Molecular Aspects of Medicine 77 (2021) 100923

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Immunometabolic control of hematopoiesis

George Hajishengallis [a,*], Xiaofei Li [a,**], Triantafyllos Chavakis [b,c,d,***]

[a] Laboratory of Innate Immunity and Inflammation, Penn Dental Medicine, Department of Basic and Translational Sciences, University of Pennsylvania, Philadelphia, PA, United States
[b] Institute for Clinical Chemistry and Laboratory Medicine, Faculty of Medicine, Technische Universität Dresden, Dresden, Germany
[c] Centre for Cardiovascular Science, Queen's Medical Research Institute, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, United Kingdom
[d] National Center for Tumor Diseases (NCT), Partner Site Dresden, Dresden Germany, and German Cancer Research Center (DKFZ), Heidelberg, Germany

ARTICLE INFO

Keywords:
Bone marrow
Hematopoietic stem cells
Metabolism
Trained immunity/myelopoiesis
Clonal hematopoiesis
Aging

ABSTRACT

Hematopoietic stem cells (HSC) lie at the center of the hematopoiesis process, as they bear capacity to self-renew and generate all hematopoietic lineages, hence, all mature blood cells. The ability of HSCs to recognize systemic infection or inflammation or other forms of peripheral stress, such as blood loss, is essential for demand-adapted hematopoiesis. Also of critical importance for HSC function, specific metabolic cues (e.g., associated with changes in energy or O_2 levels) can regulate HSC function and fate decisions. In this regard, the metabolic adaptation of HSCs facilitates their switching between different states, namely quiescence, self-renewal, proliferation and differentiation. Specific metabolic alterations in hematopoietic stem and progenitor cells (HSPCs) have been linked with the induction of trained myelopoiesis in the bone marrow as well as with HSPC dysfunction in aging and clonal hematopoiesis of indeterminate potential (CHIP). Thus, HSPC function is regulated by both immunologic/inflammatory and metabolic cues. The immunometabolic control of HSPCs and of hematopoiesis is discussed in this review along with the translational implications thereof, that is, how metabolic pathways can be therapeutically manipulated to prevent or reverse HSPC dysfunction or to enhance or attenuate trained myelopoiesis according to the needs of the host.

1. Introduction

Hematopoiesis is a hierarchical system at the top of which lie the hematopoietic stem cells (HSC), which have self-renewal and multilineage differentiation capacity. HSCs can thus continuously replenish the hematopoietic system with progressively committed progenitor cells and differentiated, mature cells, throughout an organism's lifespan (Jagannathan-Bogdan and Zon, 2013). LT-HSCs can differentiate into short-term HSCs (ST-HSCs) and multi-potent progenitors (MPPs). The latter have relatively restricted differentiation potential. Together, the aforementioned populations comprise the so-called 'hematopoietic stem and progenitor cells' (HSPCs). MPPs further differentiate into progenitors, committed towards lymphoid or myeloid cells, for instance, common lymphoid progenitors (CLP), common myeloid progenitors (CMPs), granulocyte–monocyte progenitors (GMPs) and others, which give rise to all mature cell types in the blood (Iwasaki and Akashi, 2007). During the development of the fetus, prior to the formation of mature bone marrow (BM), significant hematopoiesis occurs in the liver (Ciriza et al., 2013). In this review, we will focus on hematopoiesis in the adult BM.

The equilibrium between HSC self-renewal and differentiation along multiple lineages is a central process in blood cell homeostasis. Quiescent HSCs are able to respond to stress, such as severe infections, systemic inflammation, or metabolic and other types of stress, by increasing their proliferation rate and adapting hematopoiesis output (Laurenti and Gottgens, 2018). Different HSC states, namely quiescence, proliferation, and differentiation, have different metabolic demands and accordingly mitochondrial functions (Papa et al., 2019) and exhibit distinct gene

* Corresponding author. Laboratory of Innate Immunity and Inflammation, Penn Dental Medicine, Department of Basic and Translational Sciences, University of Pennsylvania, Philadelphia, PA, United States
** Corresponding author. Laboratory of Innate Immunity and Inflammation, Penn Dental Medicine, Department of Basic and Translational Sciences, University of Pennsylvania, Philadelphia, PA, United States
*** Corresponding author. Institute for Clinical Chemistry and Laboratory Medicine, Faculty of Medicine, Technische Universität Dresden, Dresden, Germany
E-mail addresses: geoh@upenn.edu (G. Hajishengallis), xiaofeil@upenn.edu (X. Li), Triantafyllos.Chavakis@uniklinikum-dresden.de (T. Chavakis).

https://doi.org/10.1016/j.mam.2020.100923
Received 11 September 2020; Received in revised form 24 October 2020; Accepted 30 October 2020
Available online 5 November 2020

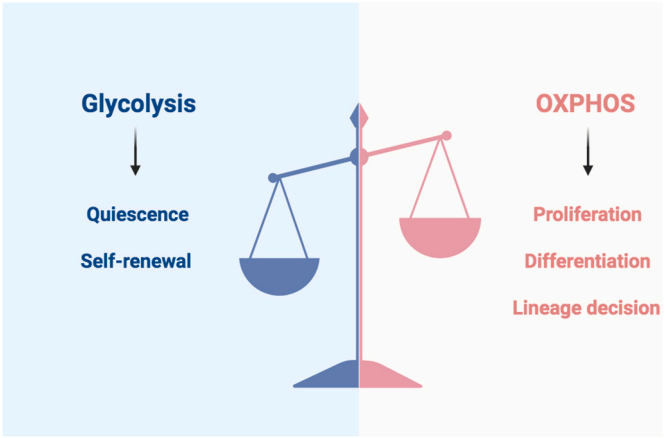

Fig. 1. The balance between glycolysis and oxidative phosphorylation (OXPHOS) and their functional outcomes in HSCs. Glycolysis promotes HSC quiescence and self-renewal, whereas OXPHOS favors HSC proliferation, differentiation and lineage decision.

expression profiles and epigenetic landscapes (Cedar and Bergman, 2011; Macarthur et al., 2009). In this regard, multiple studies have shown that specific metabolic cues are critical regulators of HSC fate decisions, such as, self-renewal versus proliferation and differentiation into specialized cell types (Ito and Suda, 2014).

Metabolic and epigenetic alterations in HSC and progenitor cells also have been identified as contributors to HSPC dysfunction in the context of aging and clonal hematopoiesis of indeterminate potential (CHIP) (Lee et al., 2020; Verovskaya et al., 2019). In addition, emerging evidence highlighted the cardinal role of metabolic adaptations in HSPCs in driving trained immunity-associated myelopoiesis (designated trained myelopoiesis hereafter) in the BM (Chavakis et al., 2019; Mitroulis et al., 2018). The maintenance and function of HSCs is facilitated by cells of a specialized microenvironment in the BM, the HSC niche. The niche consists of endothelial cells, osteolineage cells and specialized mesenchymal stromal cells, for instance, leptin-receptor-expressing cells or cells with high CXCL12 production (designated CXCL12-abundant reticular cells) (Crane et al., 2017; Ho and Mendez-Ferrer, 2020; Mitroulis et al., 2020; Pinho and Frenette, 2019). Such niche cells may orchestrate the environmental properties of the HSC niche that in turn regulate HSPC metabolism and hence their function and fate decisions. Additionally, alterations in the BM adipose tissue in the context of metabolic disease may affect the abundance of nutrients, such as fatty acids in the BM, and thereby HSPC metabolism (Li et al., 2019). However, as the main focus of our present review is the immunometabolic cell-intrinsic facets of the regulation of HSPCs and hematopoiesis, the reader is referred to recent reviews with regards to the role of the BM niche (Crane et al., 2017; Ho and Mendez-Ferrer, 2020; Mitroulis et al., 2020; Pinho and Frenette, 2019).

