Experimental Hematology

Experimental Hematology 2018;64:1–11

Hematopoietic stem cell fate through metabolic control

Kyoko Ito[a,b], and Keisuke Ito[a,b,c]

[a]*Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, Bronx, NY, USA;* [b]*Departments of Cell Biology and Medicine, Albert Einstein College of Medicine, Bronx, NY, USA;* [c]*Albert Einstein Cancer Center and Diabetes Research Center, Albert Einstein College of Medicine, Bronx, NY, USA*

(Received 14 February 2018; revised 18 May 2018; accepted 19 May 2018)

Hematopoietic stem cells maintain a quiescent state in the bone marrow to preserve their self-renewal capacity, but also undergo cell divisions as required. Organelles such as the mitochondria sustain cumulative damage during these cell divisions and this damage may eventually compromise the cells' self-renewal capacity. Hematopoietic stem cell divisions result in either self-renewal or differentiation, with the balance between the two affecting hematopoietic homeostasis directly; however, the heterogeneity of available hematopoietic stem cell-enriched fractions, together with the technical challenges of observing hematopoietic stem cell behavior, has long hindered the analysis of individual hematopoietic stem cells and prevented the elucidation of this process. Recent advances in genetic models, metabolomics analyses, and single-cell approaches have revealed the contributions made to hematopoietic stem cell self-renewal by metabolic cues, mitochondrial biogenesis, and autophagy/mitophagy, which have highlighted mitochondrial quality control as a key factor in the equilibrium of hematopoietic stem cells. A deeper understanding of precisely how specific modes of metabolism control hematopoietic stem cells fate at the single-cell level is therefore not only of great biological interest, but will also have clear clinical implications for the development of therapies for hematological diseases.

Stem cells are self-renewing and either multipotent or unipotent [1–5] and these unique capacities offer opportunities for stem-cell-based therapies in the clinical setting [6]. Past research has implied only limited contributions by hematopoietic stem cells (HSCs) to unperturbed hematopoiesis, but HSCs are still believed essential to hematopoiesis under stress conditions such as hematopoietic recovery [7–11]. Therefore, HSC transplantation has been a key therapeutic strategy in combatting hematological disorders [12,13]. Like the stem cells of other tissues, HSCs basically remain quiescent to maintain their undifferentiated state, but they also undergo cell divisions as required [2,3]. Because HSC populations are precisely controlled within certain limits in vivo, once hematopoietic recovery is complete, it is believed that HSCs return to a quiescent state (dormancy). This suspension of the cell cycle is thought to make a critical contribution to the maintenance of stem cells' self-renewal capacity and multipotency because deletion of the genes involved in quiescence often leads to HSC exhaustion due to uncontrolled proliferation [14–19]. Indeed, the regenerative potential of HSCs may be governed by their divisional history [2,3] and therefore it is believed that cell-intrinsic networks involving key cell cycle regulators and the levels of *Hox* genes or Polycomb complex protein, along with the activity of transcriptional factors, integrate and cooperate with cumulative signals from the microenvironment to fine-tune the self-renewal capacity of HSCs and maintain whole hematopoiesis [15,17,20–24]. The role of cellular metabolism in regulating HSC self-renewal capacity has thus become a focus of much current stem cell research,

Offprint requests to: Dr. Keisuke Ito, Departments of Cell Biology and Medicine, Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, 1301 Morris Park Ave. Bronx, NY 10461; E-mail: keisuke.ito@einstein.yu.edu

