of differentiation inducing determinants that are expressed in the mother cell would have to be blocked before mitosis (e.g., in the G2 phase of the cell cycle) to avoid premature loss of self-renewal.

Black, undifferentiated cells; white, differentiated cells.

function. By tracking dividing HPCs and their individual daughters via time-lapse imaging, the authors observed divisions after which only one daughter maintained eGFP expression; the decreased eGFP signal in the other daughter indicated its differentiation. In line with previous publications (Ema et al., 2000; Punzel et al., 2003), Wu et al. further showed that the frequency of these asymmetric fate outcomes can be modulated by extrinsic signals.

Although interesting, these findings do not in and of themselves prove that ACD has occurred. However, Wu et al. (2007) extended their analysis to examine the distribution of the protein Numb during precursor divisions. Numb, a negative modulator of Notch signaling, is known to asymmetrically segregate to one daughter during ACD in other cell types. Numb is therefore an attractive candidate to link the observed asymmetries in Notch activity in HPC daughters to molecular asymmetries during their division. The authors chose to analyze Numb localization in HPCs that had been fixed during mitosis and indeed found it to be frequently enriched in one of the two emerging daughter cells. Although live observations in dividing precursors would have been even more convincing, these data strongly suggest that, as in other cell populations, Numb can be asymmetrically segregated during HPC divisions, inhibiting Notch signals in only one daughter cell, correlating with and possibly inducing its differentiation (compare Figure 1E).

The demonstration of ACD in HPCs by the combined observation of asymmetric daughter cell fates and the mitotic asymmetric distribution of a potential cell fate regulator is a major step forward for the hematopoiesis field and opens new avenues toward improved understanding of the control of hematopoietic self-renewal. Detailed mechanistic analysis of how Numb-modulated Notch activity may influence self-renewal was beyond the scope of this study, and it does not clarify whether inhibition of Notch signaling is cause or consequence of differentiation. Nevertheless, it adds important new fuel to the debate about the role of Notch signaling in hematopoietic stem and progenitor cells. Although Notch activation has been postulated to maintain self-renewal in some studies, several groups have deleted various Notch receptors, RBP-J, the central mediator of all Notch signaling, as well as both Numb and Numb-like, and in each case, these molecules were not essential for HPC function (Han et al., 2002; Wilson et al., 2007). It remains to be seen how these conflicting conclusions will be reconciled in future studies. However, irrespective of the possible function of Notch signaling in HSC self-renewal decisions, Wu et al.'s development of a signal-dependent reporter of immature function in living precursors provides an invaluable tool for the analysis of hematopoiesis.

In an exciting second part of their study, Wu et al. (2007) go on to show that ACD frequency can be altered by expression of leukemogenic proteins. Interestingly, Nup98-HoxA9, which is associated with acute leukemia (reviewed in Abramovich and Humphries, 2005), alters the frequency of ACD, whereas the chronic leukemia promoting protein Bcr-Abl does not. Although the underlying molecular mechanism is unknown, this exciting observation shows that ACD frequencies can be influenced by both extrinsic and intrinsic factors and suggests that alterations in ACD of HPCs may be involved in leukemic transformation. This finding adds an important aspect to the current discussion about how different oncogenes that target specific hematopoietic maturation stages mechanistically influence the properties of a resulting leukemia.

With their study, Wu and colleagues contribute evidence for the widely predicted asymmetric cell division of hematopoietic precursors and its possible molecular control. Importantly,

these findings not only help to improve our understanding of normal hematopoiesis but also provide new insights into leukemogenesis.

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Kinship and Descent: Redefining the Stem Cell Compartment in the Adult Hippocampus

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Identifying multipotent, self-renewing neural stem cells (NSCs) within the adult hippocampus in vivo has been somewhat elusive. In this issue of *Cell Stem Cell*, Suh et al. (2007) show that *Sox2*-expressing cells in the subgranular zone (SGZ) of the dentate gyrus not only have NSC characteristics but also display an unexpected degree of heterogeneity.

A tenet of the adult stem cell niche hypothesis asserts that the tissue microenvironment regulates the cell cycle, self-renewal, and multilineage potential of stem cells. This principle leads to the widely accepted corollary that stem cells removed from their niche display cellular behaviors that may not be indicative of their normal function in vivo. In other words, it is the difference between what stem cells can do and what they normally do. For example, stem cells in vitro often display broader proliferative capacity, or can generate specific cell types in proportions that are different than those produced in vivo. Nonetheless, the peculiarities of niche-independent stem cell behavior can, to some extent, be rationalized by the notion that the niche necessarily mitigates a full stem cell repertoire in vivo due to physiological constraints.

In the adult vertebrate brain, neurogenic compartments display a strong bias in the types of cells that are generated (usually neuron production dominates), and the putative NSCs in these regions are thought to divide infrequently. Thus, it is difficult to define the in vivo identity of adult NSCs based on correlations of in vitro and in vivo behaviors. This challenge is exemplified in studies of hippocampal neurogenesis. Previously, in vitro experiments showed that multipotent, self-renewing NSCs could be isolated from the adult hippocampus, supporting the model that these NSCs resided in the SGZ, where neurogenesis normally occurs (Gage et al., 1998). However, at the time, direct in vivo evidence of NSCs within the SGZ was lacking.

Indeed, recent studies challenged this model (Seaberg and van der Kooy, 2002; Bull and Bartlett, 2005). Microdissection of distinct hippocampal-associated regions demonstrated that, although the adult dentate gyrus contained progenitor cells capable of clonal proliferation in vitro, these cells were only transiently self-renewing and separately specified to neuronal or glial fates. In contrast, clonally derived colonies from cells isolated from the surrounding periventricular subependyma displayed multilineage potential and longer-term self-renewal in vitro. Furthermore, pyramidal neurons in the hippocampal CA1 region are partially regenerated from periventricular subependymal NSCs postischemia (Nakatomi et al., 2002). These studies supported an alternative model, whereby quiescent NSCs