Molecular regulation of human hematopoietic stem cells
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Wiring diagram of the Ceriatone HRM 50 guitar amplifier to signify circuits, switches and dials that convert input signals to one of many possible sonic outputs. This system holds analogy to the mechanisms that control HSC output in terms of potential fate decisions.
Summary

Hematopoietic stem cells (HSCs) sustain the blood system over the lifetime of an organism. HSCs mostly reside in the bone marrow niche, and are endowed with a unique set of properties that enable them to continuously give rise to progenitor cells, which proliferate to replenish all mature blood cells. The hallmark property of HSCs is self-renewal, or the ability to undergo a cell division in which at least one daughter cell retains the developmental and proliferative potential of the parent cell. HSCs also have the potential to differentiate into all the different mature blood cell types that make up the blood system. They are generally quiescent, limiting the number of divisions HSCs undergo throughout life. The longevity and clonal persistence of individual HSCs gives unique perspectives to apoptosis/survival decisions under conditions of stress or damage accumulation. Overall, the maintenance of HSCs depends on the integration of signals that balance self-renewal with differentiation, quiescence with proliferation, and apoptosis with survival. Although manipulation of stem cells holds great therapeutic potential, few regulatory elements of human HSCs have been discovered. In this thesis, I examine the molecular constituents that underlie the unique properties of HSCs and discover three new regulatory axes that control the maintenance of human HSCs.

MicroRNAs represent a class of genes that exert post-transcriptional control of gene expression. Many microRNAs are dynamically expressed during HSC differentiation, suggesting their involvement in regulating hematopoiesis. One of the most HSC-enriched microRNAs is miR-126. In Chapter 2, I describe the role of miR-126 in restraining cell cycle progression of HSCs. miR-126 knockdown increased HSC proliferation without inducing exhaustion, resulting in expansion of mouse and human long-term repopulating HSC. Conversely, enforced miR-126 expression impaired cell cycle entry, leading to progressively reduced hematopoietic contribution. These studies also showed that miR-126 functions in HSCs and early progenitors by down-regulating multiple targets in the PI3K/AKT/GSK3β pathway. Activation of this pathway in response to extrinsic signals was attenuated by miR-126. Thus, miR-126 controls the HSC quiescence/activation equilibrium and governs HSC pool size, establishing the importance of microRNAs in the control of HSC function.

Like microRNAs, transcription factors have the potential to regulate many downstream targets and can provide an anchor to broadly influence gene expression programs. The process of differentiation is accompanied by the initiation of lineage-associated transcriptional programs by transcription factors. Interestingly, even in HSCs, low-level expression of lineage-associated genes has been observed. It is not known whether this process, termed lineage priming, influences HSC self-renewal. In Chapter 3, I identify a link between stemness and lineage priming through genetic modulation of ID genes as well as E47 and EBF1 B-lymphoid factors. Transcriptional profiling of ID2 over-
expression HSCs showed down-regulation of B-cell factors including EBF1 and FOXO1. There was a concomitant increase in myeloid factors such as CEBPA and GATA1, revealing myeloid commitment bias already in primitive HSC and progenitors. Mechanistically, ID2 inhibited the lymphoid transcription factor E47 to attenuate lymphoid priming in HSCs. Strikingly, attenuation of lymphoid factors enhanced HSC self-renewal and ID2 overexpression resulted in a 10-fold expansion of HSCs in serial limiting dilutions assays. Thus, early lymphoid transcription factors antagonize human HSC self-renewal, providing a link between lineage priming and maintenance of stem cell self-renewal.

In Chapter 4, I examine how HSCs respond to conditions that threaten integrity of the HSC pool. Many intrinsic and extrinsic sources of stress, including hypoxia, ROS, DNA damage, oncogene activation and nutrient fluctuation, converge to disrupt protein folding in the endoplasmic reticulum. This leads to activation of the Unfolded Protein Response (UPR), a pathway that enables a cell to either resolve the stress or initiate apoptosis. I find distinct activation of the UPR in human HSCs compared to early progenitors. HSCs initiated apoptotic signaling through the PERK branch of the UPR upon misfolded protein accumulation, causing CHOP upregulation and GADD34-mediated eIF2α dephosphorylation. This led to HSC loss under conditions where early progenitors survived. To modulate UPR signaling in HSCs, I overexpressed the co-chaperone ERDJ4, which increased the threshold of stress needed to activate apoptotic UPR signaling. Strikingly, the xenograft repopulation capacity of HSCs was increased by overexpression of ERDJ4, establishing the potential of manipulating this signaling axis to improve HSC survival. More broadly, these findings reveal how various sources of stress lead to clearance of HSCs, which are specifically predisposed to undergo apoptosis in comparison to progenitor cells. This process prevents propagation of damaged individual HSCs, providing insight into how the stem cell pool maintains clonal integrity.

Collectively, these studies have advanced our understanding of vital processes for life-long maintenance of the stem cell pool. The insights that these studies have generated are among the most detailed mechanistic descriptions of the molecular components that govern human HSC biology. Continued exploration will reveal the full complement of factors and processes that give HSCs their unique properties including self-renewal and multipotency. This will reveal opportunities to exploit the therapeutic potential of stem cells for regenerative medicine and to prevent their functional decline or malignant transformation associated with aging.