https://doi.org/10.1016/j.exphem.2018.05.005

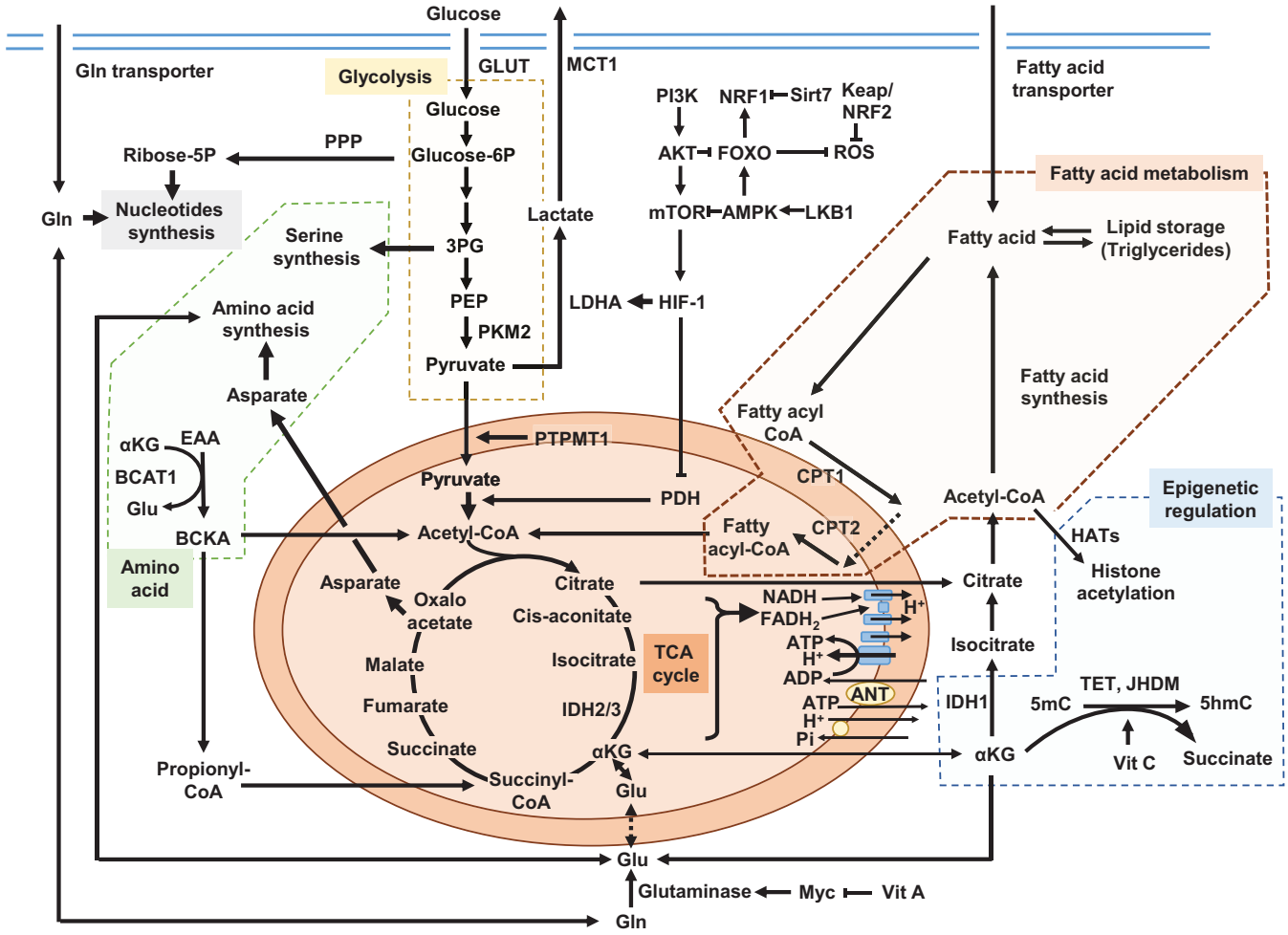

Figure 1. Overview of metabolic pathways contributing to HSC self-renewal and differentiation. HSCs rely on glycolysis (indicated by orange background). HIF-1α both promotes glycolysis and prevents pyruvate oxidation by suppressing the PDH complex. The PI3K–AKT pathway promotes ROS production by repressing FOXO. FAO (brown background) is required for HSC self-renewal by controlling cell fate decisions. HSCs are dependent on dietary valine and vitamin A and Gln is converted to Glu by glutaminase, which is partly under the control of MYC. Important contributions from BCAA metabolisms regulated by BCAT1 to myeloid leukemia have been suggested (green background). The intact mitochondrial function for HSC maintenance may include metabolism-driven epigenetic changes or code. Acetyl-CoA can be a source for histone acetylation and IDHs are a family of enzymes catalyzing the oxidative decarboxylation of isocitrate into αKG, which is a cofactor for the dioxigenase enzymes TET2 and JHDM. Vitamin C is a cofactor for the enzymatic activity of the TET family of DNA hydroxylases (blue background). Glut=glucose transporter; Glucose-6P=glucose 6-phosphate; PDH=pyruvate dehydrogenase; 3PG=3-phosphoglyceric acid; PPP=pentose phosphate pathway; PEP=phosphoenolpyruvic acid; PKM2=pyruvate kinase M2; LDHA=lactate dehydrogenase A; MCT1=monocarboxylate transporter 1; PTPMT1=PTEN-like mitochondrial phosphatase, or PTP localized to the Mitochondrion 1; TCA=tricarboxylic acid cycle; NADH=nicotinamide adenine dinucleotide; FADH=the reduced form of flavin adenine dinucleotide; ANT=adenine nucleotide translocases; Pi=inorganic phosphate; FOXO=forkhead box O; PI3K=phosphoinositide 3-kinase; AKT=protein kinase B or PKB; NRF=nuclear respiratory factor; Sirt7=sirtuin 7; LKB1=liver kinase B1; AMPK=AMP-activated protein kinase; mTOR=mammalian target of rapamycin; CoA=coenzyme A; CPT=carnitine-O-palmitoyltransferase; IDH=isocitrate dehydrogenases; Gln=glutamine; Glu=glutamate; EAA=essential amino acid (valine, leucine and isoleucine); BCAA=branched chain amino acid; BCAT1=BCAA transaminase 1; BCKA=branched chain keto acid; αKG=α-chetoglutarate; TET=ten–eleven translocation; JHDM=jmjC domain-containing histone demethylase; 5mC=5-methylcytosine; 5hmC=5-hydroxymethylcytosine; Vit C=vitamin C or ascorbic acid; hAT=histone acetyltransferase.

which has yielded many new insights [25–31] (Figure 1). In this review, we highlight recent advances in our understanding of the intriguing relationship among cellular metabolism, mitochondrial quality control, and HSC fate decisions.

Assessment of HSC fate

HSC fate decisions can be evaluated by paired daughter cell assays [14,32–34]. Their possible division options are: symmetric self-renewal expansion (symmetric division, SD, in which both daughter cells have the same function as the original cell), self-renewal maintenance (asymmetric division, AD), and

differentiation (symmetric commitment, SC, in which both daughter cells are differentiated from the original parent cell) and their eventual division pattern is determined by the in vivo repopulation capacity of their daughter cells. In cases in which at least one daughter cell is a long-term HSC (LT-HSC), the original cell must also be an LT-HSC. However, if both daughter cells are non-LT-HSCs, interpreting the resulting data can be complex because a cell's original function can affect its division pattern (Figure 2A).

Further, the homogeneity of the cell population is critical to accurate division pattern analysis. Tracking the divisions of individual cells from a heterogeneous population has proved difficult and any contamination of non-HSCs can lead to an overestimate of the rate of SC. As an example, let us consider a 30% pure population (low purity) in which three out of 10 single cells

