REVIEW

Mitochondrial control of hematopoietic stem cell balance and hematopoiesis

Massimo Bonora[1,2], Paolo Pinton[1], Keisuke Ito (✉)[2]

[1] *Department of Morphology, Surgery, Experimental Medicine University of Ferrara, Section of Pathology Oncology, Experimental Biology Laboratory for Technologies of Advanced Therapies (LTTA), Ferrara, Italy*
[2] *Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Departments of Cell Biology and Medicine, Albert Einstein Cancer Center and Einstein-Sinai Diabetes Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA*

Abstract Hematopoietic stem cells (HSCs) are stem cells from mesodermal derivation that reside in bone marrow and provide blood cells for the whole life of an adult individual, through a process called hematopoiesis. The long lasting support of HSCs for hematopoiesis is permitted by the fine regulation of quiescence and division output. Exit from the quiescent state is to produce a committed and/or stem daughter cells, in an event defined asymmetric or symmetric division. A deregulation in the proportion between asymmetric and symmetric divisions is critical in the appearance of hematological disorders ranging from bone marrow failure to hematological malignancies. Over the past years, several studies have indicated how the metabolism of HSCs is determinant in the regulation of HSC quiescence and commitment process. A metabolism shifted to the glycolytic pathway promotes HSCs quiescence and sustainment of hematopoiesis. Boosting mitochondrial respiration promotes the stem cell commitment followed by stem pool exhaustion, and minimal mitochondrial activity is required to maintain the HSCs quiescence. In the present review are discussed the most recent advances in comprehension of the roles of mitochondria in the hematopoiesis and in the division balance.

Keywords mitochondria, metabolism, hematopoiesis, hematopoietic stem cells, division balance, hypoxia inducible factor 1 alpha (Hif-1α)

Introduction

Hematopoiesis is the physiological process that allows the production of all types of blood cells through the whole life of an individual. This is a hierarchical process that begins with Hematopoietic stem cells (HSCs) originating different types of multi-potent progenitors that expand, and differentiates, finally, into mature blood cells.

A key element in the regulation of such homeostasis resides in the HSCs, and the number of HSCs must be precisely controlled. Similar to other tissue stem cells, HSCs are characterized by dual capacity, self-renewal and multi-potent differentiation. An excess of commitment of HSCs would result in the depletion of HSC pool and to the

consequent onset of a bone marrow failure, as differentiated cells have a reduced life span. In contrast, the excess of self-renewal activity would expand the HSC pool with consequent increase of undifferentiated cells, a phenomenon typical of some pathological conditions like some leukemia models and the myelodysplastic syndrome (Weissman et al., 2001). The balance between differentiation and self-renewal is therefore critical for the proper blood homeostasis, and one of the dramatic choices of the cell fate is attributed to the mitosis of HSCs. This event was suggested to generate two different daughter cells, one committed and one HSC (asymmetric division), two committed cells (symmetric commitment) or two HSCs (symmetric division). Increasing evidence implies that HSCs are precisely maintained through both asymmetric and symmetric cell divisions (Ito et al., 2012; Ito and Suda, 2014; Zimdahl et al., 2014).

The study of signaling events regulating the balance between commitment and self-renewal is of potential interest for therapeutic approaches, and one of the most extensively

studied in last years is the metabolic status of HSCs. Mitochondria have been observed to vary in amount and activity within different stages of hematopoiesis (Inoue et al., 2010; Simsek et al., 2010; Takubo et al., 2013; Wang et al., 2014). Further different genes required for proper HSCs homeostasis encode mitochondrial proteins or proteins that controls mitochondrial activity through regulation of principal metabolic processes. Of note, the emerging roles for mitochondria in regulating the differentiation of other tissue stem cells were further "driving force" for the investigation of mitochondria's contributions in HSC regulation (Rehman, 2010).

