Hematopoietic Stem Cell Metabolism during Development and Aging

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Abstract

Cellular metabolism in hematopoietic stem cells (HSCs) is an area of intense research interest, but the metabolic requirements of HSCs and their adaptations to their niches during development have remained largely unaddressed. Distinctive from other tissue stem cells, HSCs transition through multiple hematopoietic sites during development. This transition requires drastic metabolic shifts, insinuating the capacity of HSCs to meet the physiological demand of hematopoiesis. In this review, we highlight how mitochondrial metabolism determines HSC fate, and especially focus on the links between mitochondria, endoplasmic reticulum (ER), and lysosomes in HSC metabolism.

Introduction

Tissue stem cells operate in a dual system of quiescence and expansion to proficiently generate and regenerate their respective tissue systems (van Velthoven and Rando, 2019). Eukaryotic cells first acquired mitochondria through fusion to α-proteobacteria (Gray et al., 1999) and this metabolic symbiosis allowed cells to replicate (Chandel et al., 2016). However, cells in higher organisms often halt proliferation and become quiescent in order to maintain homeostasis. For example, hematopoietic stem cells (HSCs), mesenchymal stem

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cells, neural stem cells, epidermal stem cells in the hair follicle, and satellite cells in the skeletal muscle are normally quiescent. The induction of quiescence in stem cells often requires conversion from an active to an inactive cellular metabolism mainly through the suppression of aerobic respiration.

HSCs are rare and versatile cells that sustain life-long hematopoiesis and can generate all lineages of mature hematopoietic cells upon transplantation. Their development is unique among stem cell systems as HSCs originate in different tissues during development. In vertebrates, the initial wave of hematopoiesis occurs in blood islands of the yolk sac, outside of the embryo. Large primitive nucleated erythrocytes, with the occasional presence of primitive macrophages and megakaryocytes, represent the major hematopoietic output of the yolk sac. Hematopoietic cells in the yolk sac may contribute to adult hematopoiesis (Samokhvalov et al., 2007), yet definitive hematopoiesis mainly arises in a region around the ventral wall of the dorsal aorta called the aorta-gonad mesonephros (AGM) at E10.5 in mice. Definitive HSCs, which are serially transplantable and have long-term engraftment capacity, emerge alongside non-self-renewing hematopoietic progenitor cells in the AGM. HSCs next migrate to the fetal liver and spleen and eventually reside in the bone marrow (BM) (Orkin and Zon, 2008; Dzierzak and Bigas, 2018). While embryonic and neonatal HSCs rapidly proliferate and expand to supply the developing hematopoietic system, adult HSCs rarely divide (Crisan and Dzierzak, 2016; Bernitz et al., 2016). These transitions from embryonic/neonatal stage to adult hematopoiesis require drastic alterations in metabolic state (Figure 1).

"Metabolism" is derived from the word *metaballein* ("to change" in Greek), which is fitting, given that the changes in energy production from embryo to the mature organism can be drastic. As a major energy-converting organelle, mitochondria provide ATP for the survival of eukaryotic cells through the tricarboxylic acid cycle (TCA cycle) and oxidative phosphorylation (OXPHOS). The adult human body contains approximately 1×10^{16} mitochondria, which accounts for 10% of body weight (Lane, 2005). Mitochondria are also a center for vital cellular processes such as the regulation of reactive oxygen species (ROS) levels, calcium signaling, apoptosis, proteostasis, and heme synthesis (Filippi and Ghaffari, 2019). Cellular metabolism in HSCs has become an area of intense research interest (Ito and Suda, 2014; Chandel et al., 2016). Although metabolic changes during their development have been shown, the metabolic requirements of HSCs in adaptation to their niches have yet to be fully explored. This review focuses on how HSC metabolism transforms and adjusts through hematopoietic ontogenesis, with a special focus on mitochondrial function (Figure 2).

Metabolic Transition during HSC Development

During development, definitive HSCs are generated from mesoderm-derived hemogenic endothelium (HE) in the AGM region (Dzierzak and Bigas, 2018). The establishment of arterial identity is a prerequisite for endothelial to hematopoietic transition (EHT) from which definitive HSCs emerge. While transcriptional regulation of EHT has been extensively studied (Zhu et al., 2020; Dzierzak and Bigas, 2018), metabolic changes during EHT have not been fully characterized. HSC expansion during early hematopoiesis involves redox reactions such as the modulation of nitric oxide (NO) and ROS levels (Harris et al.,

2013). The EHT process depends on Notch/Wnt/Bmp signaling (Shin et al., 2009), and as both Bmp and Notch regulate NO production, EHT may be influenced through endothelial cell-derived nitric NO metabolism (Nogueira-Pedro et al., 2014). NO is a short-lived free radical implicated in cell proliferation (Villalobo, 2006). NO synthesis in HSCs facilitated by endoglin enhances definitive hematopoiesis (Frame et al., 2017; Manesia et al., 2015). It is also known that prostaglandin E2 contributes to the HSC expansion in zebrafish and mammals during development through the modification of reactive oxygen species (ROS) levels (North et al., 2007; Ludin et al., 2014).

HSCs migrate from the AGM region to the fetal liver for further expansion. Unlike adult HSCs, which require quiescence to maintain stemness, fetal liver HSCs maintain stem cell potential while proliferating (Ema and Nakauchi, 2000). Stem cell transplantation and limiting dilution methods reveal that the absolute number of competitive repopulating units (CRU) per liver increases 38-fold from E12 to 16. Embryonic HSCs largely rely on hepatoblast-expressed Scf, and its receptor, c-Kit, for expansion (Khodadi et al., 2016; Chou and Lodish, 2010). Nestin$^+$ pericytes in the fetal liver also function as a niche through the production of factors such as Scf, Cxcl12, and Spp1 (Khan et al., 2016). While thrombopoietin (Thpo) and its receptor, Mpl, maintains adult HSC quiescence and self-renewal, this signaling pathway is dispensable in fetal HSC maintenance (Qian et al., 2007).

Fetal HSCs are generally thought to contain more mitochondria with higher bioenergetic function than adult HSCs (Manesia et al., 2015; Liu et al., 2010). However, direct comparison of mitochondrial mass between these HSC populations remains technically challenging. This is because adult HSCs express two ABC transporters (Abcg2/Bcrp1and mdr-1a/b), which export the dyes Hoechst33342 and Rhodamine123. This adult HSC-specific dye efflux hinders direct comparison of mitochondria mass between fetal/neonate and adult HSCs using dye-dependent methods (Goodell et al., 1996; Uchida et al., 2004). Careful assessment of mitochondria function using different methodologies is necessary (Snoeck, 2017).

Mitochondrial metabolism is vital for fetal HSC expansion. Fetal HSCs exhibit higher mitochondrial membrane potential (Ψmt) and levels of oxygen consumption than adult HSCs (Manesia et al., 2015). Fetal HSCs also exhibit anti-oxidant protection against high mitochondria metabolism, and fetal HSCs defective in manganese superoxide dismutase 2 (Sod2) show defective erythroid hematopoiesis (Friedman et al., 2001). Deletion of components of the TCA cycle and electron transport chain (ETC), fumarate hydratase, and Rieske iron-sulfur protein (RISP) also result in failure of fetal hematopoiesis (Guitart et al., 2017; Anso et al., 2017). Additionally, deletion of an autophagy regulator, *Fip200 (200-kDa FAK-family interacting protein*), impairs fetal HSC functions through accumulation of mitochondrial mass and ROS (Liu et al., 2010).

Adult HSCs suppress protein synthesis (Signer et al., 2014), while fetal liver HSCs have higher protein synthesis levels (Sigurdsson et al., 2016). In order to counteract ER stress (described later in this review), fetal liver HSCs utilize bile acids as chemical chaperones to withstand unfolded protein response (UPR). Differing UPR levels may also affect metabolic quality control in proliferating fetal/neonatal HSCs versus quiescent adult HSCs.

Additionally, mitochondrial UPR (UPRmt) is activated upon adult HSC exit from quiescence (Mohrin et al., 2018; Luo et al., 2019). Thus, the metabolic state of fetal/neonatal HSCs is unique: high mitochondrial metabolism without ROS generation and high protein synthesis without ER stress. However, what triggers HSC metabolic changes during development remains a largely uncultivated field.

Hematopoiesis shifts to the bone marrow (BM) late in gestation and is then maintained there. HSCs acquire cell cycle quiescence around 4 weeks after birth (Bowie et al., 2006). Changes in HSC metabolism associated with emergence of the G_0 state have not been analyzed extensively and are an area of interest. Moreover, it is unclear whether neonatal HSCs are similar to adult HSCs, which are genetically and functionally heterogenous (Crisan and Dzierzak, 2016). Single-cell genetic analysis and clonal lineage tracing assays on HSCs should further characterize the shift from neonate to adult hematopoiesis.