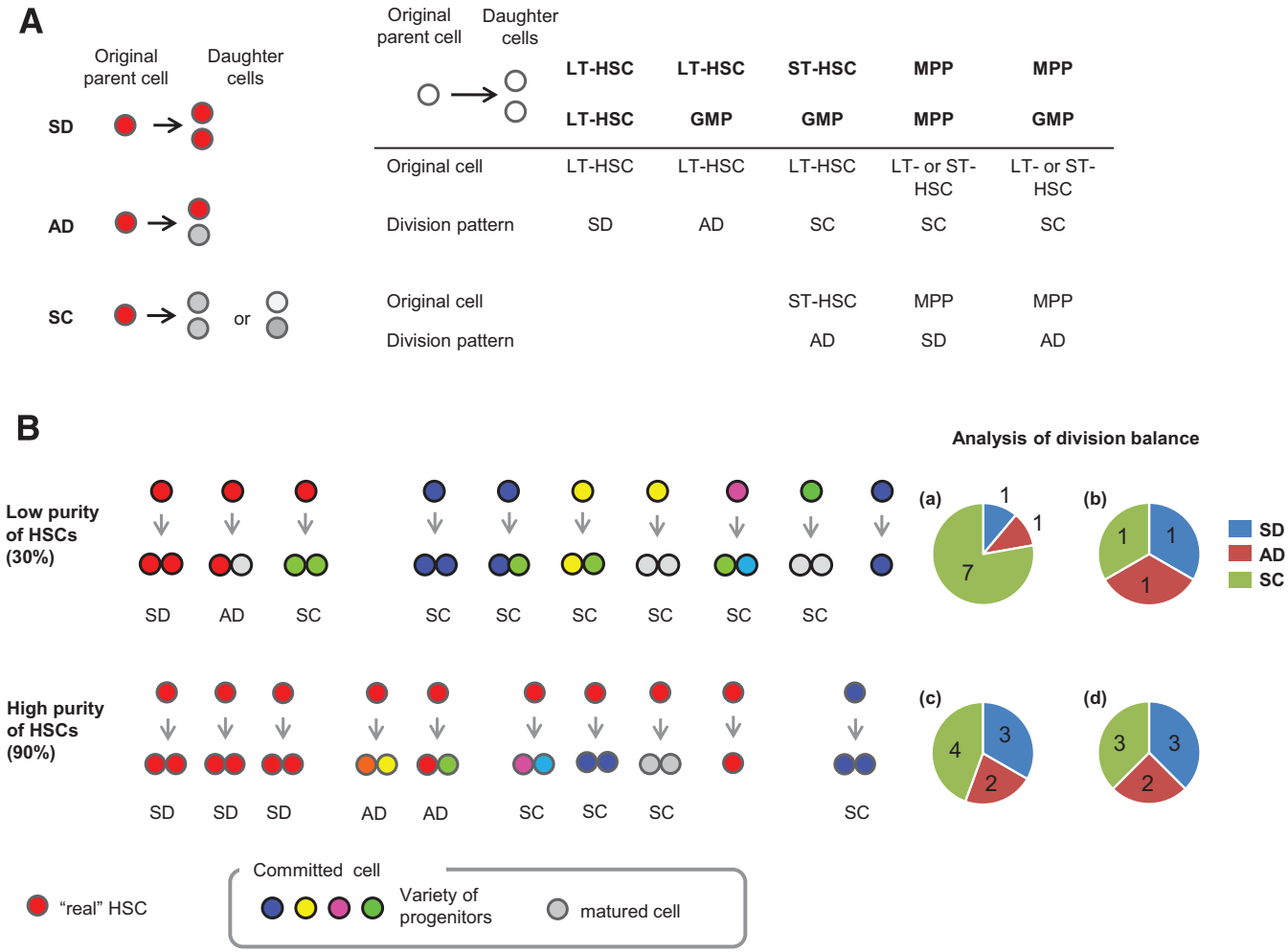

Figure 2. Division patterns by paired daughter cell assays. (A) Original cell function affects its division pattern. Shown is a schematic model of three division patterns. After SD, both daughter cells have the same function and differentiation stage as the parent cell (red), whereas both daughter cells appear as more committed cells (grey or pale grey) than the parent cells after SC (left). After initial division of the parent cell from the HSC-enriched fraction, the repopulation capacity and/or differentiation potential of the paired daughter cells is individually determined (e.g., by in vivo repopulation capacity retrospectively). Because the HSC-enriched fraction is a heterogeneous population, the immunophenotypically isolated single cells from this fraction can be hematopoietic progenitors or mature cells. Some examples of the combinations of the parent cells, their daughter cells, and their division patterns are shown at bottom right. (B) Analysis of division patterns in homogenous and heterogeneous populations. When 10 single cells are isolated from the population with 30% purity of HSCs, three are generally "real" HSCs (top). In this example, each of these three HSCs undergoes SD, AD, and SC, respectively (b), and one cell does not undergo cell division during the assay period. Because committed cells are not able to produce HSCs, the division patterns of those cells are assessed as SC. Therefore, the resulting division balance of the whole compartment will be one SD, one AD and seven SC (a) and it is difficult to extract the phenotypes of real HSCs from this low purity of HSCs. However, in the case of 90% HSC purity (bottom), the division balance of HSCs (d) can be estimated accurately from the resulting division symmetry of the isolated whole population (c). ST-HSC=short-term HSC; MPP=multipotent progenitor; GMP=granulocyte–monocyte progenitors.

in the HSC fraction must be "real" HSCs and a case in which one of these HSCs undergoes SD (33%), whereas another undergoes AD (33%) and the third undergoes SC (33%). Because committed cells cannot produce HSCs upon their division, their division patterns must be regarded as SC. The resulting division balance of the entire population would therefore be SD 11%, AD 11%, and SC 78% (Figure 2B). HSCs have been identified retrospectively after single-cell transplantation by clonal assays and these assays have demonstrated the heterogeneity of currently available HSC-enriched fractions [32,35–38]. Unfortunately, the reported frequency of HSCs in these fractions is generally lower than 30% and it is worth pointing out that, in the case described earlier (SD: AD: SC = 1: 1: 1), an HSC purity of even ~40% would be regarded as low because the overestimation of SC would lead to a significant shift in the assessed division balance (to a maximum of 44% SC in $n = 27$, and 41% SC in $n = 50$ divisions assessed, respectively. $*p < 0.05$ by Chi-squared test). However, when we have a high-purity population of real HSCs, we can more accurately determine their division pattern (Figure 2B).

To avoid this imprecision, researchers have long sought a reliable marker for individual HSCs that is strongly associated with repopulation capacity and does not fluctuate with changes in the surrounding environment and/or cell cycle. In various attempts to detect purified HSCs, recent studies have utilized combinations of cell surface markers, the reporter *Cre*-recombinase, and antibody positivity, but so far, these efforts have met with only limited success [8,39–44].

Division assays with markers for self-renewing HSCs

Until recently, HSC number and capacity were believed to decrease rather than increase with age and it has proved very challenging to expand the HSC population while maintaining stem-ness. Indeed, although division patterns in hematopoietic stem and progenitor cells (HSPCs) were thought to be controlled by the balance between SC and AD [14,32,33], advanced single-cell approaches have recently confirmed that HSCs are capable of symmetric self-renewing division (or SD) [32,45]. Analysis by the long-label retaining method with H2B-GFP (histone 2b, green fluorescent protein), for instance, has shown that HSCs can divide symmetrically at least several times throughout adult life to achieve higher density in the bone marrow [46].

Our use of *Tie2* positivity as a marker has allowed us to identify a purified population of HSCs and we have demonstrated with our local transplantation protocol that single HSCs from this population exhibit high reconstitution capacity in vivo [45,47]. Our tracking technique allowed us to determine the function of the paired daughter cells resulting from single HSC divisions, which in turn enabled us to more accurately visualize division patterns and distinguish self-renewal expansion from self-renewal maintenance. In these studies, we found that only top hierarchical HSCs underwent SD, in which both daughter cells are HSCs and retain *Tie2* positivity [45].

Because increasing evidence supports the essential contributions of metabolic control to HSC division patterns, determining the metabolic mode of purified HSCs is of crucial importance [14,28]. Single-cell gene expression assays have revealed that critical roles in HSC expansion are played by fatty acid oxidation (FAO) [45]. The mitochondria are the primary sites of FAO, in which fatty acids are broken down enzymatically [48], and because they are essential subcellular components in the metabolic process, their role in division patterns and the subsequent cell fates of HSCs is a question of great scientific interest (Figure 3). Further, research has shown that, during asymmetric division in mammary epithelial stem-like cells, older mitochondria are pushed into daughter cells fated to differentiation in order to maintain high-quality stem cell homeostasis [49]. In contrast, symmetric division requires self-clearance systems in both daughter cells because young and old mitochondria have been found to be equally distributed between both [28,45]; however, the processes involved remain among the least understood in stem cell biology.

Mitochondrial autophagy, or mitophagy, is a specific form of autophagy for the selective clearance of damaged mitochondria [50]. In depolarized mitochondria, the degradation of PTEN-induced putative kinase 1 (PINK1) is impaired, leading to the accumulation and activation of this kinase on the mitochondrial outer membrane [51–54]. PINK1 phosphorylates ubiquitin chains, which leads to the recruitment of Parkin to the mitochondria and the activation of its E3 ligase activity. Mitochondrial proteins are then polyubiquitinated and recognized by autophagy receptors to initiate autophagosomes formation [51–54]. The difference in the effects observed after chronic deletion or acute knockdown of *Parkin* implies that adaptive mechanisms for mitophagy cannot be established after acute silencing of *Parkin* (and/or *Pink1*) genes [55,56]. The impact of *Parkin/Pink1* knock down has therefore been explored in the context of HSC division patterns, which have demonstrated that enhanced clearance of damaged mitochondria by FAO is a key mechanism of the self-renewing expansion of $Tie2^+$ HSCs (Figure 3A) [45].