Taken together, in this review, we will discuss recent insights into the immunometabolic control of hematopoiesis, including the metabolic plasticity of the HSCs that facilitates their cell fate transitions, metabolic regulation of trained immunity at the HSC level, as well as how aging-related alterations in the metabolic and epigenetic activity of HSCs can precipitate their functional decline. We additionally discuss potential translational implications, such as how metabolic regulators can be therapeutically targeted to prevent or reverse HSC dysfunction.

2. Metabolic plasticity of the HSCs

Flexibility in energy metabolism, i.e., to switch between glycolysis and oxidative phosphorylation (OXPHOS), allows HSCs to adopt different cell fates, including quiescence to limit potential stress damage, self-renewal to maintain the stem cell pool, as well as lineage commitment and differentiation for replenishing the hematopoietic system with mature blood cells (Kohli and Passegue, 2014) (Fig. 1). Quiescent and self-renewing HSCs have relatively low energetic demands and utilize glycolysis to generate energy, perhaps reflecting an adaptation to the hypoxic BM environment. Metabolic transition to mitochondrial OXPHOS, and hence high ATP utilization and generation of reactive oxygen species (ROS), is linked to HSC lineage commitment and differentiation (Ito and Ito, 2018) (Fig. 1).

2.1. The complex metabolic regulation of HSC function: quiescence, lineage commitment and differentiation

HSCs are able to adapt to the changing demands of the blood system at the organismal level to cope with diverse infections, acute and chronic inflammation, bleeding and various other types of stress (Chavakis et al., 2019; Mitroulis et al., 2020). As alluded to above, demand-adapted HSCs have metabolic plasticity which enables them to switch between glycolysis and mitochondrial OXPHOS, facilitating transition from quiescence/slow cycling to an activated state. Generally, quiescent HSCs have long-term (LT) hematopoiesis reconstitution potential (hence known as LT-HSCs) and are more resistant than actively cycling HSCs to cytotoxic or genotoxic stress (UV or ionizing radiation, ROS and chemicals) (Ito et al., 2004), DNA damage checkpoint-dependent apoptosis (Mohrin et al., 2010) and replication error-induced programmed cell death (Flach et al., 2014). Actively cycling HSCs exhibit only short-term (ST) reconstitution potential, hence designated ST-HSCs.

The location of LT-HSCs within the hypoxic niche of the BM may determine their hypoxic character and metabolic status (Simsek et al., 2010). The majority of LT-HSCs engage preferentially in glycolysis for their survival and display low mitochondrial activity. Such glycolysis-dependent LT-HSC have enhanced long-term-engraftment, as compared to HSCs with high mitochondrial activity (Simsek et al., 2010). Consistently, self-renewal of HSCs can be promoted by reducing their mitochondrial activity of HSCs via uncoupling the electron transport chain (Vannini et al., 2016). These findings highlight an important feature of self-renewing HSCs, which is their capacity to reduce mitochondrial respiration, allowing them to be maintained in a quiescent state.

The metabolic state of LT-HSCs is orchestrated by the activation of hypoxia-inducible factor-1 (HIF-1)alpha (Simsek et al., 2010), which acts as the essential transcription factor that mediates metabolic adaptations to hypoxic states (Majmundar et al., 2010). The stability and activity of the HIF-1alpha subunit is regulated by various post-translational modifications, leading to HIF-1alpha degradation in normoxia via the ubiquitin-proteasome pathway (Epstein et al., 2001). In hypoxia, the HIF-1alpha subunit is stabilized and HIF-1alpha: HIF-1beta heterodimers are formed that recognize hypoxia response elements (HREs) to regulate target genes in diverse biological pathways (Semenza, 2010). HIF-1 mRNA and protein are highly abundant in LT-HSCs; HSCs derived from conditional HIF-1alpha knockout mice display impaired capacity for BM reconstitution during serial BM transplantations (Takubo et al., 2010). Moreover, in the absence of HIF-1alpha, LT-HSCs enter the cell cycle, proliferate, and display decreased tolerance to stress stimuli or conditions, such as, 5-fluorouracil or aging (Takubo et al., 2010). Thus, HIF-1alpha plays a major role in the maintenance of HSC quiescence and stress resistance.

Pyruvate deriving from glycolysis can be converted into lactate by lactate dehydrogenase A (LDHA) or decarboxylated into acetyl-CoA by the mitochondrial pyruvate dehydrogenase (PDH). In hypoxia, HSCs depend on HIF-1 for upregulating genes encoding glucose transporters, such as glucose transporter 1 (GLUT1), and enzymes involved in glucose metabolism, such as LDHA or pyruvate dehydrogenase kinase 1 (PDK1), and therefore for increased flux through the glycolytic pathway (Iyer et al., 1998). Thus, in hypoxia, HIF-1-dependent transcription activation of PDK1 inactivates the catalytic subunit of PDH, thereby preventing engagement of the Krebs tricarboxylic acid (TCA) cycle and promoting

utilization of anaerobic glycolysis as a source of energy (Takubo et al., 2013). This can also be achieved pharmacologically by suppressing the activity of PDH with an inhibitor, such as, 1-aminoethylphosphinic acid (1-AA) (Takubo et al., 2013). Moreover, the role of two enzymes involved in glycolysis, the M2 isoform of pyruvate kinase (PKM2) and of LDHA for HSC function was compared. PKM2 was found to be dispensable for hematopoiesis under homeostatic conditions and PKM2-deficient hematopoietic progenitors had only a moderate decrease in long-term reconstitution capacity in the context of regeneration conditions (Wang et al., 2014). On the other hand, deletion of LDHA resulted in reduced HSC maintenance and a substantial compromise in the long-term reconstitution capacity of HSCs due to enhanced mitochondrial respiration and ROS production. Consistently, antioxidant treatment partially reversed the HSC defects due to LDHA deficiency (Wang et al., 2014). Additionally, the PKM2 activator, DASA10, significantly increases PKM2 levels and reduces ROS accumulation in LSK under stress conditions (*e.g.*, lead exposure) (Cai et al., 2019).

Although mitochondria are rather inactive in HSCs (Filippi and Ghaffari, 2019; Simsek et al., 2010), these organelles are abundant in HSCs and can regulate HSC differentiation and lineage commitment (Filippi and Ghaffari, 2019). In comparison with the quiescent state, HSCs require higher energy inputs for differentiation and lineage commitment and, therefore, undergo metabolic rewiring from glycolysis to OXPHOS, which is a more efficient ATP generation pathway (~36 mol ATM from 1 mol glucose as opposed to 2 mol ATP from 1 mol glucose in anaerobic glycolysis) (Folmes et al., 2012). This metabolic switch to meet the higher energy demands of differentiating cells depends on adaptations of the mitochondrial network, including mitochondrial structural remodeling, increased mitochondrial DNA replication, upregulation of enzymes of the TCA cycle and of electron transport chain subunits, as well as the downregulation of glycolytic enzymes (Folmes et al., 2012; Kohli and Passegue, 2014). Cytokines and growth factors that force the HSCs out of quiescence and into cell division drive also an increase in both mitochondrial mass and membrane potential, associated with enhanced NADH levels and glucose uptake (Ho et al., 2017). Seahorse metabolic analyses assessing oxygen consumption rate (OCR) also confirmed remarkably elevated OXPHOS in activated HSCs, as compared to quiescent HSCs (Ho et al., 2017). Deficiency of the negative regulator of OXPHOS mitochondrial carrier homologue 2 (MTCH2), enhances mitochondrial OXPHOS and mitochondrial size and drives HSPCs to enter the cell cycle, leading them to exhaustion (Maryanovich et al., 2015). Additionally, initiation of HSC division is associated with enhanced intracellular Ca^{2+} levels and increased mitochondrial membrane potential. By reducing intracellular Ca^{2+} levels, HSC cell division is prolonged and their maintenance is increased (Umemoto et al., 2018). Moreover, hematopoietic cell-specific deficiency of PTPMT1, a PTEN-like mitochondrial phosphatase, led to failure of hematopoiesis due to a differentiation block in HSCs and consequent HSC pool expansion. PTPMT1 deficiency leads to defects in mitochondrial respiration and increased glycolysis. Mechanistically, the accumulation of phosphatidyl inositol phosphates (PIP), which are normally dephosphorylated by PTPMT1, resulting in enhanced UCP2 activity, underlies the mitochondrial defect (Yu et al., 2013). Mitochondrial respiration may differentially regulate proliferation and differentiation of HSCs, as exemplified by the function of the mitochondrial complex III subunit Rieske iron-sulfur protein (RISP) (Anso et al., 2017). RISP-deficiency in fetal HSCs resulted in impaired mitochondrial respiration and decreased differentiation, without affecting their proliferation, thereby resulting in anemia, while disruption of RISP in adult HSCs led to pancytopenia and death (Anso et al., 2017). Interestingly, mitochondria may also affect lineage bias of HSCs. The regulator of mitochondrial fusion mitofusin 2 (Mfn2) controls the maintenance of lymphoid-rather than myeloid-biased HSCs (Luchsinger et al., 2016).