Cell Metabolism in Adult HSCs

Glycolysis

Dormant HSCs reside in a hypoxic microenvironment that promotes glycolysis and enables them to retain a low metabolic profile (Spencer et al., 2014). Their quiescence allows them withstand premature exhaustion from cell proliferation. Adult HSCs depend upon discrete genetic and proteomic control in order to utilize anaerobic glycolysis as their main energy source (Unwin et al., 2006; Takubo et al., 2010). HSCs express high levels of hypoxia inducible factor-1 alpha (HIF1α) and *Hif1α* deletion results in loss of HSC quiescence and stem cell potential (Takubo et al., 2010; Suda et al., 2011). However, using similar conditional deletion mouse models, other studies have described HIF1α and HIF2α as dispensable for HSC maintenance (Vukovic et al., 2016; Guitart et al., 2013), necessitating further analysis of the function of HIFs during steady-state or stress hematopoiesis.

Glycolytic metabolism in HSCs is complex and interlinked with other metabolic pathways such as glutamine metabolism or branched-chain amino acid (BCAA) catabolism (Jin et al., 2018; Taya et al., 2016; Ananieva and Wilkinson, 2018). Alternative polyadenylation regulates the switching of glutaminase isoforms, which is required for HSC activation (Sommerkamp et al., 2020). The enzymes responsible for the early steps of BCAA catabolism include branched-chain aminotransferase 1 (BCAT1), which converts BCAAs into branched-chain ketoacids by transferring the BCAA amino group onto alpha-ketoglutarate (αKG) (generating glutamate). BCAT1 has been implicated in the regulation of leukemic stem cells (LSC) and knockdown of BCAT1 downregulates HIF1α activity and LSC growth (Raffel et al., 2017). BCAA metabolism, which produces αKG and TET protein, is implicated more in the regulation of proliferating HSCs and LSCs than in control of quiescent HSCs. Interestingly, mutations in isocitrate dehydrogenase (IDH) which catalyzes the oxidative decarboxylation of isocitrate into αKG, are also implicated in leukemogenesis (Dang et al., 2009; Lu et al., 2012). Mutant *IDH* enzymes produce 2-hydroxyglutarate, which leads to aberrant HSC self-renewal and leukemogenesis in part through TET2 inhibition (Rakheja et al., 2013). Ascorbic acid is a TET cofactor and facilitates TET2-mediated DNA hydroxymethylation (Agathocleous et al., 2017; Cimmino

et al., 2017). These studies offer seminal insights into how metabolites affect HSC function through the modulation of epigenetic state.

Fatty Acid Metabolism

Fatty acid oxidization (FAO) is the mitochondrial process that breaks down a fatty acid into acetyl-CoA. Acetyl-CoA enters the citric acid cycle while NADH and FADH2, which are co-enzymes, are fueled into the electron transport chain (ETC). Notably, inhibition of the FAO pathway through the deletion of *Peroxisome-proliferator activated receptor delta (Ppard)*, a regulator of fatty acid transport and oxidization that is regulated by promyelocytic leukemia (Pml), results in loss of HSC reconstitution potential (Ito et al., 2012, 2016). Interestingly, inhibition of PPARγ promotes *ex vivo* expansion of human cord blood hematopoietic stem and progenitor cells (HSPCs), while causing a metabolic shift to glycolysis (Guo et al., 2018).

Beyond their central role in fatty acid metabolism, mitochondria also participate in the biosynthesis of nucleotides, cholesterol, amino acids, as well as heme, and these roles in HSC regulation still need to be investigated.

HSC Expansion through the Modulation of Metabolism

Manipulating HSC metabolism allows the expansion of HSCs *in vitro*. A study conducted on the role of a glycolytic enzyme, pyruvate dehydrogenase kinase (Pdk), in HSCs revealed the interplay between anaerobic metabolism and aerobic metabolism (Takubo et al., 2013). Pdk inhibits pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA which fuels aerobic metabolism. Loss of Pdk2 and Pdk4 in HSCs results in loss of stem cell potential. The report highlights that Pdk agonists expand HSCs *in vitro*.

Murine HSCs in long-term culture can be maintained in quiescent states using hypoxic culture conditions (in 1% O_2 conditions), low concentrations of SCF and Thpo with the addition of 4% bovine serum albumin (BSA) as a source of fatty acids (Kobayashi et al., 2019). Although efforts in *ex vivo* cord blood expansion and preservation have shown considerable success (Chaurasia et al., 2014; Fares et al., 2014; Wagner et al., 2016), the optimal conditions of adult human HSC *ex vivo* expansion are yet to be determined. Niche scaffolds which mimic the *in vivo* bone marrow microenvironment have successfully expand HSCs through regulating of mitochondrial activity and ROS production (Bai et al., 2019). Invention of similar methods should follow.

Mitochondrial Energy Production and Synthesis

Mitochondrial Function and the Electron Transport Complex

Mitochondria contain the major enzymes that oxidize carbohydrates, proteins, and lipids to produce ATP. In this process, appropriate substrates are catabolized to acetyl-CoA, which enters the TCA cycle (tricarboxylic acid cycle, or Krebs cycle) housed in the mitochondrial matrix (Baldwin and Krebs, 1981). Subsequent enzymatic steps of the TCA cycle result in the formation of reduced cofactors NADH (nicotinamide adenine dinucleotide) and FADH2 (flavin adenine dinucleotide) and ultimately fuel the ETC on the inner mitochondria

membrane (IMM) for ATP production. Electron transfer from NADH to oxygen allows protons to be pumped out of the matrix to generate an electrochemical proton gradient in the IMM. The electron transport ends at complex V (or ATP synthase) where the electrochemical proton gradient couples the conversion of ADP +Pi to ATP (OXPHOS) (Spinelli and Haigis, 2018; Figure 3).

HSCs depend mainly on glycolysis for ATP production, leaving mitochondrial OXPHOS activity relatively inert. Nonetheless, HSCs maintain mitochondrial activity, raising the question of how mitochondrial energy production is involved in HSC maintenance and proliferation. High mitochondrial membrane potential (Ψmt) is linked to lower reconstitution capacity and affects proliferation in adult HSCs (Ansó et al., 2017; Vannini et al., 2016; Sukumar et al., 2016). Ψmt has traditionally been assayed with dyes. Tetramethylrhodamine methyl ester (TMRM) dye accumulates in the mitochondrial matrix at levels that correlate with Ψmt and cellular OXPHOS activity. Long-term HSCs (LT-HSCs) have the lowest level of TMRM staining among all hematopoietic cells (Vannini et al., 2016, 2019). Inhibition or modulation of multidrug resistance (MDR)-mediated efflux did not affect the staining intensity of Rhodamine-123 and TMRE (tetramethylrhodamine, ethyl ester) in HSPCs (Kim et al., 1998; Liang et al., 2020). On the other hand, some studies have shown that verapamil, a wide spectrum inhibitor of efflux pumps, modifies the low retention of Rhodamine-123 in human or murine HSPCs (Zijlmans et al., 1995; Wagner-Souza et al., 2008). Similar results were obtained using a Ca^{2+}-independent MDR inhibitor, Cyclosporin H (Bonora et al., 2018; Morganti et al., 2019a). Ψmt is balanced between proton pumping activity by the ETC and proton flow across mitochondrial F_1/F_O ATP synthase; HSPCs sustain a unique equilibrium of Ψmt with a high ETC complex II:complex V ratio (Bonora et al., 2018; Morganti et al., 2019a). ETC complex II maintains complex III in proton pumping, and a low coupling capacity due to minimal F_1/F_O ATP synthesis results in relatively high Ψmt in HSPCs, even though corresponding rates of respiration and phosphorylation are low (Morganti et al., 2019a). Unexpected changes in staining intensity may be detected by flow cytometry when TMRM dye levels have not yet reached perfect equilibrium. Therefore, discrepancies in TMRM staining in HSPCs could depend on staining period, which suggests that staining conditions for measuring Ψmt should be standardized (Morganti et al., 2019b). Moreover, as flow cytometry quantifies fluorescence intensity per cell, it is difficult to discern whether mitochondrial volume affects fluorescence intensity values. Combined measurement of Ψm and mitochondrial volume/morphology (e.g., 3D image-based content analysis) could help reveal mitochondrial activity in HSPCs. Furthermore, understanding the precise roles of ETC proteins, such as succinate dehydrogenase (SDH; complex II) may clarify the links between ETC complex/TCA cycle, Ψmt, and the cell cycle state of HSPCs. Moreover, ATP levels and OXPHOS activity in HSCs can also be quantified through luminescent cell viability assays and Seahorse metabolic flux analyses, respectively (Nakamura-Ishizu et al., 2018; Tan et al., 2019; Ho et al., 2017). However, these methods require a substantial number of cells for accurate analysis and cannot be used for the assessment of individual HSCs. Additional non-dye-based methodologies are needed for the precise evaluation of Ψmt in HSCs.