Mitochondria represent a unique organelle within the complex endomembrane systems that characterize any eukaryotic cell. They are the site where most of oxygen consumption occurs to sustain electron transport chain (ETC) with consequent ATP generation by the mitochondrial ATP synthase activity (Bonora et al., 2012). Mitochondria are also the site where the tricarboxylic acid (TCA) cycle occurs; this utilize acetyl-CoA as source of reduced equivalents to generate NADH or FADH2 that will act as substrate for the ETC (Bonora et al., 2012). Acetyl-CoA can also be used by TCA cycle as carbon source to provide intermediates for amino-acid or heme synthesis. This allows mitochondria to contribute to the metabolism of a cell by a complex network of catabolic or anabolic pathway. Mitochondria are also essential regulators of intracellular signaling events especially due to their contact sites with the endoplasmic reticulum (ER) (Bononi et al., 2012; Marchi et al., 2014; Vance, 2014). Specifically, mitochondria-ER communications are involved in control of inflammation, apoptosis, necrosis and metabolism (Bononi et al., 2013; Rimessi et al., 2013; Arruda et al., 2014; Bonora and Pinton, 2014; Giorgi et al., 2014, 2015).

In the present work, we will review the most recent findings about contribution of mitochondria in the metabolism of HSCs and their cell fate decision.

Mitochondria in hematopoiesis

An involvement of mitochondria in the fate of a HSC would be expected to induce alterations of some mitochondrial features between the HSCs and different stages of the hematopoietic process (i.e. alteration in mitochondrial content, membrane potential or respiration dependent ATP production). Such kind of alterations were reported for the commitment of other stem cells like embryonic or mesenchymal stem cells (Rehman, 2010; Prowse et al., 2012; Zhang et al., 2013; Ito and Suda, 2014) or some progenitor cells (Bonora et al., 2014; Vega-Naredo et al., 2014; Wanet et al., 2014).

Initial evidences about different mitochondrial pattern within hematopoietic stages were observed by Ploemacher and Brons in 1988 (Ploemacher and Brons, 1988a, 1988b) using the fluorescent marker for mitochondrial activity,

Rhodamine 123 (Rh-123). They showed that mouse bone marrow cells with lower mitochondrial activity have higher capacity to colonize the recipient's bone marrow addressing those as HSCs (Fig. 1). Later Kim and coworkers confirmed that the Rh-123 staining was inversely correlated to the "stemness"of the HSCs. They sorted Rh-123^{hi} and Rh-123^{lo} fractions in Thy1.1[low] Sca-1^{+}Lin^{−} cells and transplanted them into lethally irradiated mice to compare their reconstitution capacities *in vivo*. Rh-123^{lo} cells displayed higher repopulations then Rh-123^{hi} cells, indicating that HSCs were enriched in the Rh-123^{lo} population. As fewer HSC markers available at that time and the efficacy of the transplantation was monitored up to 12 weeks, information regarding the purity of HSC or progenitor contaminations could not be determined. Nonetheless these initial studies clearly indicate the involvement of mitochondria in the hematopoietic process. Additionally, similar observation were collected from human CD34^{+} HSCs, further supporting contribution of mitochondria in HSCs capacity (Piccoli et al., 2005; McKenzie et al., 2007; Romero-Moya et al., 2013).

In agreement with this observation, another study showed that the total cellular ATP content was almost 2 times higher in the committed progenitors, common myeloid progenitors (CMP) and common lymphoid progenitor (CLP), compared to the hematopoietic stem and progenitor cells (HSPCs) (a mixed population enriched in HSCs and multi-potent progenitor comparable to the Thy1.1[low] Sca-1^{+}Lin^{−} cells) (Inoue et al., 2010). Interestingly, the ATP levels decrease in the full differentiated population, indicating that, unlike what is observed in other differentiation process, mitochondrial activity is not strictly required for the matured blood cell, but rather for the progenitor population. Further, the same populations with high intracellular ATP levels (CMP and CLP) also display a higher proportion of cycling cells (Fig. 1). These results led authors to the preliminary conclusion that the high metabolic activity could be required to sustain the expansion of the progenitor population (Inoue et al., 2010).