As alluded to above, activation of HSCs is accompanied by a switch from aerobic glycolysis to high mitochondria activity and increased OXPHOS. However, the remodeling of the mitochondrial network is irreversible in replicating HSCs, leading to accumulation of dysfunctional mitochondria, associated with loss of function of the regulator of mitochondrial fission, Drp1. This disrupted mitochondrial morphology is not reversed when HSCs re-enter quiescence, which accounts for the reduced regenerative capacity of HSCs with high division history, representing a sort of HSC divisional memory (Hinge et al., 2020).

Although glycolysis plays a critical role in the quiescence of HSCs and their decision to undergo self-renewal rather than differentiation (Fig. 1), the diversion of glucose to the pentose phosphate pathway for nucleotide biosynthesis, which is promoted by erythropoietin, is crucial for HSC commitment to the erythroid lineage (Oburoglu et al., 2014). Moreover, the erythroid lineage specification of HSCs is entirely dependent upon glutamine transport and metabolism. Specifically, when this process is blocked (*e.g.*, by knocking down the ASCT2 glutamine transporter), HSCs differentiate into myelomonocytic fates, even in the presence of erythropoietin (Oburoglu et al., 2014). Moreover, the accumulation of cholesterol in the cell membrane (which thereby displays altered physicochemical properties), owing, for instance, to deficiency in cholesterol exporters, such as adenosine triphosphate-binding cassette (ABC) transporters, results in increased cell-surface expression of the common β-subunit of the receptor for IL-3 and GM-CSF (IL-3Rβ) and enhanced proliferation and myeloid skewing in HSCs (Yvan-Charvet et al., 2010). These findings further underscore that specific metabolic parameters contribute critically to HSC lineage specification.

The exit of HSCs from quiescence and entry into the cell cycle is also associated with the choice between asymmetric and symmetric division, which is central for HSC fate decisions. Symmetric division produces two identical daughter cells with the same fate, that is to either expand the HSC pool or give rise to lineage-committed cells. In contrast, asymmetric division produces two daughter cells with different cellular fates, promoting concomitantly self-renewal and the maintenance of the HSC pool as well as lineage differentiation (Knoblich, 2008). Fatty acid oxidation (FAO) plays an essential role in these fate decisions in HSCs. Indeed, inhibition of FAO in HSCs leads to loss of their capacity for asymmetric division, which is essential for HSC pool maintenance during stress conditions (e.g., myeloablation or systemic infection) (Ito et al., 2012). The peroxisome proliferator-activated receptor δ (PPAR-δ), which, amongst other functions, promotes mitochondrial FAO, acts as an important regulator of HSC maintenance; PPARδ gene deletion leads to HSC loss and accumulation of lineage committed progenitor cells (Ito et al., 2012).

As mentioned above, cytokines and growth factors that force the HSCs into cell division trigger also an increase in mitochondrial mass (Ho et al., 2017). Interestingly, the increase in mitochondrial mass and OXPHOS in HSCs upon infection is also caused by a rapid transfer from the stromal cells to HSCs, in a manner that involves the ROS-dependent opening of connexin channels. This mechanism allows for a rapid bioenergetics adaptation of HSCs and accelerates demand-adapted myelopoiesis toward coping with the infection (Mistry et al., 2019). Interestingly, such a process may also be operative in acute myeloid leukemia (AML), whereby ROS also trigger mitochondria transfer from bone marrow stromal cells to AML blasts, thereby increasing the survival of the latter (Marlein et al., 2017).

2.2. *Key metabolic signaling pathways governing HSC quiescence and cycling*

As a lipid and protein kinase, phosphatidylinositol 3-kinase (PI3K) can be activated downstream of diverse receptor tyrosine kinases, cytokine receptors or chemokine G protein–coupled receptors (Fruman et al., 2017). PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3), leading to the activation of PDK1 and the kinase AKT (Vanhaesebroeck et al., 2010). AKT in turn activates the mechanistic target of rapamycin (mTOR), which is a key sensor that integrates nutrient- and growth factor-derived cues to coordinate anabolic metabolism (Fruman et al.,

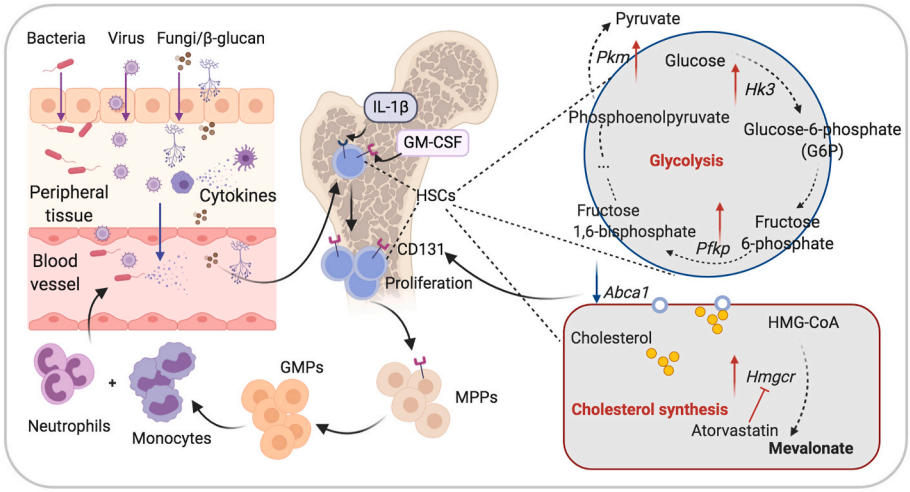

Fig. 2. Metabolic regulation of trained immunity in HSCs. HSCs sense peripheral inflammation or infection induced by bacteria, viruses, or fungi (or components thereof, such as the fungal-derived β-glucan, which is a prototypical trained-immunity-inducing agonist) and adapt by increasing proliferation and skewing toward the myeloid lineage. In β-glucan-trained mice, this agonist induces elevated innate immune mediators in the BM, such as, IL-1β and granulocyte-macrophage colony-stimulating factor (GM-CSF). HSCs from β-glucan-trained mice display upregulation of genes encoding key regulatory enzymes of the glycolytic pathway, e.g., hexokinase 3 (*Hk3*), phosphofructokinase, platelet (*Pfkp*) and pyruvate kinase (*Pkm*), as well as upregulation of genes in the cholesterol biosynthesis pathway, such as the gene encoding 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*). In contrast, HSCs from β-glucan-trained mice exhibit downregulation of the gene encoding ATP-binding cassette transporter A1 (*Abca1*), which promotes cholesterol efflux. The HMG-CoA reductase inhibitor, atorvastatin, inhibits the trained immunity-induced HSPC expansion. Increased cholesterol synthesis or downregulation of cholesterol efflux drive myeloid cell expansion by increasing HSPC expression of CD131, the common β-subunit of the receptor for IL-3 and GM-CSF.

