CHAPTER THREE

Mitochondrial calcium homeostasis in hematopoietic stem cell: Molecular regulation of quiescence, function, and differentiation

Massimo Bonora*, Asrat Kahsay, and Paolo Pinton*
Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy
*Corresponding authors: e-mail address: bnrmsm1@unife.it; pnp@unife.it

Contents

1. Introduction 112
2. Intracellular Ca^{2+} homeostasis in HSC maintenance and commitment 113
   2.1 Ca^{2+} driven commitment of HSC 114
   2.2 Ca^{2+} control of HSC quiescence 117
3. Mitochondrial calcium in HSC regulation 123
4. Calcium homeostasis deregulation in malignant hematopoiesis 125
5. Conclusion 128
Author contributions 129
Funding 129
Conflicts of interest 130
References 130

Abstract

Hematopoiesis is based on the existence of hematopoietic stem cells (HSC) with the capacity to self-proliferate and self-renew or to differentiate into specialized cells. The hematopoietic niche is the essential microenvironment where stem cells reside and integrate various stimuli to determine their fate. Recent studies have identified niche containing high level of calcium (Ca^{2+}) suggesting that HSCs are sensitive to Ca^{2+}. This is a highly versatile and ubiquitous second messenger that regulates a wide variety of cellular functions. Advanced methods for measuring its concentrations, genetic experiments, cell fate tracing data, single-cell imaging, and transcriptomics studies provide information into its specific roles to integrate signaling into an array of mechanisms that determine HSC identity, lineage potential, maintenance, and self-renewal. Accumulating and contrasting evidence, are revealing Ca^{2+} as a previously unacknowledged feature of HSC, involved in functional maintenance, by regulating multiple

International Review of Cell and Molecular Biology, Volume 362
ISSN 1937-6448
https://doi.org/10.1016/bs.ircmb.2021.05.003

actors including transcription and epigenetic factors, Ca^{2+}-dependent kinases and mitochondrial physiology. Mitochondria are significant participants in HSC functions and their responsiveness to cellular demands is controlled to a significant extent via Ca^{2+} signals. Recent reports indicate that mitochondrial Ca^{2+} uptake also controls HSC fate. These observations reveal a physiological feature of hematopoietic stem cells that can be harnessed to improve HSC-related disease. In this review, we discuss the current knowledge Ca^{2+} in hematopoietic stem cell focusing on its potential involvement in proliferation, self-renewal and maintenance of HSC and discuss future research directions.

1. Introduction

The blood system contains more than 10 different blood cell types (lineages) with various functions. All blood cell types arise from hematopoietic stem cells (HSCs) that reside mainly in the bone marrow (BM), the major site of adult hematopoiesis. The pioneering findings by Till and McCulloch revealed the regenerative potential of single BM cells, thus establishing the existence of multipotential HSCs. HSCs are the only cells within the hematopoietic system that possess the potential for both multipotency and self-renewal. BM-HSCs are functionally defined by their unique capacity to self-renew and to differentiate to produce all mature blood cell types (Song et al., 2010). Hematopoiesis is then based on the existence of hematopoietic stem cells with the capacity to proliferate and self-renew or to differentiate into specialized cells (Seita and Weissman, 2010). Multipotency is the ability to differentiate into all types of functional blood cells, while self-renewal is the ability to give rise to identical daughter without differentiation. The HSC's choice between self-renewal and differentiation must be tightly regulated to enable both the generation of differentiated cells and the accurate maintenance of the right HSC number (Ito and Ito, 2018). The cells originating from hematopoietic stem cells can commit to two lineages: the myeloid lineage, including granulocytes, erythrocytes, megakaryocytes/platelets and monocytes; and the lymphoid lineage comprising B and T lymphocytes as well as natural killer cells (Moignard et al., 2013). Leukocytes represent many specialized cell types involved in innate and acquired immunity. Erythrocytes provide O_2 and CO_2 transport, whereas megakaryocytes generate platelets for blood clotting and wound healing (Graf, 2002).

Most of the HSCs within the BM are in a quiescent state. Quiescent HSCs have the ability to self-renew indefinitely, mediating the homeostatic and continuous turnover of blood cells that organisms require throughout

their life (Arai et al., 2004; Morita et al., 2006; Seita and Weissman, 2010; Wang and Ema, 2016; Yang et al., 2007). The BM is an intricate tissue that encompasses several hematopoietic and non-hematopoietic cell types that are interconnected by a vascular and innervated network within the cavities of long bones and axial bones (Baccin et al., 2020; Coutu et al., 2017; Pinho and Frenette, 2019; Zhang et al., 2021). The stem cell niche is the essential microenvironment where stem cells reside and integrate various stimuli to determine their fate and provides special support for cell viability. Niche-specific cell populations, extracellular matrix components, varied growth factors, and cell adhesion molecules produced by niche cells are integrated together for the common goal of controlling stem cell behavior. When tissue damage occurs, niches are feedback systems for communicating information about the state of a tissue back to the related stem cells (Ellis et al., 2011; Katayama et al., 2006; Petit et al., 2002; Pinho and Frenette, 2019).