In the same study, authors explored the effect of the deletion of a wide portion of the mitochondrial DNA in the mouse hematopoietic process (Inoue et al., 2010). Mitochondrial DNA (mtDNA) is a circular DNA about 16k bp long and code specific genes for tRNA, rRNA and 13 different subunits for respiratory complex I, III and IV, and ATP synthase (Wallace and Chalkia, 2013). The mice utilized for this study carry a mtDNA with a 4696 bp deletion. This deletion results in the loss of 3 subunits of complex I, one for complex IV and 2 for ATP synthase, which causes a reduction in mitochondrial respiration and impaired ATP synthesis. This particular mouse was called "mito-mouse" and carries the mutation in 77%–83% of the mtDNA copies. Contemporary to the impaired mitochondrial activity was observed an increased amount of HSPCs in the bone marrow. These HSPCs were also faster cycling and displayed lower reconstitution capacity after *in vivo* bone marrow transplantation, while CMPs displayed the same proliferation speed as those from
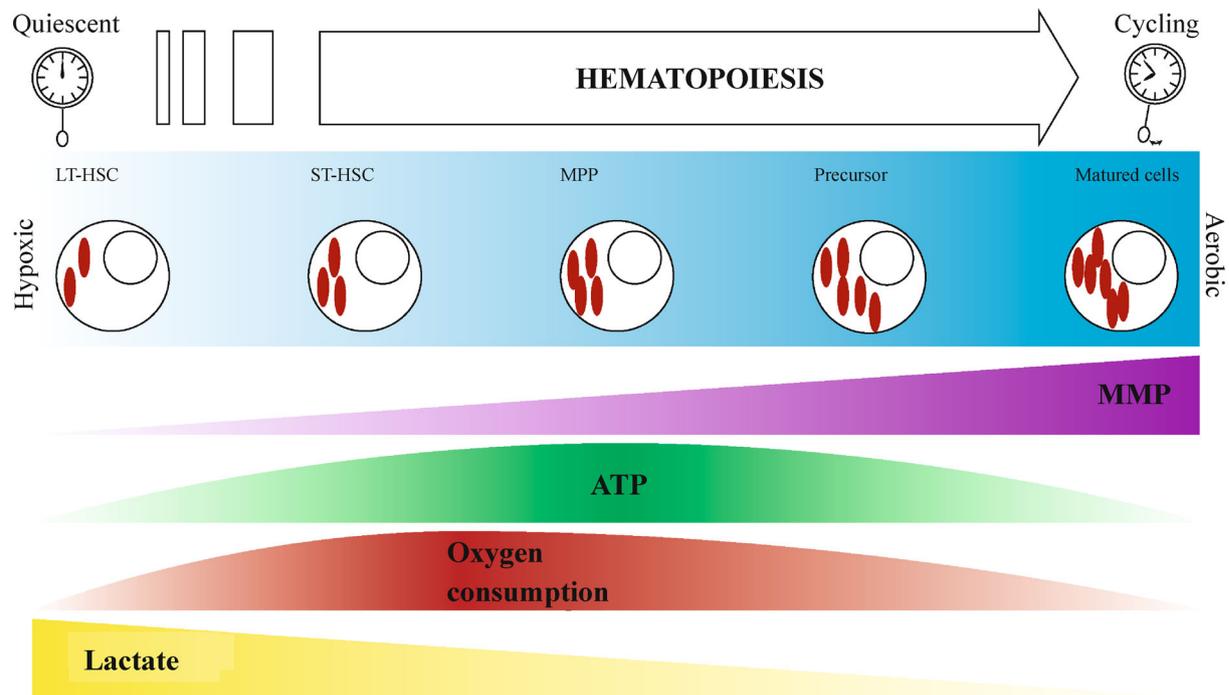
Figure 1 Simplified interpretation of hematopoiesis and metabolic features in HSC and differentiating cells. Mitochondria are displayed as intracellular brown objects. Levels of different metabolic parameters are displayed by colorized cartoons. Mitochondrial membrane potential (MMP), based on reports about Rhodamine123 measurements.

control mice. ATP levels were reduced in both HSPC and CMPs, in dissociation with the cycling activity. Overall their report indicates that mitochondrial activity is required to regulate the quiescence of HSPCs and this appears to be ATP independent.

Quiescence is a fundamental feature to ensure an HSC. In fact, mice with increased cycling of HSCs manifest the phenotype of HSC exhaustion and/or bone marrow failure. In both human and mouse, the quiescent HSCs reside in a specific location of the bone marrow, called the HSC niche (Mendelson and Frenette, 2014). This is a region characterized by a low oxygen tension that let it earn the term "hypoxic niche." HSCs have low mitochondrial activity, and it appears straightforward as an adaption to the environment in which reside (Fig. 1). This idea is supported by the observation that mouse long-term HSCs (LT-HSCs, which are highly quiescent and reside at the top of hematopoietic hierarchy) display reduced oxygen consumption and higher lactate conversion when compared to the whole bone marrow (Fig. 1). This suggests that mitochondria in LT-HSCs are either in lower amount or just unable to consume oxygen and HSCs rely on their ATP production mainly from glycolysis (Fig. 1, Simsek et al., 2010). In the same report, authors further proposed that the effect is driven by the hypoxia-inducible factor 1 alpha (Hif-1α). Hif-1α is a transcription factor that is stabilized during hypoxic conditions and degraded during normoxia. When stabilized, Hif-1α modulates expression of the many genes involved in tumor

