Connecting cell fate decision networks in hematopoiesis from the outside in

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Molecular Systems Biology 6: 418; published online 5 October 2010; doi:10.1038/msb.2010.75

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In a developing tissue, a number of cell fate decisions take place: stem cells self-renew, progenitor cells are born and proliferate or differentiate. We often consider in isolation two sets of factors regulating these cell fate decisions: extracellular influences such as ligands secreted by other cells, and cell-autonomous, intracellular factors. Cell-autonomous factors can include specific marker expression levels, or particular gene regulatory programs. Increasingly, these intracellular factors are treated as networks—signal transduction networks and gene regulatory networks. The ligands regulating tissue development also form a network (Frankenstein et al, 2006), connecting cells that secrete them and cells that they target. How these two types of networks, intracellular and extracellular, interact is still mostly unknown. In a pioneering study just published in Molecular Systems Biology, Kirouac et al (2010) connect the dots, identifying specific cytokines as well as some of the crucial intracellular network nodes involved in in vitro hematopoietic stem cell maintenance, expansion, and differentiation.

The hematopoietic ‘tissue’ is composed of stem cells (hematopoietic stem cells, HSCs), progenitor cells, and mature cells differentiated into megakaryocytes, monocytes, and erythrocytes. As long as the environment is favorable to their proliferation, stem cells self-renew and give rise to progenitor cells allowing maintenance or expansion of the tissue. To successfully expand bone marrow or umbilical cord blood in vitro to produce blood cells for clinical applications, the regenerative potential of blood stem cells—their ability to self-renew—must be maintained in a culture inevitably formed of multiple blood cell types. These multiple cell types interact through many secreted ligands and the maintenance of the regenerative potential of blood stem cells is thought to be regulated by multiple positive and negative feedbacks arising from these cell–cell interactions between blood stem cells and differentiated cells (Kirouac et al, 2009).

To identify the molecular players involved in feedback regulation of HSC fate, Kirouac et al (2010) used a multipart workflow that combined experiments, computational data analysis, and data mining and that built on their previously published dynamical model. First, they performed a differential screen that compared transcriptional and secreted ligand profiles of culture conditions leading to either stem cell expansion or stem cell depletion. This screen pointed them to ligands that may promote or hinder the self-renewal capacity of HSCs. Next, they used curated lists of ligand–receptor interactions and published transcriptional profiles of distinct mature blood cell types to assign each ligand to its source, and thus reconstruct the directionality of the cell–cell interactions. Finally, to classify each ligand by mode of action, Kirouac et al compared experimentally observed changes in the cellular composition of cultured hematopoietic tissue after adding each ligand, to theoretical dose–response model predictions. CCL3, CCL4, TGFB, CXCL10, and TNFSF9 were found to be secreted by monocytes and inhibit self-renewal and proliferation of HSCs. On the other hand, EGF, PDGF, VEGF, and 5HT1 are secreted by megakaryocytes and promote stem cell self-renewal. Finally, three other ligands also positively affect stem cell renewal (FLT3LG, KITL, THPO), but their source could not be identified (Figure 1).

If we knew the important intracellular signaling proteins responsible for the adaptation of blood stem cells to the changing landscape of tissue composition and secreted ligands, perhaps small molecule inhibitors could also be used to optimize in vitro blood stem cell culture conditions. By mining curated intracellular signaling networks, Kirouac et al identified network hubs at the cross-roads of pathways induced by the ligands in their hematopoiesis cell–cell interaction network. As hubs, these signaling proteins may act as integrators for several extracellular signals. Once more, comparing cultures treated with chemical inhibitors with model-predicted dose–responses, Kirouac et al assigned functions to intracellular signaling hubs. Inhibitors of Raf and PI3K were found to decrease the probability of the HSC self-renewal, whereas inhibitors of Akt decreased the stem cell proliferation rate. The function of MEK1 could not be defined, perhaps because it is context dependent, and, finally, inhibitors of phospholipase C (PLC) clearly increased apoptosis rates within the blood cell cultures, indicating that its activity is required for cell survival (Figure 1).
Although the signaling network identified by Kirouac et al is quite complex, it is undoubtedly a very simplified picture of the interactions between HSCs and other cells (Figure 1). In the body, ligands from solid tissues may help sustain blood stem cell regenerative potential; indeed, neurological signals have been found to influence hematopoetic stem cell fate in vivo (Spiegel et al., 2008). Interestingly, Kirouac et al found that, in vitro, the neurotransmitter serotonin (5HT1) is secreted by megakaryocytes and positively influences HSC expansion. Still, this does not rule out that in vivo there may be multiple sources of serotonin and that blood marrow-infiltrating neurons and other tissues might also secrete ligands sustaining HSC expansion.

One question left unanswered in the study by Kirouac et al is which cells are the targets of the chemical inhibitors of intracellular signaling proteins: do inhibitors directly affect stem cell behavior or do they change the cytokine secretion patterns of mature cells in the culture, indirectly affecting stem cell behavior? The answer is likely to be ‘all of the above’ and only once we can selectively inactivate these signaling proteins in individual cell types will a clearer mechanistic picture of this network be defined. In addition, the timeline of secretion and responses to each of the ligands is also still unknown—in which sequence do these ligands act on HSCs? How can we manipulate this sequence to optimize in vitro cultures of blood cells? Finally, this signaling network has only been defined in terms of how it influences whole population behavior. By adding single-cell approaches to the arsenal of methods used by Kirouac et al, we will eventually gain a much more complete understanding of the mechanisms regulating hematopoietic cell expansion and stem cell renewal.

Hematopoietic tissue, in addition to its clinical importance, is particularly amenable to experiments because the cells can be grown in well-mixed suspension cultures and their cell–cell interactions may be predominantly (although certainly not exclusively) through diffusible ligands. In other tissues, spatial organization and cell–cell contacts also influence development and expansion. Nevertheless, the workflow developed by Kirouac et al offers great potential for learning more of the topology and the logic of extra- and intracellular networks regulating the expansion and differentiation of other types of stem cells. Great challenges lie ahead, but Kirouac et al met the first one, connecting a network that governs in vitro HSC expansion, outside and in.

**Conflict of interest**

The author declares that he has no conflict of interest.
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