Components of the mitochondria electron transport complex serve as initiators of apoptosis/programmed-cell death. Cytochrome c shuttles electrons between respiratory chain

complexes II and IV and is essential for electron flow and ATP synthesis (Santucci et al., 2019). The release of cytochrome *c* from the electron transport system to the cytoplasm activates caspase signals (Santucci et al., 2019). Cytochrome *c*-triggered apoptosis is noted in proliferating hematopoietic cells (Garland and Rudin, 1998). During apoptosis, cytochrome *c* and soluble internal proteins (SIMPs) are released into the cytoplasm and interact with apoptosis protease activating factor (Apaf-1) to form apoptosomes (Marsden et al., 2002). The release of cytochrome *c* to the cytoplasm requires the peroxidization of cardiolipin, a phospholipid within the mitochondrial membrane (Yin and Zhu, 2012). Thus, cytochrome *c* is annotated as an "extreme multifunctional protein" which alters its localization, changes its conformation, and determines cell fate (Santucci et al., 2019). However, detailed analysis on how cytochrome *c* function alters with mitochondria state and whether it regulates HSCs is largely unknown.

Mitochondrial Biogenesis in Adult HSCs

Both excessive and insufficient mitochondrial function is detrimental to adult HSC homeostasis. Abnormal mitochondrial biogenesis affects HSC function (Table 1). Mammalian target of rapamycin (mTOR) complex-1 governs multiple pathways for cellular metabolism and upregulates the transcription factor PPARγ coactivator-1α (PGC-1α), which is a key regulator of mitochondrial biogenesis (Cunningham et al., 2007). mTORC1 activation causes HSC exhaustion and deletion of a component of mTORC1, Raptor, results in pancytopenia and inhibition of HSC regeneration (Kalaitzidis et al., 2012). mTOR activation also leads to HSC exhaustion through the activation of p16Ink4a and p53 (Lee et al., 2010) and is associated with high oxidative stress in HSCs (Jang and Sharkis, 2007).

Multiple molecular mechanisms have been implicated on the regulation of mTOR activity in HSCs, and these include non-coding RNAs originating from Dlk1-Gtl2 locus, tuberous sclerosis complex (TSC)-1, and ERK-dependent phosphorylation of MEK1 (pT292) (Qian et al., 2016; Chen et al., 2008; Baumgartner et al., 2018). Mitochondria in HSCs deficient of Lkb-1, which regulates cell metabolism through AMPK, exhibit low Ψmt and ATP production (Gurumurthy et al., 2010; Nakada et al., 2010; Gan et al., 2010). However, the deletion of *Pgc1a* impairs long-term reconstitution potential of HSCs in BM transplantation with minimal effects on physiological HSCs (Basu et al., 2013). Thus, mTOR and related molecules are critical in regulating HSC metabolism.

Dynamics of Mitochondria Mass and Morphology

Mitochondria Structure and Mass in Adult HSCs

Mitochondria dynamically alter their morphology in response to cellular energy demands (Seo et al., 2018). Mitochondria consist of an inner membrane folded into multiple cristae structures that contain OXPHOS machinery. Functionally active mitochondria are oblong or oval and house numerous cristae to maximize inner membrane surface area (Pernas and Scorrano, 2016). Mitochondria mass and shape alters with mitochondria fission, fusion, and mitophagy processes (Youle and van der Bliek, 2012). Mitochondrial shape also changes in response to environmental conditions, such as a hypoxia, changes in energy demand, and metabolites (Westermann, 2010). Additionally, ultrastructural electron microscopic

characteristics, including cristae number and electron density, are also related to mitochondria function (Cogliati et al., 2013; Kumar et al., 2014; Hinge et al., 2020; Nakamura-Ishizu et al., 2018). In embryonic stem cells (ESCs), individual mitochondria are small and round with infrequent cristae (Zhang et al., 2011). Although these morphologic features suggest that mitochondrial respiration in ESCs is relatively inactive, ESCs exhibit high Ψmt (Folmes et al., 2011). Mitochondrial morphology in quiescent HSCs is similar to that of ESCs, suggesting that Ψmt rather than mitochondrial morphology better reflects a primed state for differentiation and proliferation (Schieke et al., 2008). Detailed studies to understand the relationship of mitochondrial morphology to function in HSCs are needed.

HSCs contain higher number and volume of mitochondria compared to hematopoietic progenitor cells. Multiple studies suggest that a dye-based MitoTracker staining underestimates mitochondrial content in HSCs (de Almeida et al., 2017; Takihara et al., 2019; Bonora et al., 2018). In these studies, mitochondrial volume is quantified through mitochondrial DNA levels or through the assessment of mito-dendra2 transgenic mice. Evaluation of immunohistochemical staining of TOM20 (Translocase of outer membrane 20) in HSCs from transgenic mice constitutively expressing GFP in the mitochondria matrix (mtGFP mice) (Shitara et al., 2010) showed lower mitochondrial volumes in HSCs compared to progenitor cells. Further clarification and advancement in techniques such as tomography of electron microscopy are necessary to delineate the exact mitochondrial volume and morphology in HSCs.

Whether HSCs heterogeneously contain different amounts of mitochondria is another interesting topic. Through assessment of mitochondrial volume in HSCs from mito-dendra2 transgenic mice, HSCs can be fractionated into high and low mitochondrial volume subgroups, the former presenting with higher stem cell potential and quiescent characteristics (Takihara et al., 2019). However, highly proliferative and active HSCs upregulate mitochondrial volume (Nakamura-Ishizu et al., 2018; Bonora et al., 2018), indicating that mitochondrial volume does not necessarily correlate with cell cycle state.

Regulation of Mitochondrial Dynamics in Adult HSCs

Mitochondria dynamically alter their shape though fission and fusion processes (Westermann, 2010; Figure 4). Loss of mitofusin2 (Mfn2), an OMM protein which facilitates mitochondrial fusion, results in defective lymphoid lineage differentiation of HSCs (Luchsinger et al., 2016). Although structurally similar to Mfn2, the role of Mfn1 in HSC regulation has not been elucidated (Chen et al., 2003). Deletion of *Optic atrophy 1 (Opa1)*, another fusion regulator located on the IMM (Olichon et al., 2003), lowers the activity of mitochondrial ETC complex I and ATP production in neutrophils (Amini et al., 2018). Further investigation on Mfn1 or Opa1 function may reveal how mitochondrial fusion associates with HSC maintenance.

Deficiency of the mitochondrial fission regulator *Drp1* results in mitochondrial aggregation, depolarization, and subsequently loss of reconstitution potential in HSCs (Hinge et al., 2020). Drp1 activity is regulated through phosphorylation (Ser637, Ser616), small ubiquitin-like modifier proteins, or s-nitrosylation by nitric oxide (Chang and Blackstone, 2007; Foster et al., 2009; Wasiak et al., 2007; Kashatus et al., 2015). Fis1 is located on the outer

mitochondria membrane (OMM) and facilitates attachment of Drp1 for mitochondrial fission. Fis1-deficient skeletal muscle cells exhibit swollen mitochondria and a decline in mitochondrial function (Zhang et al., 2019), yet no study has addressed the role of Fis1 in HSCs.

Advances in delineating HSC function and cell fate at the single-cell level has revealed mitochondrial heterogeneity in HSCs. Observation of single asymmetric HSC divisions revealed that mitochondria are distributed in an imbalanced manner to HSC daughter cells (Loeffler et al., 2019). Another study also showed that, under stress conditions (e.g., after transplantation), HSC mitochondria aggregate and depolarize (Hinge et al., 2020). Mitochondrial dynamism and motility were also lost in post-transplantation proliferative HSCs. Mitochondrial patterns were unevenly inherited to daughter cells during asymmetric HSC division, which was frequently observed in post-transplanted HSCs. HSCs with depleted Drp1 have decreased reconstitution potential due to the accumulation of dysfunctional mitochondria by asymmetric segregation. Altered fidelity of mitochondrial morphology implies that HSCs carry dysfunctional mitochondria to track their divisional history and limit their self-renewal capacity (Hinge et al., 2020).