metastasis, angiogenesis, energy metabolism, cell differentiation and apoptosis. Specially, in regards of energy metabolism, it can promote glucose uptake and glycolytic flux, but also inhibits mitochondrial biogenesis and cellular respiration (Zhang et al., 2007; Takubo et al., 2010). LT-HSCs display higher levels of Hif-1α, and interestingly, its transcriptional levels are also under the transcriptional control of the homeobox gene *Meis1*.

A contemporary study confirmed the role of Hif-1α in HSC homeostasis (Takubo et al., 2010). Authors first confirmed that LT-HSCs are in hypoxic condition by the use of the hypoxic marker, pimonidazole. Hif-1α levels are enriched in HSC populations and the depletion of *Hif-1α* results in loss of LT-HSC quiescence. A later intriguing metabolic study (Takubo et al., 2013) conducted by the same group demonstrated that LT-HSCs displayed higher lactate levels and lactate dehydrogenase activity (the enzyme responsible for pyruvate to lactate conversion), compared to cells representing the immediate committed step, the short-term HSCs (ST-HSCs). They also observed that the pyruvate dehydrogenase kinase (Pdk), an inhibitor of mitochondrial pyruvate utilization, restored the impaired cell-cycle-status and repopulation-capacity of *Hif-1α*-deleted HSCs.

These studies supported the previous observation that LT-HSCs displayed lower "mitochondrial activity" (at least in terms of cellular respiration and ATP production) compared to the committed progenies. However, there are some phenotypical differences between Hif-1α model and mito-

mouse. The mtDNA deletion of the mito-mouse induces the reduction in mitochondrial activities, and this is associated with loss of HSC quiescence and reduced its repopulation capacity. On the other hand, though the *Hif-1α*-depletion has an increased mitochondrial activity, this depletion induces to the similar results, a loss of LT-HSC quiescence and defective repopulation capacity. Additional studies are thus required to clarify the role of Hif-1α in the control of mitochondrial activity and HSC biology.

The studies about the conditional knockout mice of *Lkb1* (*Liver kinase B1*) further increase the complexity of the picture (Gan et al., 2010; Gurumurthy et al., 2010; Nakada et al., 2010). LKB1 is a stress sensing kinase involved in the control of cell growth and energy metabolism. LKB1 is able to activate the AMPK (5′ AMP-activated protein kinase) that in turn activate the tuberous sclerosis complex (TSC), inhibitor of the mechanistic target of rapamycin (mTOR). TSC-mTOR axis as a regulator of HSCs quiescence by repressing both mitochondrial biogenesis and reactive oxygen species (ROS) production had been demonstrated (Chen et al., 2008). Nonetheless in 2010, three independent studies showed that conditional depletion of *Lkb1* in the hemato-poietic compartment (by the use of two different inducible systems, *Mx1-Cre* and *Vav1-Cre*) leads to loss of HSCs quiescence with impaired bone marrow repopulation capacity in the irradiated mice (Gan et al., 2010; Gurumurthy et al., 2010; Nakada et al., 2010). Surprisingly, this event appears to be mTOR- or AMPK-independent. Complementary observa-tions among these three studies are that loss of *Lkb1* impairs mitochondrial content (measured by mtDNA content and mito-tracker staining) and activity (measured by oxygen consumption or membrane potential). Interestingly *Lkb1*-depletion was associated with increased cell death and chromosomal instability of the cycling HSCs, which is analogous to what was observed in the mito-mouse (Nakada et al., 2010).

