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Hematopoietic stem cell fate through metabolic control

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Abstract

Hematopoietic stem cells (HSCs) 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. HSC divisions result in either self-renewal or differentiation, with the balance between the two directly impacting hematopoietic homeostasis; but the heterogeneity of available HSC-enriched fractions, together with the technical challenges of observing HSC behavior, has long hindered the analysis of individual HSCs, and prevented the elucidation of this process. However, recent advances in genetic models, metabolomics analyses and single-cell approaches have revealed the contributions made to HSC self-renewal by metabolic cues, mitochondrial biogenesis, and autophagy/mitophagy, which have highlighted mitochondrial quality as a key control factor in the equilibrium of HSCs. A deeper understanding of precisely how specific modes of metabolism control HSC fate at the single cell level is therefore not only of great biological interest, but will have clear clinical implications for the development of therapies for hematological disease.

Stem cells are self-renewing, and either multi- or unipotent[1–5], and these unique capacities offer opportunities for stem cell-based therapies in the clinic[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]. HSC transplantation has therefore been a key therapeutic strategy in combatting hematological disorders[12–14]. 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]. As 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, or dormancy. This suspension of the cell cycle is thought to make

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a critical contribution to the maintenance of stem cells' self-renewal capacity and multipotency, as deletion of the genes involved in quiescence often leads to HSC exhaustion due to uncontrolled proliferation[15–20]. 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[16,18,21–25]. The role of cellular metabolism in regulating HSC self-renewal capacity has thus become a focus of much current stem cell research, which has yielded many new insights[26–32] (Fig. 1). In this review, we will highlight the recent advances in our understanding of the intriguing relationship between cellular metabolism, mitochondrial quality control, and HSC fate decisions.

Assessment of HSC fate

HSC cell fate decisions can be evaluated by paired daughter cell assays[15,33–35]. Their possible division options are: symmetric self-renewal expansion (symmetric division, SD; both daughter cells have the same function as the original cell), selfrenewal maintenance (asymmetric division, AD) and differentiation (symmetric commitment, SC; 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 where 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, as a cell's original function can affect its division pattern (Fig. 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 ten single cells in the HSC fraction must be "real" HSCs, and a case in which one of these HSCs undergoes SD (33%), while another undergoes AD (33%) and the third undergoes SC (33%). As 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% (Fig. 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[33,36–39]. 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-square test). However, when we have a high purity population of real HSCs, we can more accurately determine their division pattern (Fig. 2B).

To avoid this imprecision, researchers have long sought a reliable marker for individual HSCs which 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[8,40–45].

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[15,33,34], advanced single-cell approaches have recently confirmed that HSCs are capable of symmetric self-renewing division (or SD)[33,46]. 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[47].

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*[46,48]. 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[46].

As increasing evidence supports the essential contributions of metabolic control to HSC division patterns, determining the metabolic mode of purified HSCs is of crucial importance[15,29]. Single cell gene expression assays have revealed that critical roles in HSC expansion are played by fatty acid oxidation (FAO)[46]. The mitochondria are the primary sites of FAO, in which fatty acids are enzymatically broken down[49], and as they are essential sub-cellular components in the metabolic process, their role in division patterns and the subsequent cell fates of HSCs is a question of great scientific interest (Fig. 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[50]. In contrast, symmetric division requires self-clearance systems in both daughter cells, as young and old mitochondria have been found equally distributed between both[29,46]; 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[51]. In depolarized mitochondria, the degradation of PINK1 (PTEN-induced putative kinase 1) is impaired, leading to the accumulation and activation of this kinase on the mitochondrial outer membrane (MOM)[52–55]. 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

poly-ubiquitinated, and these are recognized by autophagy receptors to initiate autophagosomes formation[52–55]. 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[56,57]. The impact of *Parkin/Pink1*-knockdown 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 (Fig. 3A)[46]