Metabolic control in HSC homeostasis

Mitochondria are bioenergetic and biosynthetic organelles that synthesize lipids and heme, as well as iron-sulfur clusters, amino acids, and nucleotides, and play

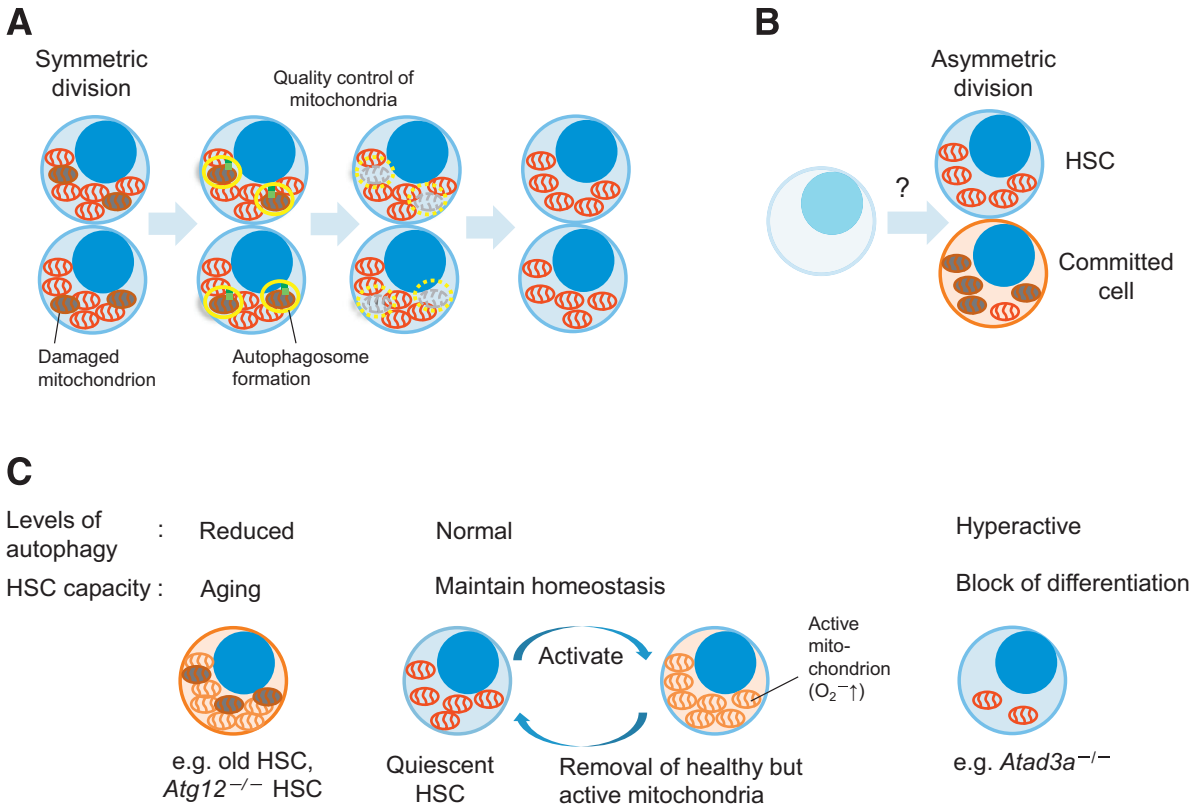

Figure 3. Quality control machineries in HSC division balance and hematopoietic homeostasis. (A) In SD, mitochondria are equally segregated into two daughter cells, although their metabolic processes may differ from those of the mother cell. Upon cell division, organelles such as mitochondria are damaged, which activates mitochondrial autophagy. This activation of mitophagy promotes mitochondrial quality control and subsequent self-renewing HSC expansion. (B) In some mammary stem-like-cell divisions, mitochondria are split unevenly between the two daughter cells and old mitochondria are apportioned primarily to the tissue-progenitor daughter, whereas newly synthesized mitochondria are apportioned to the stem-cell-like daughter. It has yet to be formally demonstrated, but asymmetric HSC division by unequal apportionment of older or damaged mitochondria could be a potential strategy for removing damaged cell components. (C) HSC activation is accompanied by mitochondria activation and a shift in metabolic activity to Oxphos (right). Healthy but active mitochondria are unselectively removed by autophagy and these active HSCs return to replicative quiescence (left). The majority (two-thirds) of HSCs from aged mice and some autophagy-deficient HSCs (e.g. *Atg12*-deficient HSCs) were not able to limit the number of active mitochondria efficiently, which drives aging phenotypes in the blood (far left). Hyperactivated mitophagy (e.g., loss of *Atad3a*) results in blocked hematopoietic lineage commitment at the progenitor stage and enlarged HSPC pools (far right).

important roles in HSC homeostasis (Figure 1) [27]. HSCs exhibit much lower baseline and maximal respiration than progenitor cells even though different levels of mitochondrial content, as measured by staining from targeted fluorescent protein, have been reported due to dye flux by xenobiotic efflux pumps [57–62]. Enhanced respiration is nevertheless detrimental to HSC maintenance and function [63–67]; for example, loss of mitochondrial carrier homolog 2 (MTCH2) increases mitochondrial respiration and intracellular ROS, triggering HSC entry into the cell cycle and compromising self-renewal capacity [68]. In contrast, lowering mitochondrial activity by chemical mitochondrial uncoupler supports sustained repopulation capacity under culture [61]. The defects in cell cycle quiescence and repopulation capacity observed in HSCs with impaired hypoxia-inducible factor (HIF)–pyruvate dehydrogenase kinase pathways are accompanied by enhanced flux of glycolytic metabolisms in the mitochondria during the tricarboxylic acid cycle [59,69]. Further, deletion of *Sirtuin 7* (*Sirt7*) increases mitochondrial unfolded protein stress, as well as mitochondrial biogenesis and respiration, leading to impaired regenerative capacity with a loss of quiescence and a shift in metabolic process that signals cellular differentiation [70,71]. When HSCs differentiate, they exit from quiescence and undergo a metabolic switch to mitochondrial Oxphos. Indeed, disrupting mitochondrial Oxphos upon the loss of *Ptpmt1*, a mitochondrial phosphatase targeting phosphatidylinositol phosphates, blocks early HSC differentiation and results in rapid hematopoietic failure in vivo [72].

The hypoxic condition has been shown to be critical to the maintenance of self-renewal, whereas stress

factors (e.g., infection or polyinosinic-polycytidylic acid, granulocyte-colony stimulating factor, or chronic blood loss) are now known to induce HSC cycling [18,73,74]. This entry into the cell cycle is associated with DNA replication, upregulated energy production via oxidative phosphorylation (Oxphos), and elevated levels of intracellular reactive oxygen species (ROS). Because quiescent HSCs are generally sensitive to increased intracellular ROS, the DNA damage that accumulates with repeated cell divisions leads to reduced self-renewal capacity and, ultimately, HSC exhaustion [25,26,75–81].

Autophagy in hematopoiesis and HSC aging

Recent studies from multiple groups have also shown that macroautophagy (hereafter called simply autophagy) [82–84] has an indirect but significant effect on HSC metabolism. Self-renewing stem cells, particularly in tissues with high cellular turnover such as the blood, counterbalance an array of stresses. HSCs in particular may combat stresses to maintain life-long hematopoiesis, so the repair or clearance of mitochondrial damage is supported by a range of mechanisms that are critical to their function. Autophagy is a lysosomal degradation pathway that maintains the quantity and quality of organelles and proteins by degrading them once they are damaged or unwanted [82–84]. The autophagy-related (Atg) conjugation systems, which contribute to the formation of double-membraned autophagosomes, are another crucial element in the proper regulation of autophagy to ensure mitochondrial maintenance. The Forkhead Box O 3a (FOXO3A)-driven pro-autophagy gene program is known to protect HSCs from metabolic stress [85] and a small-molecule inducer of autophagy has been shown to stimulate erythropoiesis [86,87]. The failure of this coordinated regulation can have a profound impact because impaired autophagy has been shown to result in HSC exhaustion and conditional depletion of *Atg7* can lead to lethal anemia [88,89].