Regulation of ROS in Adult HSCs

ROS are a byproduct of mitochondrial OXPHOS, which at high levels harms HSCs through the generation of reactive free radicals (Bigarella et al., 2014). HSCs acquire various safeguard mechanisms against cytotoxic accumulation of ROS. Loss of *Ataxia telangiectasia mutated* (*Atm*) causes accumulation of ROS and loss of quiescence in HSCs through the activation of p16Ink4a or p38MAPK(Ito et al., 2004,2006). Loss of Atm-mediated phosphorylation of Bid (a BH3-only BCL2 family member) along with loss of *Mtch2* (*Mitochondrial carrier homolog 2*) leads to less quiescence and an increase in mitochondrial oxidative stress (Maryanovich et al., 2012). As ROS production mainly derives from OXPHOS, high Ψmt is likely associated with high cellular ROS levels. This was highlighted by a study indicating that HSCs exposed to brief periods of ambient oxygen showed a rise in Ψmt and ROS levels, which lowered stem cell potential (Mantel et al., 2015). This phenomenon, named "extra physiologic oxygen shock/stress" (EPHOSS), involved oxidative stress-induced opening of mitochondrial permeability transition pore (MPTP).

Cells with damaged mitochondria, which are generally associated with low Ψmt, also exhibit high ROS levels. *Foxo3a* deficiency elevates HSC ROS levels through reduction of ROS detoxification molecules (Miyamoto et al., 2007). Despite presenting high ROS levels, *Foxo3a*$^{-/-}$ HSCs exhibit low ATP production and low Ψmt (Rimmelé et al., 2015). Aged HSCs decrease in stem cell potential but display both low Ψmt and ROS levels (Ho et al., 2017). ROS regulation in HSCs is complex, and impaired HSC function may not directly reflect ROS levels, but rather reflect the general integrity of mitochondrial function. These studies collectively support the observation that mitochondrial quality is associated with ROS production, which needs further assessment in HSCs.

Proper levels of intracellular ROS have been implicated in cell survival (Bigarella et al., 2014; Tan and Suda, 2018). ROS mediates the oxidization of target proteins at specific amino acid residues (especially cysteines or methionines) to regulate protein function (Holmström and Finkel, 2014). ROS modulates the activity of protein kinases (c-Kit, Akt, and MAPK) as well as transcriptions factors (p53, HIFs, and FOXOs) which are crucial pathways for HSC maintenance (Paulsen et al., 2011; Murata et al., 2003; Galli et al., 2008; Bigarella et al., 2014). p38MAPK activation leads to upregulation of purine metabolism to fuel HSC proliferation after stress, suggesting the involvement of oxidative stress (Karigane et al., 2016).

Mitochondria participate in anti-oxidant processes which reduce ROS levels. Mitochondrial superoxide dismutase (SOD2) or cytosolic SOD1 converts superoxide radical anion (O_2-) to hydrogen peroxide (H_2O_2) (McCord et al., 1971). While *Sod2* deficiency impairs erythroid cell production (Mohanty et al., 2013), no report has indicated its direct involvement in HSC regulation *in vivo*. Nuclear factor erythroid 2-related factor 2 (Nrf2) is known to regulate HSC quiescence and stem cell potential through modulation of oxidative stress (Murakami et al., 2017; Merchant et al., 2011). Glutathione-dependent enzymes also catalyze biological redox reactions, yet due to their redundancy in function, transgenic mice that lack glutathione-dependent enzymes are often phenotypically intact (Henderson and Wolf, 2011). However, *Microsomal glutathione transferase 1* (*Mgst1*)-deficient HSCs exhibit defective differentiation (Bräutigam et al., 2018). Collectively, while quiescent HSCs generally retain low ROS levels, low ROS levels do not necessarily indicate low mitochondrial function. Neonatal HSCs exhibit active mitochondrial metabolism but low ROS, and more importantly, are capable of expansion *in vivo*. It should be emphasized that the complexity of HSC response to oxidative stress emphasizes the potential indications of ROS levels on mitochondria and redox state.

Mitochondria and the Endoplasmic Reticulum

Mitochondria-Associated Endoplasmic Reticulum Membranes (MAMs)

Mitochondria and endoplasmic reticulum (ER) are essential organelles in eukaryotic cells and coordinate various biological pathways such as maintenance of calcium homeostasis, protein folding, and lipid metabolism. Mitochondria interact with ER to facilitate calcium and lipid exchange between the two membrane systems (Krols et al., 2016). Mitochondria and ER communicate via the formation of mitochondrial-associated membranes (MAM, or mitochondria-associated ER membranes). MAMs are formed through the juxtaposition of ER subdomains to mitochondria and defects in their formation and function alter cell survival and death. MAM proteins either directly connect ER and mitochondria or modulate tethering complexes. Mfn1 and 2 (Mfn1/2) are found in the MAM complex (de Brito and Scorrano, 2009). Mitochondrial ubiquitin ligase Mitol/March5, a MAM protein, ubiquitinates mitochondrial Mfn2, and its deletion impairs MAM domain formation results in decreased tethering of mitochondria to ER (Sugiura et al., 2013). The role of MAMs in the metabolic regulation of HSCs is yet to be explored.

Regulation of Cellular Calcium

Upregulation of mitochondrial Ca^{2+} in physiological condition activates mitochondrial enzymes, which facilitate the TCA cycle and OXPHOS. α-ketoglutarate dehydrogenase, IDH, and pyruvate dehydrogenase (PDH) are Ca^{2+}-dependent enzymes (McCormack and Denton, 1993). ATP synthase activity also depends on Ca^{2+} (Das and Harris, 1990). Thus, increased Ca^{2+} levels enhance electron transport activity in mitochondria, resulting in elevated generation of ATP (Hansford, 1994; Figure 5).

In contrast, prolonged or excessive upregulation of mitochondrial Ca^{2+} promotes apoptosis. MAMs convey mitochondrial apoptotic signals to ER, especially through the refinement of Ca^{2+} levels (Iwasawa et al., 2011). Ca^{2+} flux from ER to mitochondria stimulates Bcl-2-associated X protein (BAX) to oligomerize and permeate the mitochondrial membrane (Rostovtseva et al., 2005). Mitochondrial permeability transition pores (PTPs) are induced by high Ca^2 levels (Hunter and Haworth, 1979). PTP opening increases mitochondrial membrane permeability, leading to apoptosis by releasing cytochrome *c*, apoptosis-inducing factor (AIF), and Smac/DIABLO (Bernardi et al., 2001).

Several reports have highlighted the role of intracellular calcium levels in HSC regulation. Increase of intracellular calcium triggered HSC divisions during stress hematopoiesis induced by 5-fluouracil administration (Umemoto et al., 2018). Dampening of intracellular calcium inhibited mitochondrial functions and prolonged the cell cycle phase of HSCs which resulted in slower recovery of stress hematopoiesis. The suppression of intracellular calcium level by nifedipine, a calcium channel blocker, prolonged the cell division interval and maintained HSCs.

Further association between intracellular calcium levels and mitochondria have been highlighted through the function of Mfn2 (Luchsinger et al., 2016, 2019). Deletion of *Mfn2* in HSCs decreased intracellular calcium buffering through ER-mitochondria tethering activity. As intracellular calcium stimulated nuclear translocation and transcriptional activity of Nuclear factor of activated T cells (Nfat), Mfn2-deficient HSCs exhibited loss of lymphoid differentiation potential. Steady-state HSCs were also endowed with low intracellular calcium levels (Luchsinger et al., 2019). HSCs cultured in low concentration of calcium exhibited reduced mitochondrial respiration but not glycolysis. The study also highlighted how intracellular calcium levels regulate calpain and subsequently the activity of TET (Ten-eleven translocation) enzymes for HSC maintenance. However, high concentration of cytoplasmic calcium has also been associated with dormant HSCs (Fukushima et al., 2019).

Mitochondria fission (Drp1) and unfolded protein response have been implicated in regulation of cellular calcium (Wang et al., 2017; Favaro et al., 2019) and these should be further investigated in HSCs. Along with improved methods to accurately measure intracellular calcium in HSCs, detailed monitoring of external and intracellular regulators of calcium levels will further clarify the role of calcium in HSC maintenance. The mechanism of how cytoplasmic calcium levels affect mitochondrial activation should also be investigated.

ER Stress and Mitochondrial UPR

Increased protein synthesis may lead to the accumulation of unfolded and misfolded proteins. This accumulation triggers ER stress as a part of the unfolded protein response (UPR). Since ER stress is a multi-layered, bidirectional cellular response that results in either cell survival or death, proper management of UPR and ER stress signals are crucial for HSCs. It has been suggested that UPRmt is implicated in cellular senescence and aging (Jensen and Jasper, 2014). UPRmt is upregulated upon mitochondrial biogenesis and involves retrograde signaling from the mitochondria to the nucleus to stimulate transcription of mitochondrial chaperones, mediating enhanced mitochondrial function during cell proliferation. Mitochondrial proteostasis has been implicated in HSC regulation. HSCs induced to proliferate with polyinosinic:polycytidylic acid (pI:pC, or poly(I:C)) increased the expression of mitochondrial chaperones and protease-associated genes (Mohrin et al., 2018). The deletion of *Sirtuin 7* (*Sirt7*), a protein deacylase, elevates UPRmt, impairs regenerative potential, and compromises lymphopoiesis of HSCs (Mohrin et al., 2015). Sirt7 represses the activity of Nrf1 (nuclear respiratory factor 1) to suppress mitochondrial metabolism and cell proliferation. Mitochondrial stress may also trigger the formation of aberrant Nlrp3 (NOD-, LRR-, and pyrin domain-containing protein 3) inflammasomes in HSCs and promote HSC aging (Luo et al., 2019).

