Deep diving in the blood stem cell-ome

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Defining the functional distinctions between cells comprising the bone marrow has yielded fundamental insights into lineage ordering and drivers of blood cell production. A novel, highly granular and multi-dimensional molecular characterization of functional subsets of hematopoietic stem- and progenitor cells recently published in *Cell Stem Cell* (Cabezas-Wallscheid *et al*, 2014) will serve as a landmark and treasure trove for unanticipated insights into basic biology and the development of future targeted medicine.

See also: N Cabezas-Wallscheid *et al*

HSCs have the ability to self-renew for the lifetime of an organism and differentiate into progenitors with more restricted self-renewal and differentiation capabilities. Through a series of differentiation events, these progenitors are responsible for producing all the cells of the blood system for daily needs as well as in times of stress. While much has been learned about the cellular and molecular functions required for hematopoiesis and HSCs under various conditions (Rossi *et al*, 2012), the molecular control of self-renewal vs. differentiation is still a mystery in HSCs. The answer to this puzzle will likely inform future therapies using regenerative medicine as well as cancer therapeutic strategies, as self-renewal mechanisms are subverted in neoplasms.

In a recent publication (Cabezas-Wallscheid *et al*, 2014), the laboratory of Andreas Trumpp provides a rich resource of new data that could be used to address the mechanisms of self-renewal. They performed quantitative mass spectrometry of proteins, RNA sequencing, and Tagmentation Based Whole Genome Bisulfite Sequencing (TWGBS) for DNA methylation analysis on

immunophenotypically defined mouse adult bone marrow (BM) stem and progenitor populations under homeostatic conditions (Fig 1). Unlike embryonic stem cells grown in culture, these populations are rare and necessitate large amounts of mice to perform any protein-level analysis, explaining previous paucity of such type of analyses.

The Trumpp laboratory previously described a gating scheme that combines earlier described surface markers (Osawa *et al*, 1996; Adolfsson *et al*, 2005; Kiel *et al*, 2005) to segregate different cell types within a cell population negative for mature lineage markers, while positive for Sca1 and cKit antigens (LSK) and thus most enriched for HSC and progenitor populations (Wilson *et al*, 2008). They had found that LSK-CD34[−]CD150⁺CD48[−]CD135[−] cells are the most quiescent (~70%) and reveal here their long-term HSC activity. The closely related LSK-CD34⁺CD150⁺CD48[−]CD135[−] population is less quiescent (~40%) and cannot efficiently repopulate irradiated mice in secondary transplantations, thus termed multipotent-progenitor 1 (MPP1) by the authors (Wilson *et al*, 2008; Cabezas-Wallscheid *et al*, 2014). These functional results allowed for direct comparison of two closely related cell populations, one of which, HSC, can self-renew through multiple rounds of transplantation and the other, MPP1, cannot.

The authors first identify differentially expressed proteins in HSC and MPP1 by quantitative mass spectrometry. Previous analysis of protein levels had compared fairly heterogeneous HSPC compartments in bulk (Unwin *et al*, 2006; Klimmeck *et al*, 2012) likely due to the rarity of defined cell types. Four hundred thousand fluorescence-activated cell sorted (FACS) HSC and MPP1 cells were subjected to mass-spectroscopic analysis in triplicate, a Herculean-feat

requiring several dozens of mice per replicate. While over 4,000 proteins were detected in both cell types, only 47 were differentially expressed. HSCs displayed increased expression of proteins involved in monosaccharide metabolism, iron homeostasis and response to hypoxia, and had unique expression of cellular defense proteins. MPP1 displayed increased levels of proteins involved in cell cycle, DNA repair and cell proliferation, consistent with their relative decreased propensity to be found in G₀.

RNA sequencing revealed further differences between HSC and MPP1 with 479 genes differentially expressed. Pathway analysis largely corroborated the results from mass spectrometry experiments. By comparing fold changes in protein and RNA levels between HSC and MPP1, the authors found a strong correlation between protein and RNA level changes. However, changes in protein levels of members of the LIN28/let-7/HMGA/Igf2bp2 pathway were elevated in HSC compared to MPP1, without significant differences in RNA levels, suggesting highly elevated post-transcriptional regulation of this pathway in HSC. Specifically, the authors reported elevated HMGA1, HMGA2, and Igf2bp2 protein levels (the latter a known LIN28 target), with untouched RNA level in HSCs. Lending functional relevance to this dataset, enforced expression of LIN28 or HMGA2 in murine HSC increases their self-renewal ability *in vivo* (Copley *et al*, 2013).

RNA sequencing and TWGBS was performed on HSC, MPP1, and three newly reported LSK populations that were found to be functionally and immune-phenotypically distinct. Their analyses significantly add to the complexity of HSC-differentiation. While they demonstrate negative correlation between DNA methylation and gene expression—and thus add depth to the anticipated,

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Figure 1. Application of global profiling methods for the study of HSC biology.

Cabezas-Wallscheid *et al* (2014) performed multiple unbiased profiling methods (listed) on an HSC-enriched populations (HSC-EP) and 4 other distinct hematopoietic progenitor populations (HPC). They identified multiple pathways upregulated in HSC-EP, as well as provided a rich resource of multiple data types for stem-cell biology.

they also report a multitude of differentially methylated genes and discover new mechanisms of HSPC-differentiation. Notably, retinoic acid signaling and glycolytic pathway components were strongly increased and specific DNA repair enzymes downregulated in HSCs versus their LSKs populations. Lastly, the authors determine the expression of unique long non-coding RNAs and emphasize differential splicing events between the various HSPC populations.

Cabezas-Wallscheid *et al* have therefore provided a unique, novel and very data-rich account of adult, hematopoietic stem-cell biology. Its future mining and subsequent functional characterization by many laboratories will inform our understanding

and ultimate capabilities to therapeutically control hematopoiesis: an achievement of depth and consequence.

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