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 important roles in HSC homeostasis (Fig. 1)[28]. 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[58–63]. Enhanced respiration is nevertheless detrimental to HSC maintenance and function[64–68]; for example, loss of mitochondrial carrier homologue 2 (MTCH2) increases mitochondrial respiration and intracellular ROS, triggering HSC entry into the cell cycle and compromising self-renewal capacity[69]. In contrast, lowering mitochondrial activity by chemical mitochondrial uncoupler supports sustained repopulation capacity under culture[62]. The defects in cell cycle quiescence and repopulation capacity observed in HSCs with impaired HIF (hypoxia-inducible factor) - PDK (Pyruvate dehydrogenase kinase) pathways are accompanied by enhanced flux of glycolytic metabolisms in the mitochondria during the TCA (tricarboxylic acid) cycle[60,70]. 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[71,72]. 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*[73].

The hypoxic condition has been shown to be critical to the maintenance of selfrenewal, while stress factors (e.g., infection or polyinosinic-polycytidylic acid, granulocyte-colony stimulating factor, or chronic blood loss) are now known to induce HSC cycling[19,74,75]. 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). As 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[26,27,76–82].

Autophagy in hematopoiesis and HSC aging

Recent studies from multiple groups have also shown that macroautophagy (hereafter called simply autophagy)[83–85] 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, and therefore 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 which maintains the quantity and quality of organelles and proteins by degrading them once damaged or unwanted[83–85]. 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[86], and a small-molecule inducer of autophagy has been shown to stimulate erythropoiesis[87,88]. The failure of this coordinated regulation can have a profound impact, as impaired autophagy has been shown to result in HSC exhaustion, and conditional depletion of *Atg7* can lead to lethal anemia[89,90].

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 (Fig. 3C)[58].

Enhanced mitophagy in hematopoiesis

The impact of excessive mitophagy on hematopoiesis has also been explored (although not in purified HSC populations)[91]. Atad3a, or ATPase family AAA domain-containing protein 3a, facilitates the transportation of Pink1 from the translocase of the outer membrane (TOM) complex to the translocase of the inner membrane (TIM) complex. In healthy mitochondria, Pink1 is rapidly degraded 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 (Fig. 3C)[91]. 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[89,91,92]. Although the contributions of autophagy at different hematopoietic stages remain to be clarified, these studies collectively demonstrate that mitophagy must be precisely controlled 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)[93–97]. 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 Prdm16 (PR domain containing 16) - Mitofusin-2 (Mfn2) axis contributes to the maintenance of HSCs with lymphoid potential by buffering calcium levels through mitochondrial tethering to the endoplasmic reticulum[98,99]. In addition, intact mitochondrial function for HSC maintenance may require metabolism-driven epigenetic changes or code[100–104].

Autophagy (or macroautophagy) was originally characterized as a non-selective bulk degradative system; however, it has been shown that under certain conditions, autophagosomes engulf cytosolic materials selectively, and diverse autophagy pathways have been identified[105]. Whether selective autophagy (e.g. pexophagy, glycophagy, SQSTM1-related autophagy) or other forms of autophagy (microautophagy and 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, lipids etc) 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)[28,58,89]. A new method of assessing the dynamic content of autophagosomes, combined with genetic approaches to 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 quantitatively regulated 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[15,33,34,106,107]; virtually nothing has been observed *in vivo*. Yet *in vivo* HSC behavior certainly differs from *ex vivo*, and it has been shown that cellular metabolism can be extrinsically modulated. 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[21,108], 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[70,109–111]. 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[112,113]. Thus 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 vs. 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 multi-lineage hematopoiesis both in the steady state and during cytokine response[114,115]. Notably, phenotypic HSCs comprise a major source of the megakaryocyte/platelet lineage in steady state conditions[11,35], but these cells show "multi"-lineage 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[12–14], but also severely alters the levels of ROS and other metabolic regulators, as well as the bone marrow microenvironment[116]. 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 pre-clinical stage[116–118]. New *in vivo* genetic tools are being developed to assess hematopoiesis with three- or even five-blood lineage resolution[11,35,115], 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 directly differentiate 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)[11,33,35]. 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 sternness (for instance, 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[29]. The establishment of assay systems in which realtime markers are associated with HSC-specific functions (e.g., repopulation capacity) will enable researchers to accurately assess the division patterns of HSCs by prospectively tracking their division patterns. This development will be a breakthrough in identifying the key regulatory machineries of HSC fate decisions, and will significantly improve our understanding of the fundamental properties of HSCs.