Mitochondria Clearance

Autophagy and Mitophagy in Adult HSCs

Autophagy degrades damaged organelles and remove aggregated proteins within cells in response to stress (Shahrabi et al., 2019). Nutrient-sensing pathways, such as the AMPK pathway, activate autophagy-related genes (Atgs) to initiate formation of autophagosomes and clearance of unwanted cellular material. HSCs modulate autophagic flux to control their maintenance, differentiation, and proliferation (Mihaylova and Shaw, 2011; Figure 6). Impairment of autophagy leads to premature aging, while maintenance of autophagy has been implicated in the maintenance of various stem cells including HSCs (Revuelta and Matheu, 2017). For example, deletion of *Atg5* in hematopoietic cells causes abnormal clearance of damaged mitochondria and a decline in HSC reconstitution potential (Jung et al., 2019). Conditional knock-out of *Atg12* impaired HSC reconstitution potential and resulted in premature HSC aging (Ho et al., 2017). Interestingly, *Atg12*-deficient HSCs presented an increase in active mitochondria with high Ψmt, indicating that Atg12-mediated autophagy is involved in the clearance of healthy but active mitochondria. Similarly, deletion of *Atg7* resulted in decrease HSC function and exhaustion accompanied with increased mitochondria numbers and ROS levels (Mortensen et al., 2011).

One of the key regulators of mitophagy, Pten-induced putative kinase 1 (Pink1), interacts with the E3 ubiquitin ligase Parkin (or Park2) and directly facilitates mitochondria aggregation to form autophagosomes (Ito et al., 2016). Pink1 specifically senses depolarized and damaged mitochondria, accumulates on the surface of the mitochondria to recruit and activate Parkin (or Park2), and eventually leads to autophagic removal of mitochondria (Pickrell et al., 2015; Jin et al., 2010). Acute silencing of Parkin/Pink1 in HSCs has been shown to promote enhanced clearance of damaged mitochondria mediated through Ppar-

FAO pathway, and defective mitophagy impairs the self-renewal of *Tie2*^{+} HSCs. In addition, the studies with *Ulk1*- and *Bnip3l/Nix*- deficient mice have found defective mitochondrial clearance during terminal stages of red blood cell maturation, suggesting a potential role of an Atg5/Atg7-independent non-canonical autophagy pathway in hematopoietic cells (Honda et al., 2014).

Enhanced mitophagy also affects HSCs. The deletion of AAA+-ATPase, Atad3a, a regulator of Pink1-dependent mitophagy, hyperactivated mitophagy in HSCs (Jin et al., 2018). Atad3a locates on both IMM and OMM and serves as a mitochondria-ER organizing network protein (Yang and Suda, 2018). Its anchorage into the IMM enables it to interact with ER membranes via N terminus and regulate mitochondria-ER interactions to support steroid and lipid biosynthesis. *Atad3a*-deficient HSCs exhibited defective lineage differentiation yet expanded in number. Thus, enhanced mitophagy also induces HSC dysfunction.

Lysosomal Function

Interactions between mitochondria and lysosomes have also been implicated in HSC regulation. MITF,TFEB, orTFE3 regulate autophagosomal and lysosomal biogenesis through their interaction with ATG13 and ULK1 (Kennedy and Lamming, 2016). Quiescent HSCs exhibited abundant numbers of active lysosomes which sequestered lysosomal cargo such as mitochondria (Liang et al., 2020). The suppression of lysosomal acidification using concanamycin A (con A) greatly increased Ψmt as well as the number and the reconstitution potential of primed-HSCs. Con A-treated, primed-HSCs exhibited enlarged lysosomes with enhanced sequestration of mitochondria as well as low glucose uptake and glycolysis. Lysosomal activity therefore coordinates metabolism for the maintenance of HSCs (Liang et al., 2020). Taken together, low lysosomal activity is critical for the regulation of quiescent HSCs.

HSC Metabolism during Stress and Aging

HSC Heterogeneity and Stress Hematopoiesis

Multipotency, quiescence, and self-renewal capacity are fundamental properties that segregate HSCs from progenitor cells. Yet, HSCs are phenotypically and functionally heterogeneous, presenting variable stem cell properties. Adult HSCs have different depths of cell cycle quiescence: deeply dormant HSCs versus easily activated, primed HSCs. It is postulated that dormant HSCs require a longer time to enter the cell cycle compared to primed HSCs (Cabezas-Wallscheid et al., 2017) and primed HSCs primarily contribute to the restoration of hematopoiesis during stress. Native hematopoiesis assessed with non-transplant models revealed that expansion of multi-potent progenitors (MPPs) establishes steady-state hematopoiesis (Sun et al., 2014; Rodriguez-Fraticelli et al., 2018). These data suggest that long-lived progenitors, rather than HSCs, are the main drivers of physiological hematopoiesis in adults. Lastly, subpopulations of HSCs exhibit bias toward specific lineages, signifying their variability in multi-lineage differentiation capacity (Sanjuan-Pla et al., 2013; Yamamoto et al., 2013; Haas et al., 2015). Lineage-biased HSCs prominently emerge during stress hematopoiesis due to inflammation or recovery from myeloablation. It

is questioned whether HSPC heterogeneity (i.e., non-biased HSCs, megakaryocyte-biased HSCs, and long-lived progenitors) associates with HSC metabolic state.

During stress hematopoiesis, HSCs require ATP production for expansion and differentiation. Emergent demands for additional production of mature hematopoietic cells are often relayed through cytokine signaling (Singh et al., 2018). Deletion of the helix-loop-helix transcription factor *Id1* attenuated the response of HSCs to cytokine induced proliferation due to a reduction in mitochondrial OXPHOS. We recently reported that the cytokine Thpo upregulates mitochondrial metabolism and skews adult HSCs toward a megakaryocyte lineage-biased differentiation (Nakamura-Ishizu et al., 2018). Administration of recombinant Thpo or Romiplostim (Thpo mimetic drug) to wild-type mice rapidly upregulated HSC Ψmt and megakaryocyte differentiation. Furthermore, HSCs with a high Ψmt displayed a megakaryocyte-lineage skewed reconstitution pattern upon competitive BM transplantation. These findings suggest that HSC response to stress depends on metabolic state and may be modulated by extrinsic factors such as Thpo.

HSC Aging

Aged HSCs display several distinct phenotypes, including an increase in frequency, reduction of regenerative capacity, and differentiation bias toward myeloid-lineage (de Haan and Lazare, 2018). As described above, metabolic changes such as decrease in autophagy and reduction in proteasomal degradation along with the accumulation of DNA damage can accelerate HSC aging. Aged HSCs generally exhibit enhanced mitochondria OXPHOS and increased ROS production which presumably compromise HSC function (Ito et al., 2006; Mohrin et al., 2015). However, it should be carefully assessed whether high ROS levels are a cause or consequence of HSC dysfunction. Changes in the BM microenvironment influence stem cell aging and indeed, anatomical and functional remodeling of HSC niches accelerates myeloid-lineage cell expansion during aging (Ho et al., 2019). However, changes in the niche that promote HSC aging thorough metabolic stress are yet to be explored.

High mitochondria activity relates to alteration of epigenetic states that impact aging. Mitochondria influence and modify the epigenetic state of cells (Reid et al., 2017). The TCA cycle generates citric acid which modulates histone acetylation and gene expression through its conversion to acetyl-CoA (Reid et al., 2017). Mitochondrial FAO generates acetyl-CoA for histone modification in HSCs (Ito and Suda, 2014). These metabolites moderate histone hypoacetylation and hypermethylation. Although not bound to histones, mitochondrial DNA (mtDNA) is epigenetically regulated through methylation, yet the physiological relevance is still unknown (Matilainen et al., 2017). Nevertheless, the integrity of mtDNA influences HSC aging as exemplified in mice with a proof-reading-deficient version of mitochondrial DNA polymerase gamma (Polg) which causes an accumulation of mutations in mtDNA in HSCs (Norddahl et al., 2011).