Hematopoietic cells share this bone cavity together with other cells, such as osteoblasts, osteoclasts, macrophages, adipocytes, perivascular cells, mesenchymal stem cells, and endothelial cells. The communications among these different cells are mediated through various cytokines, growth factors, matrix proteins, and cell-cell adhesions. All these factors have been demonstrated to be HSC niche component as they are co-localized with HSCs and have the functional effects on HSC self-renewal and differentiation (Morrison and Scadden, 2014).

Considering the complex pattern of signals regulating HSC activity, it is not surprising that in the last years, investigation of Ca^{2+} signaling have started, bringing light to unexpected clues. In addition, in last decade, mitochondrial physiology have been reported as a significant regulator of HSC function, mostly by means of respiration and reactive oxygen species (ROS) production (Chen et al., 2008; Ito et al., 2019; Rimmelé et al., 2015; Tai-Nagara et al., 2014).

The purpose of this review is then to summarize the early and exciting observation reported so far that try to link mitochondrial Ca^{2+} to normal or defective hematopoiesis. Also, we will try to explain the contradictory results presented and offer a perspective on future directions.

2. Intracellular Ca^{2+} homeostasis in HSC maintenance and commitment

To date, studies investigating the role of Ca^{2+} signaling in HSC are relatively few. Still, thanks to some recent insight we can start delineating

some pictures. Intriguingly the evidence now available are partially contradicting and could support two different models. This section then groups the evidence according to the models which are most likely to occur in hematopoiesis.

2.1 Ca^{2+} driven commitment of HSC

Direct measurements of cytoplasmic Ca^{2+} concentration, $[Ca^{2+}]_c$, at resting conditions by Indo-1 in flow cytometry, indicates that mouse HSC have extremely low free cytosolic Ca^{2+} (between 20 and 30 nM). The stimulation with stromal cell-derived factor 1 (SDF-1, also known as CXCL12) activates the G protein-coupled protein receptor C-X-C chemokine receptor 4 (CXCR4) inducing an Ip3 mediated release of Ca^{2+} from ER via inositol trisphosphate (Ip3) receptors (Ip3R) with transient elevation of $[Ca^{2+}]_c$ (Cancilla et al., 2020; Hadad et al., 2013; Xiang et al., 2002) (Fig. 1), which still reaches concentration as low as 100 nM (Luchsinger et al., 2019).

Ca^{2+} levels significantly increase along with hematopoietic commitment. Both basal $[Ca^{2+}]_c$ and SDF-1 induced cytosolic transients are augmented in multipotent (MPP) and lineage-restricted progenitors (CP). In agreement with Indo-1 measurement, analysis of mitochondrial motility (considered as a readout of $[Ca^{2+}]_c$ due to the activity of Miro) displayed high movement frequency in the most primitive hematopoietic compartment compared to progenitors. To explain a so low $[Ca^{2+}]_c$ Luchsinger et al. observed that HSC expressed the highest levels of plasma membrane Ca^{2+} ATPase isoform 4 (PMCA4) among all the tested hematopoietic populations and that its activity is inversely proportional to their differentiation status.

These measurements suggest that low $[Ca^{2+}]_c$ is required for HSC commitment. In agreement, agents which induces HSC activation (exit from quiescence status and commitment) are reported to elicit transient $[Ca^{2+}]_c$ elevation. Several cytokines are known to induce HSC proliferation and commitment for the physiological stimulation of blood production. Among many, the most characterized are probably IL-3, IL-6, Il-7, EPO and GM-CSF (Abkowitz et al., 2003; Ogawa, 1993; Zhao et al., 2014). All these cytokines have been shown to stimulated rapid and transient elevation of $[Ca^{2+}]_c$ in HSPC maintained *in vitro* on stromal cells (Paredes-Gamero et al., 2008). Authors further characterized the signaling induced by IL-3 and GM-CSF and observed that *in vitro* co-administration of Ca^{2+} chelator, BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid) or the Ip3R