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. As 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[119,120], and the longterm survival rate among blood cancer patients remains stubbornly low, as 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[119–125]. 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[126,127]. It is no surprise that the metabolic requirements of leukemogenesis and LSC function have therefore become a focus of much current research[101,102,104,128–131], 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[132]. 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[133,134]. As the many metabolic pathways involved are conserved in human hematopoiesis, identifying the key metabolic cues that precisely control LSC fate and maintain stem-ness upon division could provide effective targets in strategies to enhance LSC commitment, and will therefore be of high clinical importance.

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Highlights

- Specific modes of metabolism play important roles in HSC self-renewal.
- Heterogeneity and technical challenges prevented the elucidation of HSC behavior.
- Recent advances highlighted mitochondrial quality control as a key HSC fate factor.
- A deeper understanding of HSC fate via metabolic control has clinical implications.

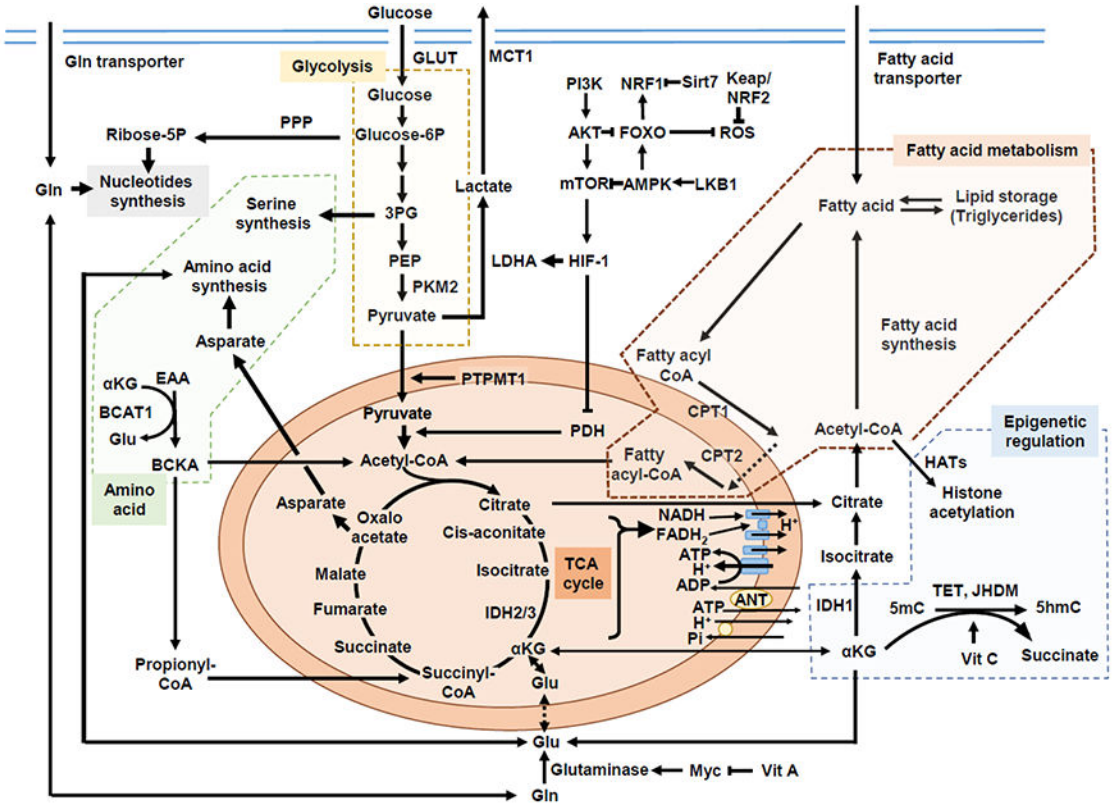

Figure 1. Overview of metabolic pathways contributing to HSC self-renewal and differentiation.