These phenotypes display similarities with the once of mice defective for macroautophagy. Macroautophagy (herein "autophagy") is a degradation pathway primarily regulated by nutrient starvation (Galluzzi et al., 2014). During the process a double membrane vesicle (autophagosome) encloses a portion of cytoplasm, long-lived proteins or organelles and then fuses with a lysosome for the degradation and recycling of its contents. Autophagy is also considered a major mechanism for the removal of "aged/damaged" mitochondria and required for maintenance of total mitochondria hemo-stasis within cells (Patergnani and Pinton, 2015). Specially the block of selective incorporation of mitochondria into autophagosomes (an event called mitophagy) leads to accumulation of mitochondria with reduced membrane potential and ATP synthase activity to which follow the reduction of the oxidative metabolism of a cells (Green et al., 2014). Homogyzous and conditional depletion of the master regulator, *Atg7*, in hematopoietic cells (by the use of a Cre recombinase under the control of *Vav1* promoter) lead to

lethal anemia due to loss of quiescence and increased proliferation of HSC and progenitors. This event is associated with an increase in content of mitochondria (Mortensen et al., 2010). Additionally, RB1CC1/FIP200, a component of the autophagy-initiating complex, also has a critical role in regulation of hematopoiesis as its conditional depletion (in this case by controlling Cre recombinase through the *Tie2* promoter) also leads to perinatal lethality and severe anemia accompanied with mitochondria accumulation (Liu et al., 2010). It could be speculated that defects in autophagic process in HSCs leads to accumulation of malfunctioning mitochondria that produce a metabolic phenotype close to the one observed in the *Lkb1* conditional knockout models.

The increased cell-death in HSCs with defective mitochon-dria could lead to the hypothesis that impaired oxidative phosphorylation induces the escape of HSCs from the quiescent status but the failure in their commitment, finally resulting in apoptosis (Fig. 2). This hypothesis has been explored in the recent study, where mitochondrial respiratory activity was impaired by conditional deletion of the gene *Ptpmt1* (*Pten-like mitochondrial phosphatase*). This gene encodes for a mitochondrial Pten-like protein tyrosine phosphatase. Ptpmt1-targets have not been extensively characterized, but its deletion has been reported to reduce the intracellular ATP synthesis in immortalized insulin producing cells (Pagliarini et al., 2005). Conditional knockout of *Ptpmt1* results in reduction of oxygen consumption rate in hematopoietic progenitors (LSK, Lin^{neg}Sca-1$^+$c-Kit$^+$) and an increase in the extracellular acidification rate (used as readout of aerobic glycolysis). *Ptpmt1*-deletion causes the loss of HSCs quiescence with consequent expansion of the HSC population, but impairs their ability to form colonies in differentiating conditions (Yu et al., 2013). Though further studies will measure apoptosis levels in this model, these observations support that defective mitochondrial activity results in loss of HSC quiescence and impaired differentiation process.

Mitochondria in cell fate determination

As previously mentioned, the balance between self-renewal and multi-potent differentiation in HSCs is critical for correct hematopoietic homeostasis. In addition to symmetric division and symmetric commitment, the existence of asymmetric division of HSCs has been suggested; paired daughter cell assays can track different destinies of the corresponding daughter cells from the same parent cell that is purified from HSC fraction both in mouse (Suda et al., 1984; Leary et al., 1985; Ito et al., 2012) and human HSCs-enriched fractions (Giebel et al., 2006). More complex resides in the molecular definition of the symmetry or asymmetry. Three hypothetical models have been proposed; the cell fate choice in the daughter cells is 1) accidentally obtained after the division, 2) depends on post mitotic extrinsic mechanisms as adhesion to
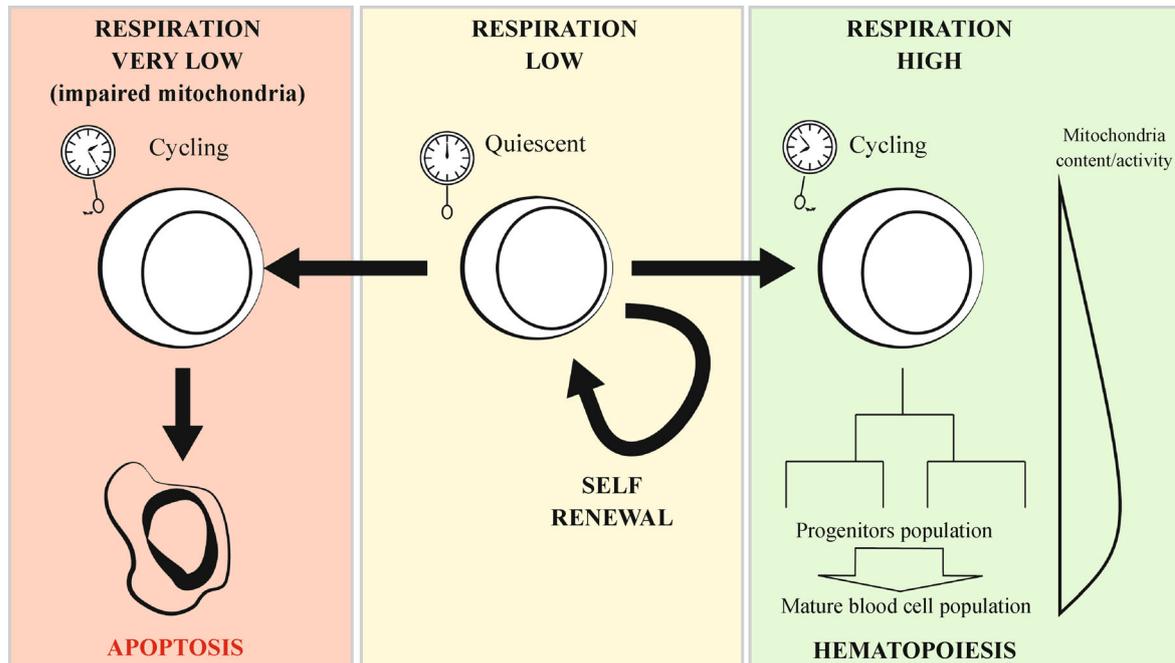
Figure 2 Schematic representation of mitochondria activity and the consequent cellular respiration on cellular state of a long-term hematopoietic stem cell.