2017). AKT can activate mTOR via direct phosphorylation and, indirectly, via inhibition of the negative regulator of mTOR, the tuberous sclerosis complex 2 (TSC2); these signaling events coordinate HSC growth, metabolism and autophagy (Meng et al., 2018). Constitutively active AKT signaling promotes accelerated proliferation and impaired engraftment of HSCs (Kharas et al., 2010), while AKT deletion (involving both AKT1 and AKT2 isoforms) leads to long-term functional defects of LT-HSCs, which are associated with increased quiescence (Juntilla et al., 2010). Inactivation of TSC1 leads to reduced quiescence of HSCs and their cell cycle entry, accompanied by enhanced mitochondrial biogenesis and higher ROS, resulting in decreased HSC self-renewal and hematopoiesis defects (Chen et al., 2008). Moreover, mTOR complex 1 (mTORC1)-associated signaling facilitates the translation of mitochondria-related transcripts and mitochondrial biogenesis in the context of erythropoiesis (Liu et al., 2017). Inhibition of PI3K-mTOR signaling by ncRNAs produced from the Dlk1-Gtl2 locus suppresses mitochondrial biogenesis in LT-HSCs, protecting them from ROS and promoting their function (Qian et al., 2016). Interestingly, the mTOR pathway may represent a therapeutic target in AML. Inhibition of mTORC1 and mTORC2 signaling reduced proliferation of AML blasts and promoted their apoptosis (Willems et al., 2012).

The forkhead transcription factor FOXO3 (also called FOXO3a) plays a crucial role for HSC self-renewal. HSCs deficient in FOXO3 had reduced potential for long-term repopulation of hematopoiesis, as revealed by competitive transplantation assays, and displayed defective quiescence, associated with elevated ROS levels (Miyamoto et al., 2007). Consistently, another study found that inactivation of FOXO3 leads to elevated ROS and enhanced DNA damage in HSPC. FOXO3 deficiency also induces defects in the DNA repair machinery of HSPC. Hence, FOXO3 is a gatekeeper of HSC genomic stability (Bigarella et al., 2017). Interestingly, FOXO3 links mitochondrial metabolism to HSC function. Along this line, FOXO3-deficient HSCs display defective long-term reconstitution capacity, associated with impaired mitochondrial metabolism (Rimmele et al., 2015).

The 5′ adenosine monophosphate-activated protein kinase (AMPK) senses the cellular energy status (specifically the AMP/ATP ratio). When ATP levels are low, this enzyme is switched on and activates catabolic processes (glucose and fatty acid uptake and oxidation and autophagy), while it inhibits ROS production, thereby promoting quiescence and self-renewal of HSCs (Kohli and Passegue, 2014). The ability of AMPK to increase resistance to oxidative stress depends, in large part, on its capacity to activate FOXO transcription factors, which induce autophagy. AMPK can also be activated under hypoxia, perhaps even prior to energy depletion, which is thought to serve as an early adaptation mechanism (Dengler, 2020). The tumor suppressor liver kinase B1 (LKB1) is a serine/threonine kinase that positively regulates AMPK activity (Nakada et al., 2010). HSCs with *Lkb1* deletion fail to mediate long-term reconstitution of hematopoiesis upon transplantation and show increased division leading to their rapid depletion and pancytopenia. Interestingly, HSCs show greater dependence on LKB1 for cell cycle regulation and survival than other hematopoietic cells (Nakada et al., 2010). These findings were corroborated by another study that also demonstrated a crucial role of LKB1 in HSC homeostasis. Inactivation of LKB1 decreased HSC quiescence and survival, resulting in their exhaustion and decreased repopulation capacity, in a manner that was independent of mTORC1 and associated with dysregulated mitochondrial biogenesis. These two studies defined LKB1 as a major regulator of hematopoiesis (Gan et al., 2010). In conclusion, the LKB1–AMPK pathway acts as a metabolic checkpoint, which promotes cell growth arrest under conditions of low intracellular ATP levels and thereby HSC quiescence (Kohli and Passegue, 2014; Shackelford and Shaw, 2009).

Therefore, the functions of the LKB1–AMPK pathway contrast with those of the PI3K–mTOR pathway, which is activated under conditions of nutrient abundance, increases anabolic processes and ROS levels, and thereby promotes HSC proliferation and differentiation (Kohli and Passegue, 2014). Not surprisingly, the two opposing pathways are cross-regulated. The LKB1–AMPK pathway inhibits mTOR activity through AMPK phosphorylation of TSC2 and of regulatory associated protein of mTOR (Raptor) (Hardie, 2011; Shackelford and Shaw, 2009). Conversely, the PI3K–mTOR pathway antagonizes the LKB1–AMPK pathway through AKT inhibition of FOXO activity (Zhao et al., 2017).

3. Metabolic regulation of trained immunity at the HSC level

Emerging evidence points to the ability of HSPCs to sense peripheral inflammation or infection, either directly via their pattern recognition receptors, or indirectly via reacting to proinflammatory cytokines induced in response to infection. This process leads to HSC proliferation and expansion and enhanced differentiation toward the myeloid lineage (Chavakis et al., 2019). The inflammatory adaptation of HSPCs also

underlies the concept of trained innate immunity, which can thus be initiated in the BM and contribute to the generation of 'trained' or hyper-reactive myeloid progeny (Christ et al., 2018; Kaufmann et al., 2018; Mitroulis et al., 2018; Penkov et al., 2019). Systemic treatment of mice with fungal-derived β-glucan, a prototypical trained-immunity-inducing agonist, induces the expansion of myeloid-biased CD41$^+$ LT-HSCs and progenitors of the myeloid lineage, in a manner dependent on IL-1β- and granulocyte-macrophage colony-stimulating factor (GM-CSF)–mediated signaling, and on elevated glucose metabolism and cholesterol biosynthesis (Mitroulis et al., 2018). Specifically, RNA sequencing (RNA-seq) of LT-HSCs after β-glucan administration showed significant upregulation of genes encoding key regulatory enzymes of the glycolytic pathway (*Hk3, Pfkp*, and *Pkm*) and of the rate-limiting enzyme of the pentose phosphate pathways (*G6pdx*). Moreover, increased extracellular acidification rate (ECAR) was observed in BM progenitor cells from β-glucan-treated mice as compared to control mice. Consistently, co-treatment of β-glucan-administered mice with 2-Deoxy-D-glucose (2-DG), a glycolysis inhibitor, inhibited the β-glucan-dependent expansion of HSPCs, leading to reduced numbers of GMPs and decreased proportion of GMPs among myeloid progenitors, as compared to β-glucan administration alone (Mitroulis et al., 2018) (Fig. 2).

Cellular cholesterol homeostasis and the mevalonate pathway are also involved in β-glucan-induced trained innate immunity. Indeed, the genes encoding (i) the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (*Hmgcr*), which catalyzes the conversion of HMG-CoA to mevalonate that is required for cholesterol biosynthesis, and (ii) the low-density lipoprotein (LDL) receptor (*Ldlr*), which regulates LDL uptake in LT-HSCs, were significantly upregulated, whereas (iii) the gene encoding ATP-binding cassette transporter A1 (*Abca1*), which mediates cholesterol efflux, was significantly downregulated in LT-HSCs from β-glucan-treated mice (Mitroulis et al., 2018). Therefore, HSC-based trained immunity induced by β-glucan appears to increase the demand for cell cholesterol as well as promoting its biosynthesis and retention in LT-HSCs. In line with this, the HMG-CoA reductase inhibitor atorvastatin reduced the numbers of LSKs and MPPs and decreased the frequency of GMPs in β-glucan-trained mice, as compared to β-glucan administration alone (Mitroulis et al., 2018) (Fig. 2).