More recently, the analysis of the roles of autophagy in the hematopoietic system has extended to the context of the aging. One-third of HSCs from older mice exhibit high levels of autophagy activity and these HSCs show higher repopulation capacity. Defective autophagy by the ablation of *Atg12* accelerates blood aging phenotypes, with myeloid-biased lineage distribution and elevated Oxphos. The unselective removal of "active and healthy" mitochondria by autophagy contributes to reducing oxidative metabolism, which is essential for maintaining replicative quiescence in HSCs (Figure 3C) [57].

Enhanced mitophagy in hematopoiesis

The impact of excessive mitophagy on hematopoiesis has also been explored (although not in purified HSC populations) [90]. ATPase family AAA domain-containing protein 3a (Atad3a) facilitates the transportation of Pink1 from the translocase of the outer membrane complex to the translocase of the inner membrane complex. In healthy mitochondria, Pink1 is degraded rapidly after its import by mitochondria peptidases. Conditional deletion of *Atad3a* in adult hematopoietic cells leads to the accumulation of Pink1 and the enhancement of mitophagy. *Atad3a* conditional knockout mice exhibited blocked hematopoietic lineage commitment at the progenitor stage and enlarged HSPC pools. Ablation of *Pink1* in these mice rescued defective mitophagy, which was in turn associated with the rescue of some defective hematopoietic phenotypes found in *Atad3a*-deficient mice (Figure 3C) [90]. Interestingly, high mitochondrial turnover capacity was found in the progenitor stages and both defective and enhanced mitophagy led to blocked erythoid differentiation at terminal erythrocyte maturation and erythroid progenitor differentiation, respectively [88,90,91]. Although the contributions of autophagy at different hematopoietic stages remain to be clarified, these studies collectively demonstrate that mitophagy must be controlled precisely to ensure maintenance of HSPCs and their appropriate differentiation.

Key open questions

Beyond generating ATP for cellular energy, mitochondria are required for mtDNA maintenance and intracellular calcium homeostasis, produce key metabolites that are utilized to synthesize macromolecules (e.g., lipids and nucleotides), and function as signaling organelles (e.g., for apoptosis) [92–96]. They are also known to form networks and can change shape through the combined actions of fission, fusion, and movement along cytoskeletal tracks. These dynamics likely affect cell fate choice through multiple mechanisms, but we are only beginning to understand the mitochondrial requirements for stemness. Indeed, recent studies have shown that the PR domain containing 16 (Prdm16)–Mitofusin-2 (Mfn2) axis contributes to the maintenance of HSCs with lymphoid potential by buffering calcium levels through mitochondrial tethering to the endoplasmic reticulum [97,98]. In addition, intact mitochondrial function for HSC maintenance may require metabolism-driven epigenetic changes or code [99–103].

Autophagy (or macroautophagy) was originally characterized as a nonselective bulk degradative system; however, it has now been shown that, under certain conditions, autophagosomes engulf cytosolic materials selectively and diverse autophagy pathways have been identified [104]. Whether selective autophagy (e.g.

pexophagy, glycophagy, or SQSTM1-related autophagy) or other forms of autophagy (e.g., microautophagy or chaperone-mediated autophagy) participate in HSC homeostasis remains to be determined, but it will be interesting to explore how the controlled turnover of macromolecular components and nutrients (e.g., amino acids, metals, and lipids) by autophagy contributes to the self-renewal capacity of HSCs. It is already clear that specific autophagy activity is required at various periods of life (e.g., developmental, perinatal young and adult hematopoiesis, as well as blood aging) [27,57,88]. A new method of assessing the dynamic content of autophagosomes, combined with genetic approaches for elucidating the selective forms of autophagy, will enrich our understanding of the roles of autophagy in the precise control of HSC fate decisions. Another open question of high importance is how the quantitative balance between selective autophagy and other catabolic pathways is controlled, as in the case of depolarized mitochondria, which are specifically degraded by Parkin-mediated mitophagy but might also be removed by bulk nonselective autophagy. Analysis of how each pathway is regulated quantitatively and the detailed contributions of mitophagy to the physiological aging of HSCs await future investigation.

Technical challenges to study HSC division balance

Our limited knowledge of division symmetry in HSCs and progenitor cells has so far come almost exclusively from in vitro studies [14,32,33,105,106]; virtually nothing has been observed in vivo. However, in vivo HSC behavior certainly differs from ex vivo behavior and it has been shown that cellular metabolism can be modulated extrinsically. A complete model of the bone marrow environment in vitro (i.e., oxygen levels, cell–cell interactions, cellular components of the niche, cytokines, and buffer milieu) has not yet been achieved [20,107] and it is known that the metabolic modes of HSCs are dramatically changed once cells are placed ex vivo: for instance, HSCs are known to adapt their mitochondrial metabolism in the hypoxic niche [69,108–110]. When bone marrow is harvested and maintained in a hypoxic environment, greater numbers of phenotypically defined HSCs can be obtained than can be collected in ambient air, but this beneficial effect is lost rapidly (in as little as 30 min) after exposure to normoxia [111,112]. Therefore, the key metabolic pathways obtained from in vitro assays cannot reflect in vivo functional states. The development of new platforms to assess the division balance of single HSCs in vivo will provide a deeper understanding of both the metabolic and molecular basis of HSC fate decisions in vivo.

Reporter systems are powerful tools for the characterization of fundamental HSC properties in vivo, with the functionality of the labeled cells validated retrospectively by clonal assays after single-cell transplantation. Theories differ regarding the contributions of HSCs to unperturbed homeostasis versus tissue recovery conditions [7,8,11] and technical considerations may influence conclusions derived from transplantation experiments; nevertheless, several studies have described murine and human HSCs as the major contributors to multilineage hematopoiesis both in the steady state and during cytokine response [113,114]. Phenotypic HSCs comprise a major source of the megakaryocyte/platelet lineage in steady-state conditions [11,34], but these cells show multilineage differentiation capacity once they are transplanted into irradiated recipient mice [11]. These data imply potential differences in fate decision mechanisms between steady state and hematopoietic recovery. Perhaps most importantly in terms of our understanding of HSC metabolism, myeloablative preconditioning such as irradiation and high-dose chemotherapy is commonly used to create space in the niche for HSC engraftment [12,13], but also severely alters the levels of ROS and other metabolic regulators, as well as the bone marrow microenvironment [115]. These genotoxic effects remain a substantial barrier to further clinical translation of this approach and have raised concerns about whether transplantation results accurately reflect the true situation of the physiological metabolic mode of HSCs. A non-genotoxic method has long been sought as an alternative to current regimens, especially in the treatment of non-malignant blood diseases, and these efforts have met with some success at the preclinical stage [115–117]. New in vivo genetic tools are being developed to assess hematopoiesis with three- or even five-blood lineage resolution [11,34,114] and, in light of these advances, the technical challenges of exploring native HSC fate decisions will remain critical to future research.