Hematopoietic stem cells (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. Fatty acid oxidation (brown background) is required for HSC selfrenewal 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 dioxigenase enzymes, TET2 and JHDM. Vitamin C is a co-factor for the enzymatic activity of the TET family of DNA hydroxylases (blue background). Abbreviations: HIF-1α, hypoxia-inducible factor 1α; 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; ROS, reactive oxygen species; 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 acetyltransferases;

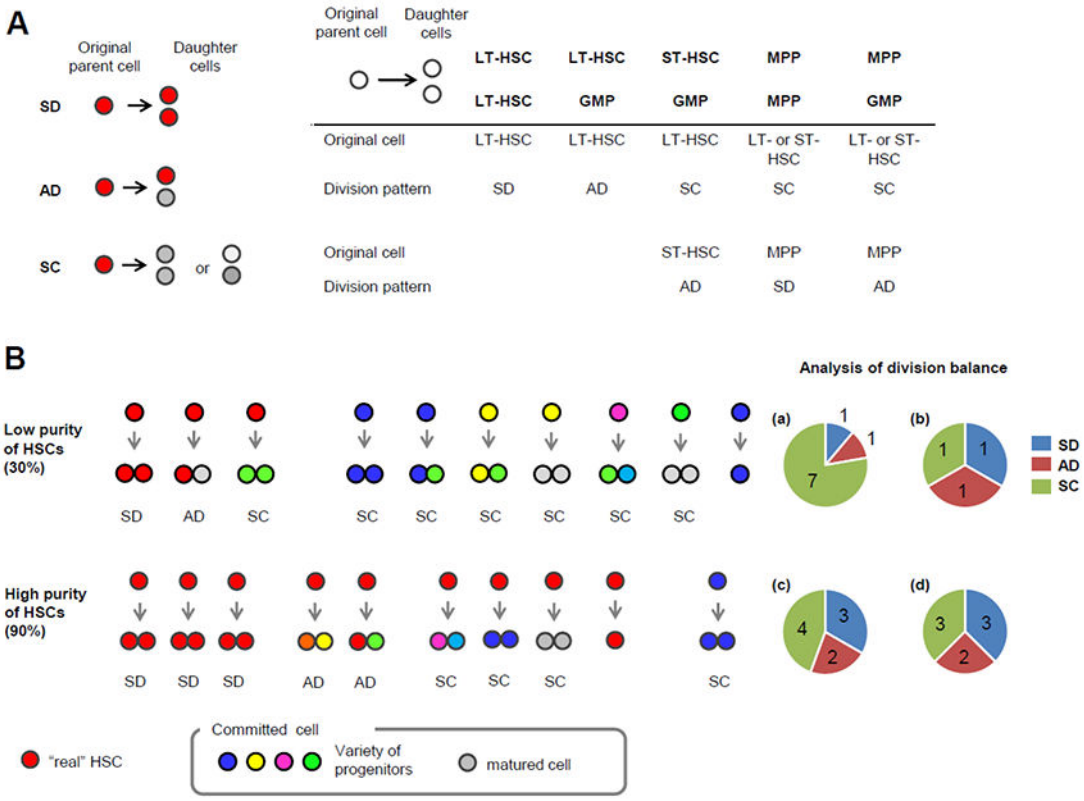

Figure 2. Division patterns by paired daughter cell assays.