Antibody-mediated blockade of GM-CSF in mice trained with β-glucan also reduced the numbers of hematopoietic progenitors, as compared to β-glucan injection alone. Interestingly, the disruption of cholesterol efflux drives myeloid cell expansion by increasing, in LT-HSCs and MPPs, the surface expression of CD131, which is the common β-subunit of the receptor for IL-3 and GM-CSF (IL-3Rβ) (Murphy et al., 2011; Yvan-Charvet et al., 2010). Consistent with these earlier studies, treatment of mice with β-glucan resulted in significant increase in CD131$^+$ LSKs, CD131$^+$ MPPs, and CD131$^+$ LT-HSCs and enhanced signaling downstream of CD131, as compared to control-treated mice (Mitroulis et al., 2018). Therefore, the myeloid bias caused by β-glucan-induced trained immunity is causally associated with changes in lipid metabolism that promote increased frequency of CD131-expressing HSPCs. Overall, the effects of β-glucan-induced trained immunity on HSPCs are associated with an integral network of IL-1β signaling, increased glycolysis and cholesterol biosynthesis, and upregulation of the GM-CSF/CD131 axis (Mitroulis et al., 2018) (Fig. 2).

Through inflammatory signals, the gut microbiota promotes myelopoietic activity in the BM to a level sufficient for successful control of systemic infection with invading pathogens (Khosravi et al., 2014). This protective primed state of the BM associated with the presence of the gut microbiota might involve HSPC training although this possibility was not addressed. On the flip side, the gut microbiota also promotes the number of aged neutrophils in the circulation, which can exacerbate inflammation-induced organ damage as in the context of septic shock (Zhang et al., 2015a). In what appears to be a homeostatic mechanism to mitigate such consequences, aged neutrophils upregulate the expression of the chemokine receptor CXCR4 to home back to the BM for efferocytic clearance by resident macrophages, a process that in turn promotes the circadian release of progenitor cells to the blood circulation (Casanova-Acebes et al., 2013).

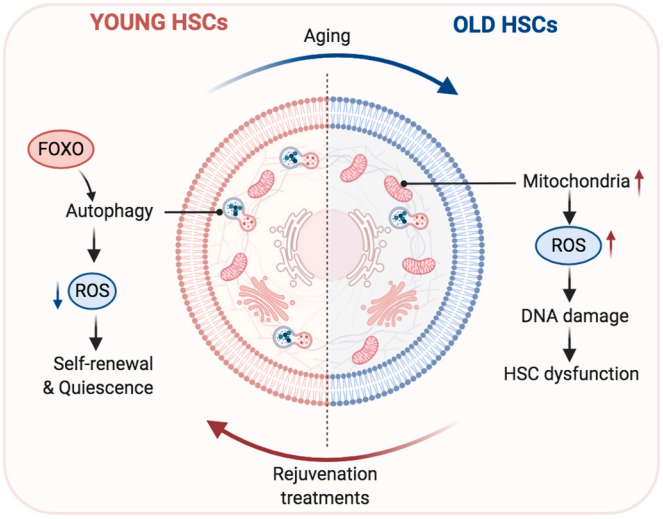

Fig. 3. The metabolic alterations in aging HSC and strategies for HSC rejuvenation. In young HSCs, FOXO promotes autophagy to clear mitochondria thus controlling the numbers of the mitochondria and suppressing ROS levels; this activity promotes the self-renewal and quiescence of HSCs. In contrast, old HSCs exhibit a relative higher level of oxidative metabolism and higher numbers of mitochondria associated with decreased autophagic activity, consequently leading to increased production of ROS and hence ROS-induced DNA damage and HSC dysfunction (e.g. diminished regenerative potential, impaired capability to return to quiescence and unbalanced blood cell production). However, the mTOR inhibitor (rapamycin), activators of AMPK (e.g., A769662, metformin and AICAR) and sirtuin 7 (SIRT7), a histone deacetylase involved in the regulation of mitochondrial metabolism and homeostasis, might be productively exploited therapeutically for HSC rejuvenation.

4. Metabolic alterations in the aging HSCs

Chronic stressful stimuli (such as, frequent infections, chronic inflammatory conditions or blood loss) that force HSCs to exit their homeostatic quiescent state can result in DNA damage and functional attrition of the HSCs, thereby contributing to accelerated aging of the hematopoietic and immune system (Walter et al., 2015). Although the number and frequency of HSCs in the BM of both mice and humans increases with aging, this quantitative increase does not compensate for the aging-associated functional defects of HSCs (Geiger et al., 2013). Thus, for instance, under regenerative stress associated with serial transplantations, aged HSCs exhibit diminished regenerative potential due to their defective long-term self-renewal capacity (Geiger et al., 2013). Moreover, aged HSCs show a bias towards increased myeloid lineage differentiation and decreased capacity for lymphoid differentiation (Dykstra et al., 2011). It should be noted that single-cell transcriptomic analysis revealed cellular heterogeneity regarding the onset of HSC aging, with the aged subpopulations exhibiting pro-proliferative JAK/STAT signaling and p53-associated functional decline (Kirschner et al., 2017). For a comprehensive review of aging-associated intrinsic alterations in HSCs, the reader is referred to a recent excellent review (Mejia-Ramirez and Florian, 2020). Here we will focus on salient metabolic alterations that affect the aging HSCs.

As alluded to above, upon activation, glycolytic and quiescent young HSCs shift towards oxidative metabolism, which can be efficiently reversed (towards glycolysis) as the cells return to quiescence. Although young HSCs generally have a low metabolic rate, aged HSCs exhibit a relative higher level of oxidative metabolism, accompanied by increased production of ROS and hence ROS-induced oxidative stress, an important cause of DNA damage and HSC dysfunction