the niche, or 3) is induced by the symmetric or asymmetric segregation of intracellular materials from the mother cell in a sort of molecular imprinting (Morrison and Kimble, 2006; Schroeder, 2007).

One of the initial evidence for asymmetric segregations of material in human HSCs compartments was from the study of Beckmann and coworkers, and they observed the asymmetric distribution of four different surface markers (CD53, CD62L/ L-selectin, CD63/lamp-3, and CD71/transferrin receptor) in cells fixed in late mitotic phase (Beckmann et al., 2007). In the same year, using an analogous approach, another groups demonstrated that Numb displays uneven repartition in mouse hematopoietic progenitor cells (LSK) (Wu et al., 2007). In 2012, Ting and coworkers observed the clear asymmetric segregation of Ap2a2, an adaptor protein involved in AP-2 clathrin-mediated endocytosis of trans-membrane receptors, using CD150$^+$CD48$^-$ mouse HSC-enriched fraction (Ting et al., 2012). Ap2a2 enhances HSC activity both *in vitro* and *in vivo* without expanding HSC population, suggesting that it could preferentially stimulate the self-renewal divisions of HSCs. Should be noted that authors reported the asymmetric Ap2a2 segregation even in presence of symmetric Numb distribution. A more recent paper further provided the live imaging of asymmetric Numb segregation in HSCs. Numb segregation depends on Lis1, also known as Pafah1b1, a dynein binding protein that anchors the mitotic spindle to the cellular cortex. Authors also showed that loss of *Lis1* leads to accelerated HSC maturation and impairs correct development of the hematopoietic system (Zimdahl et al., 2014).

We have recently proposed the intriguing concept that mitochondrial activity might regulate the division balance of HSCs (Ito et al., 2012). In this project, we used an immunophenotyping assay to characterize the symmetry of daughter cells after HSC division. Loss of the *Peroxysome proliferatior-activate receptor delta* (*Ppard*) causes a reduction of frequency of the asymmetric division with an increase of the symmetric commitment. The ablation of *Ppard* reduces the reconstitution capacity, while its pharmacological activation promotes *in vivo* reconstitution capacity and restores the division balance of *Ppard*-deleted HSCs. PPARδ is a member of nuclear receptor family and an important regulators of fatty acid oxidation (FAO) (Takahashi et al., 2007). FAO is the catabolic decomposition of long chain fatty acid to "two carbon bricks" represented by acetyl-CoA. Acetyl-CoA is transported into mitochondria and feed the TCA cycle to promote respiration and ATP production or to provide carbons for other biochemical processes. Intriguingly, when a pharmacological inhibition of FAO is induced by exposure to Etomoxir (inhibitor of Carnitine Palmytoyltransferase I, a key enzyme in the FAO process), the frequency of symmetric commitment is increased while asymmetric division is decreased. The consequent exhaustion of stem cell poolwas observed in BMT experiment showing a reduced capacity of HSCs treated with Etomoxir (Ito et al., 2012). These data support the FAO's role to control the cell fate of HSCs and thus their self-renewal capacity, although the further examinations are still needed to understand the precise mechanisms of FAO regulating the division balance of HSCs.