Clonal Hematopoiesis of Indeterminate Potential (CHIP)

The presence of age-associated clonal hematopoiesis, CHIP (or age-related clonal hematopoiesis, ARCH), is a seminal finding in the study of HSC aging. A small number of HSC clones that disproportionally contribute to peripheral blood production emerge with

aging and are detected in 10% of persons older than 65 years of age (Genovese et al., 2014). The majority of these clones present with mutations principally affecting epigenetic modifiers DNMT3A, ASXL2, and TET2. Among 10%–40% of cases, the clone will progress to meet the diagnostic criteria for CHIP (variant allele frequency [VAF] above 2%), which is associated with an increased risk of hematological cancer. TET2 and DNMT3a enzymes regulate metabolic activation and hematopoietic differentiation. Inactivation of these enzymes allows LSCs to utilize OXPHOs without impairing self-renewal (Sperling et al., 2017; Testa et al., 2016). Proteomic analysis of aged HSPCs revealed significant alterations in the expression of specific proteins involved in DNA repair, cell proliferation, and metabolism (Hennrich et al., 2018). Among these pathways, HSPCs uniquely exhibited the elevation of glucose metabolic enzymes upon aging, which led to the hypothesis that the upregulation of glycolytic enzymes may influence the development of CHIP. Modulation of metabolism to affect CHIP development and transition to hematopoietic malignancy is a topic of considerable therapeutic interest.

Concluding Remarks

HSCs are capable of maintaining homeostasis and meeting demand for hematopoiesis through control of their cell cycle status and metabolism. Interestingly, HSCs in fetal liver cells and neonatal bone marrow are engaged in active cycling, while adult HSCs remain in G_0. Over the past decade, an abundance of new genetic models and -omics techniques have expanded our knowledge of both respiratory and non-respiratory functions of the mitochondria in adult HSCs. However, our understanding of metabolic regulation during embryonic hematopoiesis is still limited. This fact holds particularly true for human hematopoiesis; it is difficult to recapitulate the emergence of HSCs from pluripotent stem cells *in vitro*. The development of HSC capacity begins in the embryo with a series of transient hematopoietic waves. A clear description of how this development occurs, with metabolic adaptation across discrete anatomic sites that shift as the embryo evolves, is of fundamental importance.

Cellular metabolism can be extrinsically modulated. Yet, as the complete recapitulation of bone marrow physiology *in vitro* is difficult, findings obtained from cultured cells may not reflect *in vivo* metabolic state. Self-renewing division, which yields two functional HSCs, is believed to be infrequent after birth, as HSCs undergo symmetric self-renewal only 4–6 times across the entire lifespan of a mouse (Wilson et al., 2008). Upon stress, HSCs begin to divide and gradually lose regenerative capacity. This observation leads to many open questions. Why are self-renewing divisions rare in adult HSCs? How do the mitochondria, ER, and lysosomes cooperatively transform the metabolism of HSCs as they exit or re-enter quiescence? New analytical tools that can map spatial distributions of biomolecules (i.e., 4D digital imaging mass spectrometry) will identify novel interactions between HSCs and their niche. This will allow a range of interventions to tailor metabolic events that determine the equilibrium between quiescent and proliferating HSCs. Furthermore, unraveling the mechanism of HSC quiescence may provide insights into rejuvenation and prolonging life of an individual organism. Recently, it was shown that Polycomb complex members (CBX7) are upregulated during diapause state in killifish, a model of short-lived vertebrates (Hu et al., 2020). Diapause state suspends development and helps organisms survive extreme stress.

A multi-disciplinary view of dormancy or hibernation may stimulate research and novel concepts in metabolism in organisms and dormant tissue stem cells.

It is unclear whether HSC metabolism during self-renewing cell divisions differs from that of divisions associated with differentiation and cell commitment. Metabolic comparisons between these division patterns provokes high scientific interest; *ex vivo* HSC expansion from limited donor cells is required for therapeutic application. Although the number of cells necessary for full metabolomics analysis limits analysis of rare populations, recent advances in continuous live imaging and tracking have highlighted an imbalanced apportioning of mitochondrial parameters between HSC daughter cells during asymmetric division (Loeffler et al., 2019). The reliable measurement of single-cell metabolomics in paired daughter cells will contribute to unveil specific metabolic modes to HSC fate decisions (Zenobi, 2013).

Elucidating the mechanisms of HSC fate control is a central goal of ongoing research in the field. However, HSC expansion *ex vivo* has had only limited success (Wilkinson et al., 2019; Bai et al., 2019) and HSC culture systems must be improved to fully realize the potential of HSC-based therapy. Interestingly, while adult HSCs and embryonic/neonatal HSCs both exhibit low mitochondrial ROS levels, adult HSCs exhibit a low metabolic and embryonic/neonatal HSCs exhibit high mitochondrial activity. Comparative analysis of mitochondrial contributions between adult and embryonic hematopoiesis could help clarify the underlying mechanism behind developmental changes and achieve an *in vitro* model of the physiological BM microenvironment. This will in turn illuminate the key metabolic requirements of healthy stem cell aging and may be applied to the invention of novel therapies for hematological disorders.

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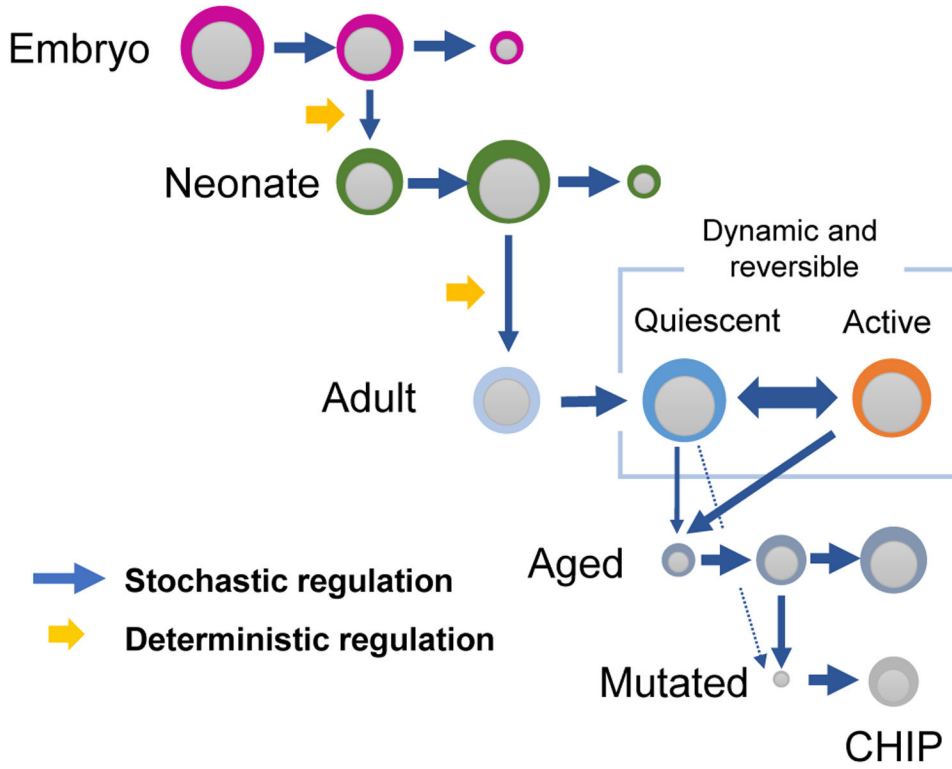
Figure 1. Schematic Representation of HSC Dynamics during Development
HSCs of different developmental state (embryonic, neonatal, adult, and aged HSCs) clonally expand through stochastic processes. HSCs give rise to differential clones during development through a deterministic process possibly through the modification of hematopoietic environment. Adult HSCs maintain a quiescent state which may be reversed to an active proliferative state upon stress. Aged HSCs eventually accumulate genetic mutations leading to the expansion of abnormal clones (CHIP).