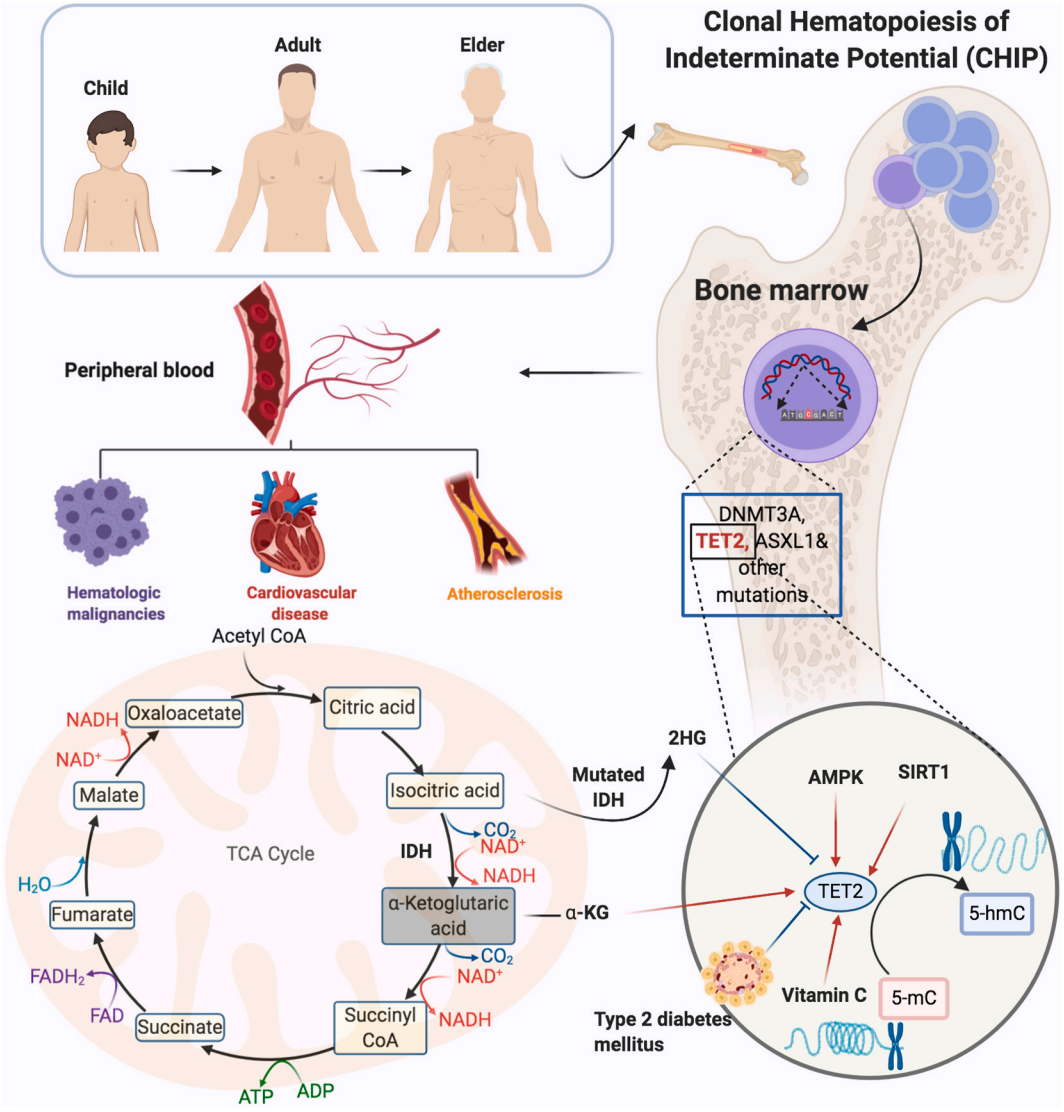

Fig. 4. Effect of metabolism on epigenetic enzymes affecting clonal hematopoiesis. Mutations in genes encoding the epigenetic regulators TET2 (ten-eleven translocation 2), DNMT3 (DNA nucleotide methyltransferase 3A), and ASXL1 (the addition of sex combs like 1) frequently appear during aging. These frequent mutations, together with other less frequent ones, characterize the aging-related phenomenon known as clonal hematopoiesis of indeterminate potential (CHIP). CHIP is associated with an increased risk of developing hematologic malignancies as well as cardiovascular disease and atherosclerosis. TET2, which promotes DNA demethylation by converting 5-methyl-cytosine (5-mc) to 5-hydroxymethyl-cytosine (5-hmc), plays a critical role in HSC maintenance. TET2 loss-of-function mutations in CHIP result in profound expansion of hematopoietic and myeloid progenitors. Vitamin C acts as a co-factor of Fe2(+) and α-KG-dependent dioxygenases, such as, TET2, and can enhance the activity of the functional TET2 protein from a nonmutated allele. The activity of TET2 is also regulated by the TCA cycle intermediate, α-ketoglutarate (α-KG), which is a required cofactor for TET2's dioxygenase function. Isocitrate dehydrogenase (IDH) converts citrate to α-KG but mutated IDH converts citrate to 2-hydroxyglutarate dehydrogenase (2-HG), which directly inhibits TET2 activity. AMPK phosphorylates and stabilizes TET2, but the activity of this enzyme is decreased in type 2 diabetes mellitus, which thus adversely affects TET2 and leads to decreased 5-hmc levels. The NAD-dependent deacetylase enzyme SIRT1 and vitamin C also upregulates the activity of TET2 in HSPCs and is considered as a therapeutic target for treating HSPC disorders.

(Geiger et al., 2013; Ho et al., 2017). HSCs with low levels of ROS maintain a higher self-renewal potential in serial transplantation (Jang and Sharkis, 2007). FOXO proteins are critical for the ability of HSCs to cope with physiologic oxidative stress and suppress ROS levels, and thus FOXO proteins foster HSC quiescence and enhanced survival as well as long-term regenerative potential (Tothova et al., 2007) (Fig. 3). Elevated level of ROS in HSCs from young adult animals, as seen in FOXO-deficient HSCs, result in phenotypes very similar to those seen in aging, for instance, defective long-term repopulating activity with myeloid skewed differentiation.

Autophagy, a lysosomal degradation pathway induced by metabolic stress, clears active mitochondria (mitophagy) and thus leads to suppression of ROS levels, thereby promoting quiescence and the regenerative capacity of HSCs. Conditional deletion of the essential autophagy gene *Atg7* in HSCs leads to increased numbers of mitochondria, elevated ROS production, increased proliferation and DNA damage (Mortensen et al., 2011). Autophagy is critical for the survival of old HSCs. HSCs express a FOXO3A-dependent gene network that primes them to induce autophagy rapidly under starvation conditions (Warr et al., 2013). Nevertheless, the majority of HSCs in aged mice display reduced level of autophagy, thus leading to an elevated metabolic state, which drives increased myeloid differentiation of HSCs at the expense of their self-renewal activity and regenerative potential (Ho et al., 2017). Consistently, autophagy-deficient young HSCs show an accelerated aging phenotype, characterized by loss of quiescence and preferential myeloid-biased differentiation (Ho et al., 2017). Autophagy is regulated by nutrient sensor pathways, such as mTOR, which inhibits autophagy, and AMPK, which activates autophagy. Thus, reduced autophagy in aged

HSCs could, in part, be attributed to the enhanced mTOR activity in HSCs from old mice as compared to HSCs from young mice (Chen et al., 2009). In line with this, mTOR activation through conditional inactivation of *Tsc1* (which encodes for a component of the TSC1/TSC2 complex that negatively regulates PI3K-mTOR signaling, as discussed above) in the HSCs of young mice mimics the phenotype of HSCs from aged mice; that is, there is a relative reduction in generation of lymphocytes and impaired capacity for hematopoiesis reconstitution in the context of transplantation (Chen et al., 2009). In contrast, the mTOR inhibitor rapamycin restores the self-renewal and hematopoietic capacity of HSCs in old mice (Chen et al., 2009). Therefore, rapamycin, which also increased the life-span of treated mice (Chen et al., 2009), appears to be a promising pharmacological agent for HSC rejuvenation. In addition to the hyperactivation of the PI3K-mTOR signaling pathway, cellular aging also involves disruption of AMPK activity (Khorraminejad-Shirazi et al., 2018). In this context, activators of AMPK (e.g., A769662, metformin, or 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside [AICAR]) have the potential to improve stem cell-based transplantation therapies in old age (Burkewitz et al., 2014; Khorraminejad-Shirazi et al., 2018) (Fig. 3).

A study using 'POLG mtDNA mutator' mice (a model based on proofreading-defective mitochondrial DNA polymerase) showed that accumulating mitochondrial DNA mutations in HSCs drive certain hematopoietic alterations (e.g., skewed myeloid differentiation at the expense of lymphoid differentiation) that mimic premature HSC aging, without, however, affecting the hematopoietic stem cell pool (Norddahl et al., 2011). Thus, although proper mitochondrial function is required for HSC homeostasis, mitochondrial DNA mutations can only partly account for the HSC aging phenotype as occurs physiologically.

It is becoming increasingly appreciated that the aging of HSCs may, in large part, entail dysregulation of cellular homeostatic programs. This notion suggests that reinstating such protective cellular programs could rejuvenate HSCs. For instance, sirtuin 7 (SIRT7), a histone deacetylase involved in the regulation of different cellular processes including mitochondrial activity (Ryu et al., 2014), might be productively exploited for HSC rejuvenation. Indeed, whereas SIRT7 was shown to promote HSC maintenance and prevent myeloid skewing, its expression is reduced in aged HSCs; importantly, however, the overexpression of SIRT7 improved the regenerative capacity of aged HSCs (Mohrin et al., 2015) (Fig. 3).

Furthermore, maintenance of HSC integrity requires clearance of HSCs exposed to stress, including ROS and related DNA damage. A strong activation of the unfolded protein response in human HSCs, but not in progenitors, drives the former towards apoptosis, thereby preventing the propagation of damaged HSCs (van Galen et al., 2014). Together, a complex network of mechanisms regulates homeostasis of young and old HSCs.