Other recent studies have proposed an additional differentiation model in which HSCs can differentiate directly into lineage-restricted progenitors while bypassing the multipotent progenitor stage during acute conditions that demand the rapid replenishment of mature cells (e.g., respond to ablation stress) [11,32,34]. These findings suggest another possibility in which, as is often the case with other cell types, the first HSC divides symmetrically and then one of its daughter cells stochastically loses its stemness (e.g., through the availability of niche positions or interaction with cytokines), which yields two daughter cells with distinct fates: one HSC and one differentiated hematopoietic cell [28]. The establishment of assay systems in which real-time markers are associated with HSC-specific functions (e.g., repopulation capacity) will enable researchers to assess the division patterns of HSCs accurately by tracking their division patterns prospectively. This development will be a breakthrough in identifying the key regulatory machineries of HSC

fate decisions and will improve our understanding of the fundamental properties of HSCs significantly.

Conclusion and perspectives

HSC fate control is certain to be a central focus of ongoing research and it is thus essential to expand our knowledge of both the mitochondrial and molecular basis of HSC fate decisions. The metabolic comparison between SD and other division modes and the subsequent identification of specific metabolites as HSC fate determinant will be of particularly high interest because inducing SD may prove key to therapeutic applications for transplantation cases in which HSC expansion ex vivo is required with a limited number of donor cells. Better understanding of the molecular mechanisms and cross-links between all three division options will make possible the manipulation of HSC cell fate decisions. Because the rarity of HSCs is a major hurdle for metabolic or epigenetic studies that depend on purified HSC populations, novel metabolomics and epigenomics approaches adapted to small numbers of HSCs certainly bear further exploration.

A disturbed division balance causes hematological disorders [118,119] and the long-term survival rate among blood cancer patients remains stubbornly low because most patients who have achieved remission eventually relapse. Leukemia stem cells (LSCs, also known as leukemia-initiating cells) are believed to not only drive disease initiation, progression, and drug resistance, but also contribute to relapse [118–124]. Elimination of every single LSC is therefore essential to a long-term cure. Upon division, LSCs can either self-renew or commit to differentiation and shifting their division balance away from renewal and toward commitment holds great promise as a therapeutic strategy [125,126]. It is no surprise that the metabolic requirements of leukemogenesis and LSC function have therefore become a focus of much current research [100,101,103,127–130] and the discovery of contributions to leukemogenesis by metabolism, mitochondrial biogenesis, and cytoprotective autophagy support the notion that mitochondrial quality control by autophagy may be a key determinant of division balance [131]. Tracking the division pattern of individual LSCs has, however, proved challenging and the development of new techniques of single LSC assay is critical to achieving a better understanding of the molecular basis of LSC fate choice [132,133]. Because the many metabolic pathways involved are conserved in human hematopoiesis, identifying the key metabolic cues that control LSC fate and maintain stem-ness precisely upon division could provide effective targets in strategies to enhance LSC commitment and will therefore be of high clinical importance.

Acknowledgments

The authors thank the members of the Ito laboratory and Einstein Stem Cell Institute and T. Suda for their comments on HSC self-renewal.

KI is supported by grants from the National Institutes of Health (R01DK98263, R01DK115577, and R01DK100689) and the New York State Department of Health as Core Director of Einstein Single-Cell Genomics/Epigenomics (C029154). We apologize to the investigators whose work could not be cited due to space limitations.

References

1. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiat Res. 1960;13:115–125.
2. Weissman IL, Anderson DJ, Gage F. Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol. 2001;17:387–403.
3. Visvader JE, Clevers H. Tissue-specific designs of stem cell hierarchies. Nat Cell Biol. 2016;18:349–355.
4. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell. 1997;88:287–298.
5. Ramalho-Santos M. and Willenbring H. On the origin of the term "stem cell". Cell Stem Cell. 2007;1:35–38.
6. Daley GQ, Goodell MA, Snyder EY. Realistic prospects for stem cell therapeutics. Hematology Am Soc Hematol Educ Program. 2003: 398–418.
7. Sun J, Ramos A, Chapman B, et al. Clonal dynamics of native haematopoiesis. Nature. 2014;514:322–327.
8. Busch K, Klapproth K, Barile M, et al. Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. Nature. 2015;518:542–546.
9. Sawai CM, Babovic S, Upadhaya S, et al. Hematopoietic stem cells are the major source of multilineage hematopoiesis in adult animals. Immunity. 2016;45:597–609.
10. Ito K, Frenette PS. HSC contribution in making steady-state blood. Immunity. 2016;45:464–466.
11. Rodriguez-Fraticelli AE, Wolock SL, Weinreb CS, et al. Clonal analysis of lineage fate in native haematopoiesis. Nature. 2018;553:212–216.
12. Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med. 2007;357:1472–1475.
13. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006;354:1813–1826.
14. Ito K, Carracedo A, Weiss D, et al. A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat Med. 2012;18:1350–1358.
15. Wilson A, Laurenti E, Oser G, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008;135:1118–1129.
16. Cheng T, Rodrigues N, Shen H, et al. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science. 2000;287:1804–1808.
17. Pietras EM, Warr MR, Passegue E. Cell cycle regulation in hematopoietic stem cells. J Cell Biol. 2011;195:709–720.
18. Trumpp A, Essers M, Wilson A. Awakening dormant haematopoietic stem cells. Nat Rev Immunol. 2010;10:201–209.
19. Nakamura-Ishizu A, Takizawa H, Suda T. The analysis, roles and regulation of quiescence in hematopoietic stem cells. Development. 2014;141:4656–4666.

20. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014;505:327–334.
21. Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature. 2008;453:306–313.
22. Frenette PS, Pinho S, Lucas D, Scheiermann C. Mesenchymal stem cell: keystone of the hematopoietic stem cell niche and a stepping-stone for regenerative medicine. Annu Rev Immunol. 2013;31:285–316.
23. Scadden DT. The stem-cell niche as an entity of action. Nature. 2006;441:1075–1079.
24. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000;287:1427–1430.
25. Ito K, Suda T. Metabolic requirements for the maintenance of self-renewing stem cells. Nat Rev Mol Cell Biol. 2014;15:243–256.
26. Shyh-Chang N, Daley GQ, Cantley LC. Stem cell metabolism in tissue development and aging. Development. 2013;140:2535–2547.
27. Chandel NS, Jasper H, Ho TT, Passegue E. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. Nat Cell Biol. 2016;18:823–832.
28. Ito K, Ito K. Metabolism and the control of cell fate decisions and stem cell renewal. Annu Rev Cell Dev Biol. 2016;32:399–409.
29. Wilkinson AC, Yamazaki S. The hematopoietic stem cell diet. Int J Hematol. 2018;107:634–641.
30. Ansó E, Weinberg SE, Diebold LP, et al. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. Nat Cell Biol. 2017;19:614–625.
31. Cabezas-Wallscheid N, Buettner F, Sommerkamp P, et al. Vitamin a-retinoic acid signaling regulates hematopoietic stem cell dormancy. Cell. 2017;169:807–823. e19.
32. Yamamoto R, Morita Y, Ooehara J, et al. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell. 2013;154:1112–1126.
33. Suda T, Suda J, Ogawa M. Disparate differentiation in mouse hemopoietic colonies derived from paired progenitors. Proc Natl Acad Sci U S A. 1984;81:2520–2524.
34. Yamamoto R, Wilkinson AC, Ooehara J, et al. Large-scale clonal analysis resolves aging of the mouse hematopoietic stem cell compartment. Cell Stem Cell. 2018;22:600–607. e4.
35. Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science. 1996;273:242–245.
36. Benveniste P, Cantin C, Hyam D, Iscove NN. Hematopoietic stem cells engraft in mice with absolute efficiency. Nat Immunol. 2003;4:708–713.
37. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005;121:1109–1121.
38. Dykstra B, Kent D, Bowie M, et al. Long-term propagation of distinct hematopoietic differentiation programs in vivo. Cell Stem Cell. 2007;1:218–229.
39. Gazit R, Mandal PK, Ebina W, et al. Fgd5 identifies hematopoietic stem cells in the murine bone marrow. J Exp Med. 2014;211:1315–1331.
40. Sanjuan-Pla A, Macaulay IC, Jensen CT, et al. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. Nature. 2013;502:232–236.
41. Acar M, Kocherlakota KS, Murphy MM, et al. Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. Nature. 2015;526:126–130.
42. Challen GA, Boles NC, Chambers SM, Goodell MA. Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. Cell Stem Cell. 2010;6:265–278.
43. Chen JY, Miyanishi M, Wang SK, et al. Hoxb5 marks long-term haematopoietic stem cells and reveals a homogenous perivascular niche. Nature. 2016;530:223–227.
44. Carrelha J, Meng Y, Kettyle LM, et al. Hierarchically related lineage-restricted fates of multipotent haematopoietic stem cells. Nature. 2018;554:106–111.
45. Ito K, Turcotte R, Cui J, et al. Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. Science. 2016;354:1156–1160.
46. Bernitz JM, Kim HS, MacArthur B, Sieburg H, Moore K. Hematopoietic stem cells count and remember self-renewal divisions. Cell. 2016;167:1296–1309. e10.
47. Turcotte R, Alt C, Runnels JM, et al. Image-guided transplantation of single cells in the bone marrow of live animals. Sci Rep. 2017;7:3875.
48. Kunau WH, Dommes V, Schulz H. beta-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: a century of continued progress. Prog Lipid Res. 1995;34:267–342.
49. Katajisto P, Döhla J, Chaffer CL, et al. Stem cells. Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. Science. 2015;348:340–343.
50. Youle RJ, Narendra DP. Mechanisms of mitophagy. Nat Rev Mol Cell Biol. 2011;12:9–14.
51. Stolz A, Dikic I. PINK1-PARKIN interplay: down to ubiquitin phosphorylation. Mol Cell. 2014;56:341–342.
52. Sekine S, Youle RJ. PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol. BMC Biol. 2018;16:2.
53. Lazarou M, Sliter DA, Kane LA, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature. 2015;524:309–314.
54. Okatsu K, Saisho K, Shimanuki M, et al. p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. Genes Cells. 2010;15:887–900.
55. Williams JA, Ni HM, Haynes A, et al. Chronic deletion and acute knockdown of Parkin have differential responses to acetaminophen-induced mitophagy and liver injury in mice. J Biol Chem. 2015;290:10934–10946.
56. Dawson TM, Ko HS, Dawson VL. Genetic animal models of Parkinson's disease. Neuron. 2010;66:646–661.
57. Ho TT, Warr MR, Adelman ER, et al. Autophagy maintains the metabolism and function of young and old stem cells. Nature. 2017;543:205–210.
58. Simsek T, Kocabas F, Zheng J, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell. 2010;7:380–390.
59. Takubo K, Nagamatsu G, Kobayashi CI, et al. Regulation of glycolysis by Pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. Cell Stem Cell. 2013;12:49–61.
60. de Almeida MJ Luchsinger LL, Corrigan DJ, Williams LJ, Snoeck HW. Dye-independent methods reveal elevated mitochondrial mass in hematopoietic stem cells. Cell Stem Cell. 2017;21:725–729. e4.
61. Vannini N, Girotra M, Naveiras O, et al. Specification of haematopoietic stem cell fate via modulation of mitochondrial activity. Nat Commun. 2016;7:13125.
62. Romero-Moya D, Bueno C, Montes R, et al. Cord blood-derived CD34+ hematopoietic cells with low mitochondrial mass are enriched in hematopoietic repopulating stem cell function. Haematologica. 2013;98:1022–1029.
63. Gan B, Hu J, Jiang S, et al. Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. Nature. 2010;468:701–704.
64. Gurumurthy S, Xie SZ, Alagesan B, et al. The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. Nature. 2010;468:659–663.