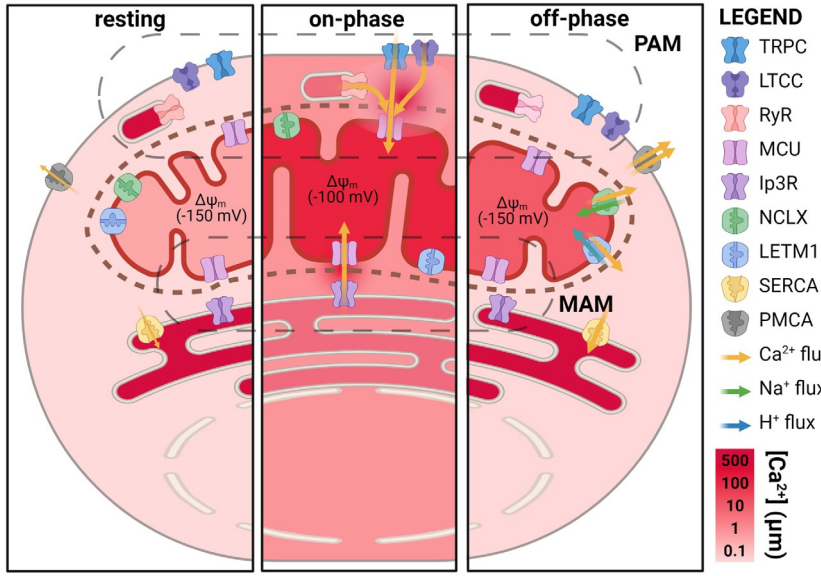

Fig. 1 Mitochondrial calcium homeostasis. At resting conditions (left panel), highest $[Ca^{2+}]$ is found within lumen of Ca^{2+} stores (e.g., ER), guaranteed by the constant activity of SERCA. Its concerted activity with the once of PMCA ensures that $[Ca^{2+}]_c$ is maintained at very low levels (most often within 0.1–0.2 μM range). The strong ΔΨm should be sufficient to induce accumulation of Ca^{2+} within mitochondrial matrix, but the low affinity for Ca^{2+} of the MCU ensures that $[Ca^{2+}]_m$ is kept within the range 0.2–0.5 μM, close to the values of $[Ca^{2+}]_c$. In the on-phase, the opening of Ip3R or RyR (Ip3-operated and voltage-operated channels, respectively) on ER membrane causes the depletion of intracellular stores, that pours its Ca^{2+} in cytoplasm (which reaches 1–2 μM). Due to the strong gradient of $[Ca^{2+}]$ between ER and cytosol, Ca^{2+} is released in the cytoplasm faster than its diffusion generating a microdomain with high $[Ca^{2+}]_c$. At contact sites between mitochondria and ER (MAM) the MCU is submerged into a $[Ca^{2+}]_c$ sufficient to induces its opening therefore the accumulation. A comparable mechanism occurs at mitochondria associated to plasma membrane (PAM) where $[Ca^{2+}]_c$ microdomain are achieved by the opening of ligand-operated or voltage-operated PM channels (e.g., TRPCs and LTCC, respectively). Finally, elevation of $[Ca^{2+}]_m$ activates exchangers on IMM that ensures the extrusion of Ca^{2+} into cytoplasm and engaging the off-phase. These are the Ca^{2+}/Na^+ exchanger, NCLX, and LETM1, the putative Ca^{2+}/H^+ exchanger. Concomitantly, activity of SERCA and PMCA reaches their maximal rates, restoring $[Ca^{2+}]_c$ and $[Ca^{2+}]_{er}$ to the values of resting.

inhibitor, 2APB, significantly impair proliferation in the forming colonies, suggesting the involvement of Ip3-Ca^{2+} signaling in IL-3- and GM-CSF-induced HSC commitment. In the same study authors shown that P2X receptor agonists ATP, ADP, and UTP elicits $[Ca^{2+}]_c$ waves higher than the once induced by cytokine and that their effect on *in vitro* differentiation was partially

reverted by BAPTA. Extracellular nucleotides (especially ATP) can be released in the BM by HSC mobilizing factor (e.g., G-CSF) to induce sterile inflammation (Ratajczak et al., 2018). Intraperitoneal injection of ATP in mice, induces mobilization of HSC and isolated HSC exposed to ATP displayed diminished reconstitution capacity (Barbosa et al., 2011). In agreement, mobilization stimulated by C-GSF is strongly inhibited in mice lacking the purinergic receptor P2X7 (Adamiak et al., 2018).

HSC can be induced to exit dormancy also by genotoxic stress. Indeed, agents which induces DNA damage (e.g., 5-FU or γ radiation) mostly affect cycling cells in the BM leading to their apoptotic cell death (Høyer and Nielsen, 1992). After an initial reduction of BM cellularity, some HSCs re-enter cell cycle undergoing commitment rather than self-renewal to replenish the dying populations (Schoedel et al., 2016). Interestingly two independent report displayed increase in $[Ca^{2+}]_c$ in active HSCs isolated from mice exposed to 5-FU (Fukushima et al., 2019; Umemoto et al., 2018).