5. Influence of metabolism on epigenetic enzymes affecting clonal hematopoiesis

As discussed above, ROS-triggered DNA damage accumulation is a common characteristic of aging HSCs, although multiple mechanisms may in principle protect their genome, e.g., via enhancing DNA repair (Hakem, 2008). DNA damage, genomic instability, impaired autophagy and epigenetic and metabolic alterations are considered as important and interconnected intrinsic causes of premature aging of HSCs (Mejia-Ramirez and Florian, 2020; Pilzecker et al., 2017). HSCs from mice deficient in DNA repair components show reduction in self-renewal capacity and repopulation ability (Li et al., 2020; Rossi et al., 2007). A series of point mutations have been reported in humans as they age, such as, mutations in the epigenetic regulators TET2 (ten-eleven translocation 2), DNMT3 (DNA nucleotide methyltransferase 3A), and ASXL1 (the addition of sex combs like 1), which appear frequently even in healthy elderly. These frequent mutations, together with other less frequent ones, characterize the aging-related phenomenon known as clonal hematopoiesis of indeterminate potential (CHIP) (Dorsheimer et al., 2019; Fuster et al., 2017; Genovese et al., 2014; Jaiswal et al., 2014) (Fig. 4), which is briefly outlined below.

In old age, HSCs exhibit increased resistance to DNA damage-induced apoptosis in comparison with their young counterparts, and therefore 'old' HSCs may survive despite DNA damage (Gutierrez-Martinez et al., 2018). This allows the 'old' HSCs to accumulate mutations, which may lead to aging-associated hematopoietic alterations, including malignancies or other types of dysfunction (Gutierrez-Martinez et al., 2018). This aging-related, progressive accumulation of somatic mutations in HSCs that confers a proliferative advantage to the mutant cell, yielding clones of mutant leukocytes in the peripheral blood, is designated CHIP (Dorsheimer et al., 2019; Fuster et al., 2017; Genovese et al., 2014; Jaiswal et al., 2014). CHIP is associated with an increased risk of developing hematologic malignancies and cardiovascular disease/atherosclerosis and perhaps other inflammatory diseases (Chavakis et al., 2019; Steensma, 2018b). As mentioned above, CHIP-related mutations occur most frequently in three genes encoding for the epigenetic regulators *TET2*, *DNMT3*, and *ASXL1* (Buscarlet et al., 2017; Busque et al., 2012; Genovese et al., 2014; Jaiswal et al., 2014; Steensma, 2018a) (Fig. 4), which are discussed in greater detail below.

TET2 is a member of the Ten-Eleven Translocation (TET) family of enzymes, which are evolutionarily conserved dioxygenases responsible for catalyzing the conversion of 5-methyl-cytosine (5-mc) to 5-hydroxymethyl-cytosine (5-hmc), thereby promoting DNA demethylation (Tahiliani et al., 2009) (Fig. 4). In line with this, TET2 deficiency or inactivation causes DNA hypermethylation within active enhancer and promoter regions (Rasmussen et al., 2015; Yamazaki et al., 2015). Moreover, the ability of TET2 to oxidize mRNA is associated with regulation of emergency myelopoiesis (Shen et al., 2018).

TET2 plays a significant role in HSC maintenance. Indeed, conditional *Tet2* loss in the hematopoietic cells causes increased stem cell self-renewal *in vivo*, resulting in pronounced competitive advantage over time. Specifically, TET2 loss (e.g., loss-of-function mutations associated with CHIP) leads to a profound expansion of the hematopoietic and myeloid progenitors. Consistent with aberrant myelopoiesis, transcriptome analysis revealed that *Tet2*$^{-/-}$ LSK cells display a significant enrichment of a CMP-associated gene expression signature (Moran-Crusio et al., 2011). The competitive expansion advantage of *TET2*-mutant clones over normal HSC clones is particularly pronounced in an inflammatory environment, as in the setting of inflamm-aging (Cai et al., 2018; Kovtonyuk et al., 2016). In this context, systemic inflammation induced by TLR2 ligands or by disseminated bacteria (owing to disruption of the gut barrier) drive IL-6-dependent pre-leukemic myeloproliferation in *Tet2*$^{-/-}$ mice (Meisel et al., 2018).

Interestingly, patients with poorly controlled Type 2 diabetes mellitus (T2DM) have significantly lower 5-hmc levels in their circulating white blood cells as compared to well-controlled T2DM or healthy individuals (Pinzon-Cortes et al., 2017). In line with this, high level of glucose results in significant reduction in TET2 phosphorylation and enhanced TET2 turnover in vitro (Wu et al., 2018). This can be explained as follows. AMPK can directly phosphorylate TET2 at Ser99 (of the catalytic domain) and thereby stabilize TET2; however, the activity of AMPK is reduced in T2DM, hence adversely affecting TET2 (Wu et al., 2018). In other words, high glucose levels impair the ability of AMPK to phosphorylate and stabilize TET2, in essence mimicking the CHIP-related dysfunction of TET2. Consistent with this notion, preclinical studies showed that diabetes induces myelopoiesis and significantly increases atherogenic monocytes (Nagareddy et al., 2013). Importantly, metformin, a drug that is prescribed for T2DM and acts as an AMPK activator, promotes AMPK-mediated phosphorylation of the catalytic domain of TET2, thereby restoring TET2 stability and 5-hmc levels (Wu et al., 2018) (Fig. 4).

The NAD-dependent deacetylase enzyme SIRT1 also upregulates the activity of TET2 in HSPCs (Sun et al., 2018). The mechanism involves deacetylation of TET2 at conserved lysine residues in its catalytic domain. In HSCs associated with the myelodysplastic syndrome (MDS),

Table 1
Selected pharmacological compounds for potential therapeutic applications relevant to HSC function.

Compound	Target	Mechanisms	Therapeutic applications	References
Metformin AICAR[a] A769662	AMP kinase	Activate AMPK; improve regenerative capacity of HSCs, correct aging-related HSC dysfunction, restore TET2 stability.	Stem cell transplantation; HSCs aging therapies; CHIP-related conditions treatment	(Burkewitz et al., 2014; Khorraminejad-Shirazi et al., 2018; Wu et al., 2018)
Rapamycin	mTOR	Inhibits mTOR activity; restores the self-renewal and hematopoietic capacity of aging HSCs, promotes HSC rejuvenation.	HSC aging-related therapies	Chen et al. (2009)
1-AA[b]	Pyruvate dehydrogenase	Competes with pyruvate to suppress PDH enzymatic activity; promotes cell number, survival and transplantation capacity of LT-HSCs.	Stem cell transplantation	Takubo et al. (2013)
DASA10	Pyruvate kinase M2	Pyruvate kinase M2 activator; alleviates lead-induced HSCs aging.	HSCs aging-related therapies	Cai et al. (2019)
Statins	HMG-CoA reductase	Inhibit the activity of HMG-CoA reductase; inhibit *de novo* cholesterol synthesis and the mevalonate pathway, inhibit cholesterol-induced proliferation and mobilization of HSPCs.	Inhibit the cholesterol -induced effects on HSPCs	(Mitroulis et al., 2018; Oguro, 2019)
Resveratrol SRT3025	SIRT1[c]	Activate SIRT1; promote deacetylation and activation of TET2, increases the frequency and total numbers of normal HSCs in the BM	HSPC disorders treatment	(Rimmele et al., 2014; Sun et al., 2018; Zhang et al., 2015b)
GW9662	PPAR-γ[d]	PPAR-γ antagonist, expands human hematopoietic stem and progenitor cells by enhancing glycolysis.	Stem cell transplantation	Guo et al. (2018)

[a] 5-aminoimidazole-4-carboxamide ribonucleotide.
[b] 1-aminoethylphosphinic acid.
[c] NAD-dependent deacetylase sirtuin-1.
[d] Peroxisome proliferator-activated receptor gamma.