(A) Original cell function affects its division pattern. Schematic model of 3 division patterns; after SD, both daughter cells have the same function and differentiation stage as the parent cell (red), while 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). As 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 right bottom. **(B)** Analysis of division patterns in homogenous and heterogeneous populations. When 10 single cells are isolated from the population with 30% purity of HSCs, 3 are generally “real” HSCs (top). In this example, each of these three HSCs undergoes SD, AD and SC, respectively (b), and 1 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. Thus, the resulting division balance of the whole compartment will be 1 SD, 1 AD and 7 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 accurately estimated from the resulting division symmetry of the isolated whole population (c). SD, symmetric division; AD, asymmetric division; SC, symmetric commitment; LT-HSC, long-term hematopoietic stem cell; ST-HSC, short-term HSC; MPP, multi-potent progenitor; GMP, granulocyte-monocyte progenitors.

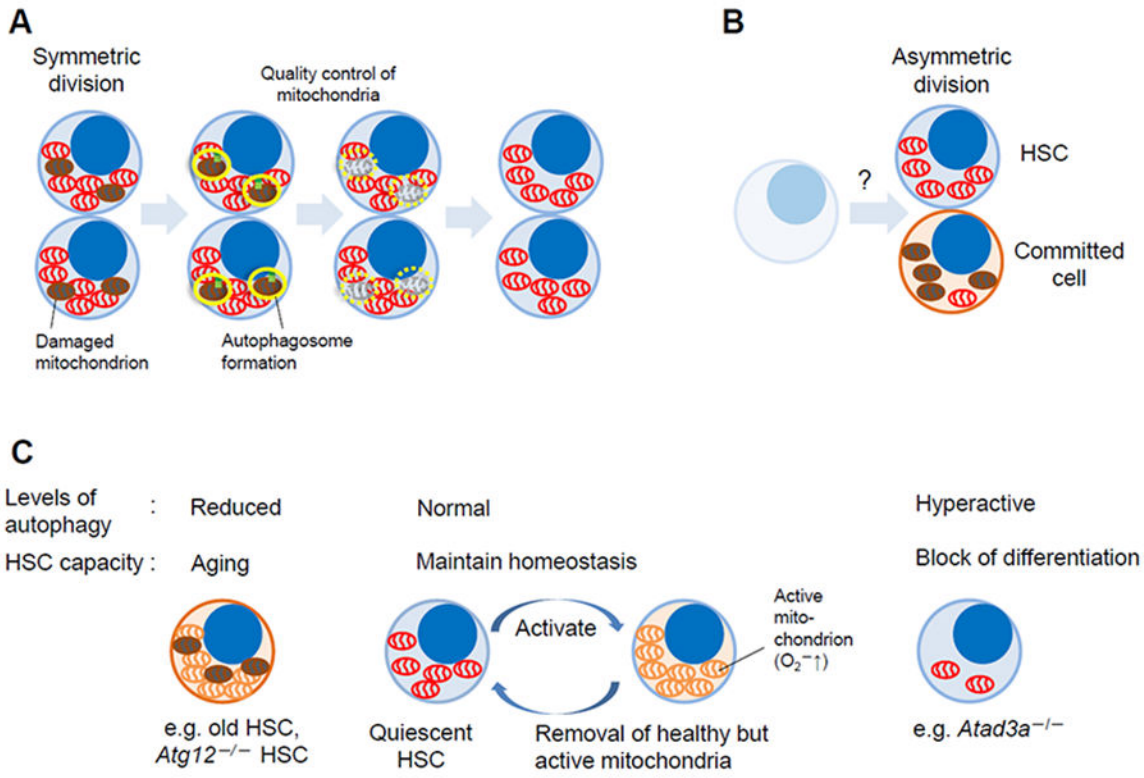

Figure 3. Quality control machineries in HSC division balance and hematopoietic homeostasis.

(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 as well as some autophagy-deficient HSCs (e.g. *Atg12*-deficient HSCs) were not able to efficiently limit the number of active mitochondria, 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). HSPC, hematopoietic stem and progenitor cell.