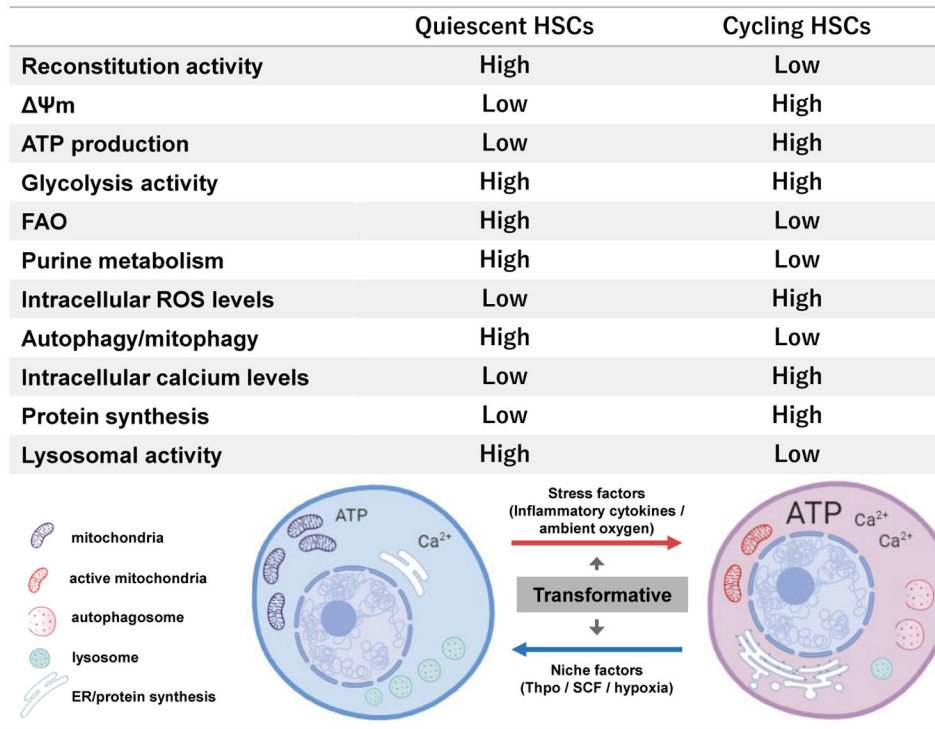|	Quiescent HSCs	Cycling HSCs
Reconstitution activity	High	Low
ΔΨm	Low	High
ATP production	Low	High
Glycolysis activity	High	High
FAO	High	Low
Purine metabolism	High	Low
Intracellular ROS levels	Low	High
Autophagy/mitophagy	High	Low
Intracellular calcium levels	Low	High
Protein synthesis	Low	High
Lysosomal activity	High	Low

Figure 2. Metabolic Characteristics of Quiescent and Cycling HSCs

Quiescent adult HSCs exhibit high reconstitution potential and differ in organelle (mitochondria, ER, lysosome, and autophagosome) content compared to cycling HSCs. The difference in organelle activity reflects the overall metabolic state (Ψm, ATP production, protein synthesis, autophagy, glycolysis, FAO, purine metabolism, ROS levels, and calcium levels).
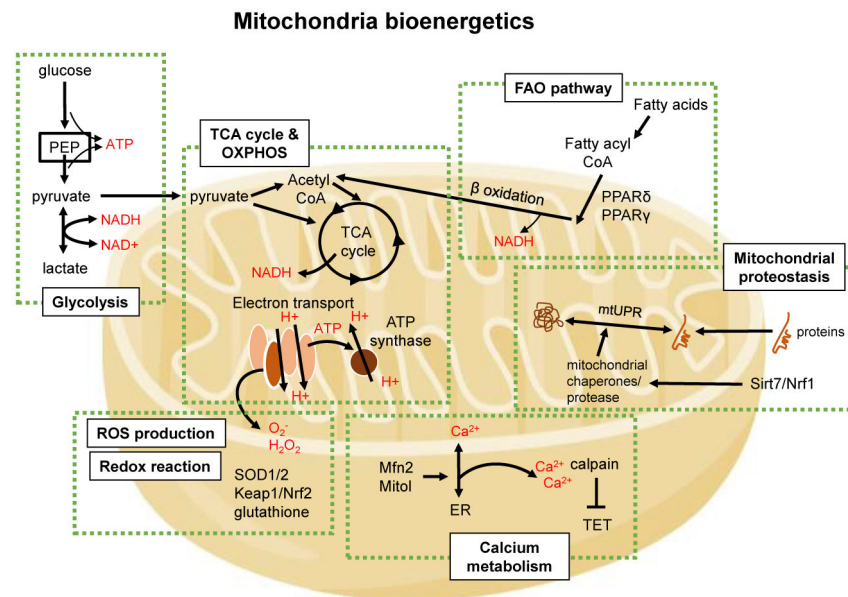
Figure 3. Mitochondria Bioenergetics in HSCs

Mitochondria bioenergetics pathways implicated in HSC metabolism. Other than ATP production through the TCA cycle and electron transport system, HSC mitochondria produce energy through FAO (beta-oxidation). ROS is generated as a byproduct of oxidative phosphorylation and is detoxified by cystolic redox reaction. Mitochondria also home specific pathways for proteostasis and process unfolded proteins through chaperone proteins and proteases. Intracellular calcium levels are regulated by the mitochondria through crosstalk with ER.
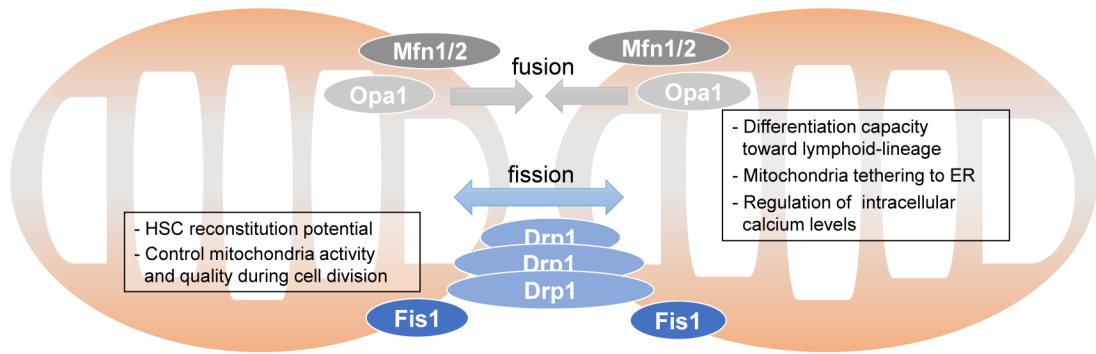
Figure 4. Mitochondria Dynamics through Fission and Fusion
Mfn1/2 located on the OMM and Opa1 on the IMM stimulate mitochondria fusion. Deletion of Mfn2 affect HSC mitochondria function and stem cell potential. Drp1 and Fis1 stimulate mitochondria fission. Fis1 is implicated to affect HSC mitochondria segregation during division and stem cell functions.
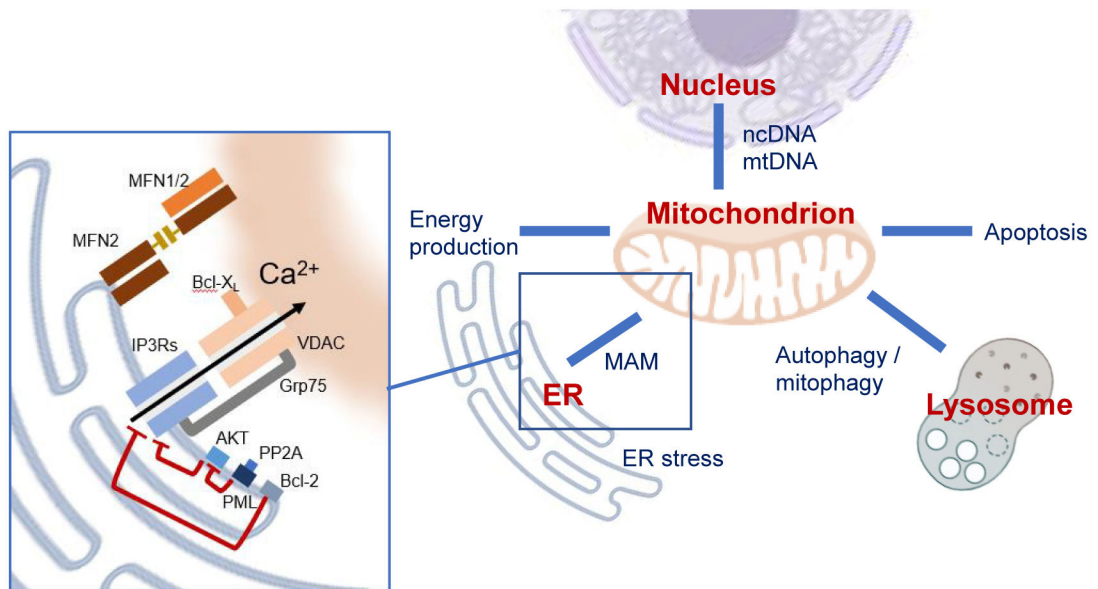
Figure 5. Metabolic Crosstalk between Cellular Organelles in HSCs