SIRT1 is downregulated leading to impaired TET2 function, in turn promoting the self-renewal and growth of the MDS-associated HSPCs (Sun et al., 2018). In this regard, resveratrol, which activates SIRT1 (hence in essence promoting deacetylation and activation of TET2), increases the frequency and total numbers of normal HSCs in the BM, and thus could be useful for the treatment of HSPC disorders (Rimmele et al., 2014) (Fig. 4).

The majority of individuals with CHIP-related conditions display monoallelic loss-of-function *TET2* mutations (Abdel-Wahab et al., 2009; Jaiswal and Ebert, 2019; Jankowska et al., 2009; Moran-Crusio et al., 2011); this haploinsufficiency is reproduced in heterozygous $Tet2^{+/-}$ mice, which similar to $Tet2^{-/-}$, exhibit increased HSC self-renewal and myeloproliferation *in vivo* (Moran-Crusio et al., 2011). Importantly, it was shown that vitamin C, a co-factor of Fe2(+) and α-KG-dependent dioxygenases such as the TET family enzymes, can promote the activity of the functional TET2 protein from a nonmutated allele. The vitamin C-mediated enhancement of TET2 activity increases 5-hmc levels and prevents HSC expansion in the BM (Agathocleous et al., 2017; Cimmino et al., 2017) (Fig. 4).

The activity of TET2 is also regulated by the TCA cycle, since one of its intermediate metabolites, α-ketoglutarate (α-KG), is a required cofactor for TET2's dioxygenase function (Tahiliani et al., 2009). Given that α-KG is synthesized from citrate through the action of isocitrate dehydrogenase (IDH) enzyme, IDH is critical for maintaining the function of TET2. In fact, mutated IDH converts citrate not to α-KG but, alternatively, to 2-hydroxyglutarate dehydrogenase (2-HG), which can directly inhibit TET2 activity and, accordingly, decrease 5-hmc levels (Xu et al., 2011). Mutations in the isoforms of IDH (IDH1, IDH2) are implicated in acute myeloid leukemia in concert with additional mutations, such as those affecting DNMT3A (Im et al., 2014) (Fig. 4).

DNMT3A is responsible for the transfer of a methyl group to specific CpG structures in DNA (Spencer et al., 2017). DNMT3A also has been shown to play a crucial role in maintaining HSC homeostasis. Conditional ablation of DNMT3A leads to progressive impairment of HSC differentiation and concomitant expansion of HSC numbers following serial transplantation. Moreover, DNMT3A deficiency in HSCs upregulates multipotency genes and downregulates differentiation-associated genes, suggesting that DNMT3A acts as a key epigenetic modulator of HSC regulatory genes and contributing to efficient differentiation of HSCs (Challen et al., 2011). ASXL1 acts as a scaffolding protein that physically interacts with Polycomb Repressive Complex 2 (PRC2), which is involved in trimethylation of lysine 27 of histone 3 (H3K27me3) (Abdel-Wahab et al., 2012). ASXL1 has been shown to play a role in balancing HSC self-renewal and differentiation, as hematopoietic-specific deletion of *Asxl1* results in increased numbers of HSPCs. Serial transplantation of ASXL1-deficient hematopoietic cells leads to a lethal myelodysplastic disorder. Furthermore, deletion of *Asxl1* results in a global reduction of H3K27 trimethylation and dysregulated expression of regulators of hematopoiesis (Abdel-Wahab et al., 2013). Unlike TET2, however, it is uncertain whether and how DNMT3A or ASXL1 are regulated by metabolic factors.

6. Conclusions, therapeutic potential and outlook

HSCs have metabolic flexibility that promotes their functional plasticity. This property facilitates their switching between different states, namely, quiescence and self-renewal, and proliferation and lineage commitment, depending on organismal needs. For instance, in case of life-threatening infection, the ability to swiftly replenish the hematopoietic system with mature blood cells takes priority over limiting potential stress damage. Contributing to this metabolic and functional plasticity are nutrient-sensitive signaling pathways, such as the reciprocally antagonistic PI3K–mTOR and LKB1–AMPK pathways. The same nutrient sensor pathways also regulate the balance between quiescence and proliferation of HSCs during aging, when increased ROS-mediated damage can compromise HSC self-renewal (Fruman et al., 2017; Geiger et al., 2013; Hardie, 2011; Ho et al., 2017; Ito and Ito, 2018; Knoblich, 2008; Kohli and Passegue, 2014; Laurenti and Gottgens, 2018). The expansion of HSPCs and hence the efficacy of hematopoietic stem cell transplantation can be improved by enhancing glycolysis using antagonists of PPARγ (e.g., GW9662) (Guo et al., 2018). The efficacy of hematopoietic stem cell transplantation can also be improved by mitigating HSC exposure to ambient O_2 ('oxygen shock') by collecting and processing the cells in the presence of cyclosporin A, or other blockers, of the mitochondrial permeability transition pores (Mantel et al., 2015). Moreover, prolonged fasting, via reduction of IGF-1 levels, promotes self-renewal of LT-HSCs and reversed the age-related myeloid lineage bias of HSCs, pointing to a possible regenerative approach (Cheng et al., 2014). Thus, therapeutic harnessing of HSC metabolism may offer new options for hematopoietic transplantation as well as for promoting HSC rejuvenation (*i.e.*, restoring their functionality) in aging and associated diseases.

Given the significant involvement of metabolism in regulating TET2, hyperglycemia or other metabolic dysfunction may influence HSCs by impairing the activity of TET2. This would affect proper epigenetic modifications thereby contributing to phenotypes that might mimic CHIP-associated TET2 mutations (even in young individuals or those without CHIP). Manipulating metabolic pathways may thus provide potential therapies to improve TET2 function in certain disorders. Although clinical studies investigating the potential benefits of vitamin C or resveratrol in cardiovascular disease have met with little or no success (Moser and Chun, 2016; Zortea et al., 2016), similar intervention strategies could be more effective in the context of T2DM where TET2 activity is decreased (Pinzon-Cortes et al., 2017; Wu et al., 2018). Restoring defective TET2 function in HSCs could also be explored through SIRT1 activation (e.g., by resveratrol or SRT3025) (Rimmele et al., 2014; Zhang et al., 2015b), especially in the context of MDS, since such treatments were shown to restore TET2 function and to disrupt the maintenance of MDS-associated HSPCs (Sun et al., 2018).

Although induction of trained immunity (e.g., by administering β-glucan) can be beneficial for fighting microbial infections and tumors or coping with myelosuppression (Mitroulis et al., 2018; Kalafati et al., 2020), it can also have detrimental effects in the context of chronic inflammatory or autoimmune disorders (Chavakis et al., 2019). In this context, a protective approach would be to inhibit trained immunity, for instance by inhibiting cholesterol biosynthesis (*e.g.*, by using HMG-CoA reductase inhibitors (Mitroulis et al., 2018; Oguro, 2019). As such approaches may entail significant risk with regard to compromising host immunity, nanoparticles are being considered for targeted delivery of therapeutic compounds to the appropriate progenitor or mature myeloid cell type (Mulder et al., 2019). A summary of selected pharmacological compounds relevant to the translational approaches discussed here is shown in Table 1.

Further studies investigating how HSPCs are affected by metabolic pathways, especially in the context of aging and of systemic disorders such as T2DM and obesity, will not only contribute to better understanding of these conditions but might offer more nuanced options for therapeutic interventions.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

The authors' research is supported by grants from the U.S. National Institutes of Health (DE024153, DE024716, DE029436 to GH; DE026152 and DE028561 to GH and TC) and the German Research Foundation (SFB1181, SFB-TR127 to TC). The figures were created using Biorender.com. The authors regret that several important studies could only be cited indirectly through comprehensive reviews, owing to space and reference number limitations.

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