65. Nakada D, Saunders TL, Morrison SJ. Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. Nature. 2010;468:653–658.
66. Chen C, Liu Y, Liu R, et al. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med. 2008;205:2397–2408.
67. Xiao N, Jani K, Morgan K, et al. Hematopoietic stem cells lacking Ott1 display aspects associated with aging and are unable to maintain quiescence during proliferative stress. Blood. 2012;119:4898–4907.
68. Maryanovich M, Zaltsman Y, Ruggiero A, et al. An MTCH2 pathway repressing mitochondria metabolism regulates haematopoietic stem cell fate. Nat Commun. 2015;6:7901.
69. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell. 2011;9:298–310.
70. Mohrin M, Shin J, Liu Y, et al. Stem cell aging: A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging. Science. 2015;347:1374–1377.
71. Mohrin M, Widjaja A, Liu Y, Luo H, Chen D. The mitochondrial unfolded protein response is activated upon hematopoietic stem cell exit from quiescence. Aging Cell. 2018;17: e12756.
72. Yu WM, Liu X, Shen J, et al. Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. Cell Stem Cell. 2013;12:62–74.
73. Essers MA, Offner S, Blanco-Bose WE, et al. IFNalpha activates dormant haematopoietic stem cells in vivo. Nature. 2009;458:904–908.
74. Walter D, Lier A, Geiselhart A, et al. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature. 2015;520:549–552.
75. Rossi DJ, Jamieson CH, Weissman IL. Stems cells and the pathways to aging and cancer. Cell. 2008;132:681–696.
76. Ito K, Hirao A, Arai F, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med. 2006;12:446–451.
77. Ito K, Hirao A, Arai F, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004;431:997–1002.
78. Maryanovich M, Gross A. A ROS rheostat for cell fate regulation. Trends Cell Biol. 2013;23:129–134.
79. Miyamoto K, Araki KY, Naka K, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell. 2007;1:101–112.
80. Liang R, Ghaffari S. Mitochondria and FOXO3 in stem cell homeostasis, a window into hematopoietic stem cell fate determination. J Bioenerg Biomembr. 2017;49:343–346.
81. Testa U, Labbaye C, Castelli G, Pelosi E. Oxidative stress and hypoxia in normal and leukemic stem cells. Exp Hematol. 2016;44:540–560.
82. He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet. 2009;43:67–93.
83. Galluzzi L, Pietrocola F, Levine B, Kroemer G. Metabolic control of autophagy. Cell. 2014;159:1263–1276.
84. Ueno T, Komatsu M. Autophagy in the liver: functions in health and disease. Nat Rev Gastroenterol Hepatol. 2017;14:170–184.
85. Warr MR, Binnewies M, Flach J, et al. FOXO3A directs a protective autophagy program in haematopoietic stem cells. Nature. 2013;494:323–327.
86. Doulatov S, Vo LT, Macari ER, et al. Drug discovery for Diamond-Blackfan anemia using reprogrammed hematopoietic progenitors. Sci Transl Med. 2017;9. eaah5645.
87. Liu F, Lee JY, Wei H, et al. FIP200 is required for the cell-autonomous maintenance of fetal hematopoietic stem cells. Blood. 2010;116:4806–4814.
88. Mortensen M, Ferguson DJ, Edelmann M, et al. Loss of autophagy in erythroid cells leads to defective removal of mitochondria and severe anemia in vivo. Proc Natl Acad Sci U S A. 2010;107:832–837.
89. Riffelmacher T, Simon AK. Mechanistic roles of autophagy in hematopoietic differentiation. FEBS J. 2017;284:1008–1020.
90. Jin G, Xu C, Zhang X, et al. Atad3a suppresses Pink1-dependent mitophagy to maintain homeostasis of hematopoietic progenitor cells. Nat Immunol. 2018;19:29–40.
91. Sandoval H, Thiagarajan P, Dasgupta SK, et al. Essential role for Nix in autophagic maturation of erythroid cells. Nature. 2008;454:232–235.
92. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324:1029–1033.
93. Chandel NS. Evolution of mitochondria as signaling organelles. Cell Metab. 2015;22:204–206.
94. Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol. 2012;13:566–578.
95. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science. 2012;337:1062–1065.
96. Chen H, Chan DC. Mitochondrial dynamics in regulating the unique phenotypes of cancer and stem cells. Cell Metab. 2017;26:39–48.
97. Aguilo F, Avagyan S, Labar A, et al. Prdm16 is a physiologic regulator of hematopoietic stem cells. Blood. 2011;117:5057–5066.
98. Luchsinger LL, de Almeida MJ, Corrigan DJ, Mumau M, Snoeck HW. Mitofusin 2 maintains haematopoietic stem cells with extensive lymphoid potential. Nature. 2016;529:528–531.
99. Raffel S, Falcone M, Kneisel N, et al. BCAT1 restricts alphaKG levels in AML stem cells leading to IDHmut-like DNA hypermethylation. Nature. 2017;551:384–388.
100. Tefferi A, Lasho TL, Abdel-Wahab O, et al. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. Leukemia. 2010;24:1302–1309.
101. Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010;18:553–567.
102. Agathocleous M, Meacham CE, Burgess RJ, et al. Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. Nature. 2017;549:476–481.
103. Cimmino L, Dolgalev I, Wang Y, et al. Restoration of TET2 function blocks aberrant self-renewal and leukemia progression. Cell. 2017;170:1079–1095. e20.
104. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008;451: 1069–1075.
105. Wu M, Kwon HY, Rattis F, et al. Imaging hematopoietic precursor division in real time. Cell Stem Cell. 2007;1:541–554.
106. Hoppe PS, Schwarzfischer M, Loeffler D, et al. Early myeloid lineage choice is not initiated by random PU.1 to GATA1 protein ratios. Nature. 2016;535:299–302.
107. Passaro D, Abarrategi A, Foster K, Ariza-McNaughton L, Bonnet D. Bioengineering of humanized bone marrow microenvironments in mouse and their visualization by live imaging. J Vis Exp. 2017;126.

108. Spencer JA, Ferraro F, Roussakis E, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. Nature. 2014;508:269–273.
109. Nombela-Arrieta C, Pivarnik G, Winkel B, et al. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. Nat Cell Biol. 2013;15:533–543.
110. Piccoli C, Agriesti F, Scrima R, Falzetti F, Di Ianni M, Capitanio N. To breathe or not to breathe: the haematopoietic stem/progenitor cells dilemma. Br J Pharmacol. 2013;169:1652–1671.
111. Mantel CR, O'Leary HA, Chitteti BR, et al. Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock. Cell. 2015;161:1553–1565.
112. Sjostedt S, Rooth G, Caligara F. The oxygen tension of the blood in the umbilical cord and the intervillous space. Arch Dis Child. 1960;35:529–533.
113. Sawai CM, Babovic S, Upadhaya S, et al. Hematopoietic stem cells are the major source of multilineage hematopoiesis in adult animals. Immunity. 2016;45:597–609.
114. Upadhaya S, Reizis B, Sawai CM. New genetic tools for the in vivo study of hematopoietic stem cell function. Exp Hematol. 2018;61:26–35.
115. Palchaudhuri R, Saez B, Hoggatt J, et al. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. Nat Biotechnol. 2016;34:738–745.
116. Waskow C, Madan V, Bartels S, Costa C, Blasig R, Rodewald HR. Hematopoietic stem cell transplantation without irradiation. Nat Methods. 2009;6:267–269.
117. Taya Y, Ota Y, Wilkinson AC, et al. Depleting dietary valine permits nonmyeloablative mouse hematopoietic stem cell transplantation. Science. 2016;354:1152–1155.
118. Zimdahl B, Ito T, Blevins A, et al. Lis1 regulates asymmetric division in hematopoietic stem cells and in leukemia. Nat Genet. 2014;46:245–252.
119. Ito T, Kwon HY, Zimdahl B, et al. Regulation of myeloid leukaemia by the cell-fate determinant Musashi. Nature. 2010;466:765–768.
120. Corces MR, Chang HY, Majeti R. Preleukemic hematopoietic stem cells in human acute myeloid leukemia. Front Oncol. 2017;7:263.
121. Sarkozy C, Gardin C, Gachard N, et al. Outcome of older patients with acute myeloid leukemia in first relapse. Am J Hematol. 2013;88:758–764.
122. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3:730–737.
123. Huntly BJ, Gilliland DG. Cancer biology: summing up cancer stem cells. Nature. 2005;435:1169–1170.
124. Shlush LI, Mitchell A, Heisler L, et al. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. Nature. 2017;547:104–108.
125. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006;441:1068–1074.
126. Kharas MG, Lengner CJ, Al-Shahrour F, et al. Musashi-2 regulates normal hematopoiesis and promotes aggressive myeloid leukemia. Nat Med. 2010;16:903–908.
127. Ward PS, Patel J, Wise DR, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell. 2010;17:225–234.
128. Agathocleous M, Meacham CE, Burgess RJ, et al. Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. Nature. 2017;549:476–481.
129. Jiang Y, Nakada D. Cell intrinsic and extrinsic regulation of leukemia cell metabolism. Int J Hematol. 2016;103:607–616.
130. Kuntz EM, Baquero P, Michie AM, et al. Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. Nat Med. 2017;23:1234–1240.
131. Sumitomo Y, Koya J, Nakazaki K, et al. Cytoprotective autophagy maintains leukemia-initiating cells in murine myeloid leukemia. Blood. 2016;128:1614–1624.
132. Duarte D, Hawkins ED, Akinduro O, et al. Inhibition of endosteal vascular niche remodeling rescues hematopoietic stem cell loss in AML. Cell Stem Cell. 2018;22:64–77. e6.
133. Hawkins ED, Duarte D, Akinduro O, et al. T-cell acute leukaemia exhibits dynamic interactions with bone marrow microenvironments. Nature. 2016;538:518–522.