PPARδ was further associated to the promyelocytic

leukemia (PML) tumor-suppressor protein, which is also implicated in HSC maintenance (Ito et al., 2008; Carracedo et al., 2013). Loss of *Pml* leads to reduction in FAO activity, and, interestingly, *Pml* knockout HSCs also displayed similar alteration in division proportions to *Ppard*-deleted HSCs, and the impaired reconstitution capacity of *Pml* knockout HSCs was partially recovered by the pharmacological activation of PPARδ with the exposure to the PPARδ agonist GW501516. Even though mitochondrial activity has not been directly explored yet, the tight connection between mitochondria and FAO implies an essential roles or mitochondria in correct maintenance of HSCs through proper control of their division balance.

Concluding remarks

HSCs are the source for all hematopoietic cells in adult mammalians, through tight regulation of quiescence, division and commitment balance. The mechanisms regulating their maintenance or engagement of differentiation are under intense study, since are considered as potential target for the control of hematological disorders. Mitochondria have been recently highlighted as one of the most interesting regulators fine-tuning HSCs function, due to recent observations about critical roles of metabolism in HSCs homeostasis and hematopoiesis.

A series of interesting studies reviewed in this work underline mitochondria's roles in both HSC quiescence and differentiation process. Quiescent HSCs are dependent on glycolysis while keeping cellular respiration at low levels, that is consistent with the hypoxic properties of the niche in which reside. This phenomenon is assisted by the master regulator of hypoxia, Hif-1α. Coherently, activation of mitochondrial respiration induces HSCs to exit from their quiescence and to engage the commitment process. However, mitochondrial respiration could not be simply suppressed, as pathways controlling FAO also contributes HSC maintenance. Indeed, impairment of mitochondrial respiration (as repetitively demonstrated by the use of conditional knockout animal models) induces HSCs to exit their quiescent state and to engage apoptotic cell death possibly because they are unable to complete their differentiation process.

HSC resides in a hypoxic niche within the bone marrow. It was recently observed that the most quiescent HSC are in close association with small arterioles preferentially found in endosteal bone marrow, in a region called arteriolar niche (Kunisaki et al., 2013). Intriguingly recent measurements of local oxygen concentration within the bone marrow suggest that the oxygen levels in proximity of arteriole is higher than the average of marrow and decrease progressively moving closer to the sinusoids (Spencer et al., 2014). These new findings suggest that a minimum amount of oxygen is required for maintenance of HSC quiescence and support the idea that proper mitochondrial activity is necessary for HSC

quiescence in their niche.

Proposed mechanisms for the regulation in number of functional HSCs is to control HSC fate decision through division balance control. Evidences indicate that mitochondrial activity could participate in determining division pattern of HSCs (Ito et al., 2012). To date, asymmetric segregation of mitochondria has been observed in yeast (Higuchi et al., 2013), insect gonial cells (Staiber, 2007) and mouse oocyte (Dalton and Carroll, 2013), but it has not been extensively tested in mouse or human HSC yet. A possible role for mitochondria as cell fate determinants was proposed by Mantel and coworkers (Mantel et al., 2010), that observed high correlation between mitochondrial mass (measured by the mitotracker) with the marker CD34 in LSK population. CD34[Hi] LSK (ST-HSC) has higher mitochondrial mass compared to CD34[Lo] (LT-HSC). Since a net separation was observed in mitotracker intensity between LT- and ST-HSCs, they proposed that the CD34[Hi]Mitotracker[Hi] LSK can be generated from the division of a CD34[Lo]Mitotracker[Lo] LSK through the asymmetric segregation of mitochondria. This is just a speculation and this idea will be proved by further analyses, and additional stemness marker(s) will be required to obtain data selective for the LT-HSC population.

Investigation of metabolic roles in stem cells has only just begun. Further studies will more clarify the detailed mechanisms on the control of hematopoiesis by mitochondria and if the mitochondria act as cell fate determinants of HSCs. Nonetheless a clarifying picture is now being drawn, unequivocally identifying the role of mitochondria in HSC function.

Compliance with ethics guidelines

Massimo Bonora, Paolo Pinton and Keisuke Ito declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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