Mitochondria interact with various cellular organelles. These interactions alter the metabolic and cellular fate of HSCs. The nucleus provides nuclear (nc) DNA encoded proteins, while mitochondria possess mitochondria (mt) DNA for the production of mitochondria proteins. Mitochondria also initiate apoptosis. Mitochondria clearance is regulated through a specific form of autophagy, mitophagy. Mitochondria are firmly associated with ER to which they tether and control intracellular calcium levels.
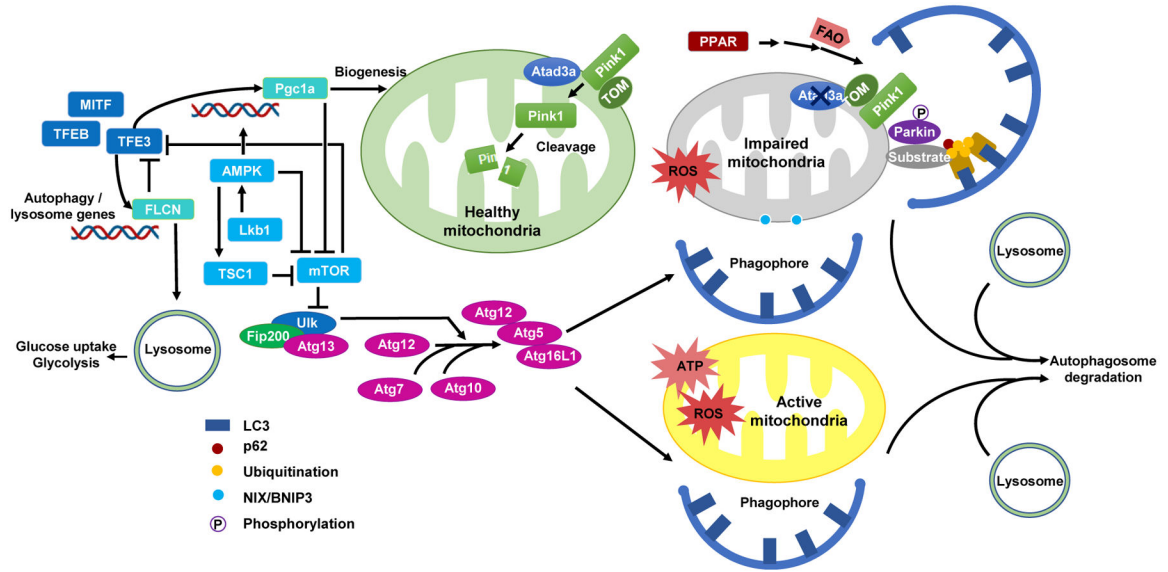
Figure 6. Regulation of Autophagic Clearance of Active and Damaged Mitochondria in HSCs
The removal of active and damaged mitochondria through autophagy maintains HSCs in a low metabolic, quiescent state. Active mitochondria with high ATP production and ROS levels are cleared through macroautophagy. Mitochondria with impaired function, such as deletion of Atad3a, may provoke Pink/Parkin pathway for mitophagy. Phosphorylated Parkin promotes the ubiquitination of mitochondrial substrates, recruits p62, and links mitochondria to LC3 for mitophagy. The presence of Atad3a on healthy mitochondria promotes the degradation of Pink1 via protease cleavage in the mitochondria matrix. Autophagy and mitophagy are regulated through a cascade of reactions involving Atg (Atg5, 7, 10, 12, 13, 16L1), Ulk1, FIP200, and other autophagy-related proteins. Autophagic pathways are interlinked with mitochondria biogenesis (mTOR, AMPK, LKB1, TSC2, and PGC1a) along with lysosomal pathways (FLCN, TFEB, TFE3, and MITF).

Table 1.

Mitochondrial Regulators Associated with HSC Function

Genes	Mitochondria-Related Function	HSC Phenotype in Transgenic Mice	Mitochondria Phenotype	References
Mitochondria Biosynthesis Regulators				
mTOR	upregulates mitochondria synthesis through PGC-1α	activation causes HSC expansion, exhaustion, and leukemogenesis; deletion causes pancytopenia and impairs HSC regeneration	activation results in high oxidative stress	Kalaitzidis et al. (2012); Lee et al. (2010); Jang and Sharkis (2007); Qian et al. (2016)
Ppargc1a (Pgc1a)	peroxisome proliferator-activated-γ coactivator-1α; regulator of mitochondrial biogenesis	deletion impairs reconstitution potential of HSCs but does not affect physiological HSCs	deletion reduces MMP and mitochondrial mass in bone marrow cells	Basu et al. (2013)
Tsc1	tuberous sclerosis complex; inhibits mTOR	deletion results in loss of HSC quiescence	deletion causes increased mitochondrial biogenesis and elevated ROS	Chen et al. (2008)
Src3	p160 SRC family of nuclear receptor coactivators; increases Pgc-1α expression	deletion results in defective HSCs	deletion results in high MMP and high ROS; deletion increases Pgc-1α expression	Hu et al. (2018)
Lkb1/Stk11	Lkb1 tumor suppressor; activates AMPK and TSC1 to inhibit mTOR	deletion of Lkb1 causes loss of HSC quiescence and repopulation potential	deletion results in the impairment of mitochondrial function and biogenesis with the reduced MMP and ATP levels	Gan et al. (2010); Gurumurthy et al. (2010); Nakada et al. (2010)
Mitochondria-Localized Proteins				
Ptpmt1	PTEN-like mitochondrial phosphatase	deletion causes BM failure due to defective HSC differentiation and expansion	deletion causes decrease in oxygen consumption and extracellular acidification rates, indicating defective mitochondrial bioenergetics as well as glycolysis	Yu et al. (2013); Liu et al. (2015)
Uqcrfs1	Rieske iron-sulfur protein (RISP) in mitochondrial complex III	deletion causes loss of HSC quiescence and severe pancytopenia	deletion impairs respiration resulting in a decreased NAD+/NADH ratio; also leads to DNA hypermethylation and histone hypoacetylation through the increases in 2-hydroxyglutarate (2HG)	Ansó et al. (2017)
Mortalin (Hspa9)	mitochondria heat shock protein	knock down of Hsp9 led to loss of HSC quiescence and reconstitution potential	knock down of Hsp9 leads to increased ROS in HSCs	Tai-Nagara et al. (2014)
Mitochondria Fusion/Fission				
Mfn2	mitochondria fusion	deletion causes defective lymphoid lineage differentiation of HSCs	deletion reduces mitochondria tethering to ER and alters intracellular calcium levels	Luchsinger et al. (2016)
Drp1	mitochondria fission	deletion causes loss of reconstitution potential	depletion causes aggregation and depolarization of mitochondria	Hinge et al. (2020)
Mitophagy/Autophagy				
Atad3a	AAA+-ATPase regulator of Pink1-dependent mitophagy	deletion causes HSCs to expand in number with defective lineage differentiation	deficient HSPCs have the reduced mitochondrial content due to hyperactivated mitophagy by Pink1 accumulation	Jin et al. (2018)

Genes	Mitochondria-Related Function	HSC Phenotype in Transgenic Mice	Mitochondria Phenotype	References
Atg7	Autophagy related 7	Atg7-deficient mice show expansion of HSPCs with defective reconstitution potential	depletion causes aberrant increase in mitochondrial mass, higher mitochondrial ROS level, and increased DNA damage	Mortensen et al. (2011); Gomez-Puerto et al. (2016)
Atg5	Autophagy related 5	deletion from hematopoietic lineage cells cause lymphopenia and anemia; impaired reconstitution potential	deficiency causes impaired clearance of damaged mitochondria	Jung et al. (2019)
Parkin/Pink1	PTEN-induced putative kinase 1 (Pink1, or Park6), Parkinson protein 2, E3 ubiquitin protein ligase (Parkin, or Park2)	acute silencing causes the impaired self-renewing expansion of Tie2+ HSCs	–	Ito et al. (2016)
FIP200	200-kDa FAK-family interacting protein	deletion in fetal HSCs increased mitochondrial mass and ROS levels	deletion causes perinatal death due to anemia; fetal HSCs increase proliferation and lose reconstitution potential	Liu et al. (2010)
Atg12	Autophagy related 12	deletion results in decreased HSC reconstitution potential with myeloid skewing	deficient HSCs present increase of active mitochondria with high MMP	Ho et al. (2017)
Other Functions				
Pml/Ppard	FAO; Pml regulates Ppard, a regulator of fatty acid transport and oxidization	loss of HSC potential	–	Ito et al. (2012)
Polg	integrity of mtDNA; proof-reading-deficient version of mitochondrial DNA polymerase gamma	defective Polg causes an accrue of mutations in mtDNA and compromises HSC differentiation in early lymphoid and erythroid lineages	mutations in mtDNA lowered MMP	Norddahl et al. (2011)
Pparg	peroxisome proliferator-activated receptor gamma	PPARγ antagonist promotes expansion of human cord blood HSPCs	PPARγ antagonism enhances glycolysis without compromising mitochondrial metabolism	Guo et al. (2018)