Also, some molecular effectors downstream Ca^{2+} are involved in HSC differentiation. Elevation of $[Ca^{2+}]_c$ in HSC have been linked to Nuclear factor of activated T-cells, cytoplasmic 2 (NFAT) activation. Sustained increase in $[Ca^{2+}]_c$ activates calcineurin, which dephosphorylates NFAT and promotes its translocation to the nucleus (Beals et al., 1997; Hogan et al., 2003). Noncanonical Wnt signaling, mediated by Fmi-Fz8, restricts NFAT nuclear translocation controlling intracellular Ca^{2+} level, potentially through inhibition of L-type Ca^{2+} channels (LTCC) (Sugimura et al., 2012). 5-FU stimulation induces NFAT nuclear translocation where up regulates the transcription of Interferon gamma (IFNγ) and Cox2 transcription in HSC. INFγ in turn is reported to instruct HSC to exit quiescent status (Baldridge et al., 2010).

In addition to NFAT signaling a Ca^{2+}-Calpain axis was proposed to control HSC function. Calpains are Ca^{2+}-regulated cysteine proteases and, according to GEXC database, HSC express at least 3 of the 14 isoforms. Calpains activity was detectable in cultured HSC that are usually maintained in media with high $[Ca^{2+}]$. On opposite, Ca^{2+} deprivation from media caused significant inhibition of Calpain activity. Interestingly, among the many predicted Calpains target is Ten-eleven translocated (TET) enzymes. These converts 5-methylcytosine (5mC) to 5-hydroxymethyl cytosine (5hmC), an epigenetic marker required for the demethylation of 5mC (Cimmino and Aifantis, 2017; Rasmussen and Helin, 2016; Zhang et al., 2016). TET2 plays a major role in HSCs by suppressing the expression of lineage-specific genes, therefore favoring HSC maintenance (Zhang et al., 2016). $Tet2^{-/-}$ HSCs

show progressive myeloid bias *in vivo* that ultimately leads to myeloid malignancy (Ko et al., 2011; Li et al., 2011). Calpains inhibition by small molecules, overexpression of its endogenous inhibitor (calpastatin) or low calcium media, increased TET protein expression and 5hmC deposition in chromosomal DNA. In agreement, Tet2$^{-/-}$ HSCs did not benefit from culturing in low Ca^{2+} media (Luchsinger et al., 2019).

Take together, this evidence indicates that HSCs requires low $[Ca^{2+}]_c$ to maintain their quiescent status and function, on opposite, stimuli which instruct HSC proliferation and commitment, elevate $[Ca^{2+}]_c$ to induce NFAT signaling and Tet2 degradation (Fig. 2). Also, as $[Ca^{2+}]_c$ is a function of intraluminal Ca^{2+} concentration of ER, $[Ca^{2+}]_{er}$, it would be of interest to test if $[Ca^{2+}]_{er}$ have adapted to this mechanism.

2.2 Ca^{2+} control of HSC quiescence

The previous model is nonetheless oversimplified as additional evidence indicates a far more complex participation of Ca^{2+} in HSC function.

One of the earliest evidence calling for Ca^{2+} investigation in HSC was the dependence of HSC function on the calcium-sensing receptor (CasR). This is a G-protein-coupled receptor activated by extracellular Ca^{2+}, $[Ca^{2+}]_o$, mostly known for its participation in parathyroid hormone response to Ca^{2+} levels in tissue milieu (Chavez-Abiega et al., 2020). CasR$^{-/-}$ develop early hypercalcemia and do not survive longer then 7–10 days after birth. CasR$^{-/-}$ mice analyzed within 72 h from birth display significant BM hypocellularity and limited amount of hematopoietic stem and progenitor cells (HSPC). Also, fetal liver HSC (FL-HSC, a common source of HSC from non-vital mouse strains) displayed altered maintenance capacity *in vitro* and impaired capacity to reconstitute the hematopoietic system (reconstitution capacity) in competitive bone marrow transplantation (BMT, a gold standard assay for HSC function). Interestingly HSC CasR$^{-/-}$ also displayed aberrant localization in the BM, suggesting alteration in the recognition of their niche (a process usually termed homing) (Adams et al., 2006). This hypothesis was further supported by the observation that isolated HSC CasR$^{-/-}$ have impaired adhesion on collagen coated culturing device. Stimulation of isolated HSPC with the CasR agonist, Cinacalcet, induces elevation of $[Ca^{2+}]_c$, promotes cellular adhesion *in vitro* and favored reconstitution capacity in competitive BMT (Lam et al., 2011). FL-HSC are not quiescent and support hematopoiesis in developing fetus. Only after birth, FL-HSC, migrates to BM, were install in their niche and exit cell cycle in favor of quiescence (Arai et al., 2005; Bowie et al., 2006).

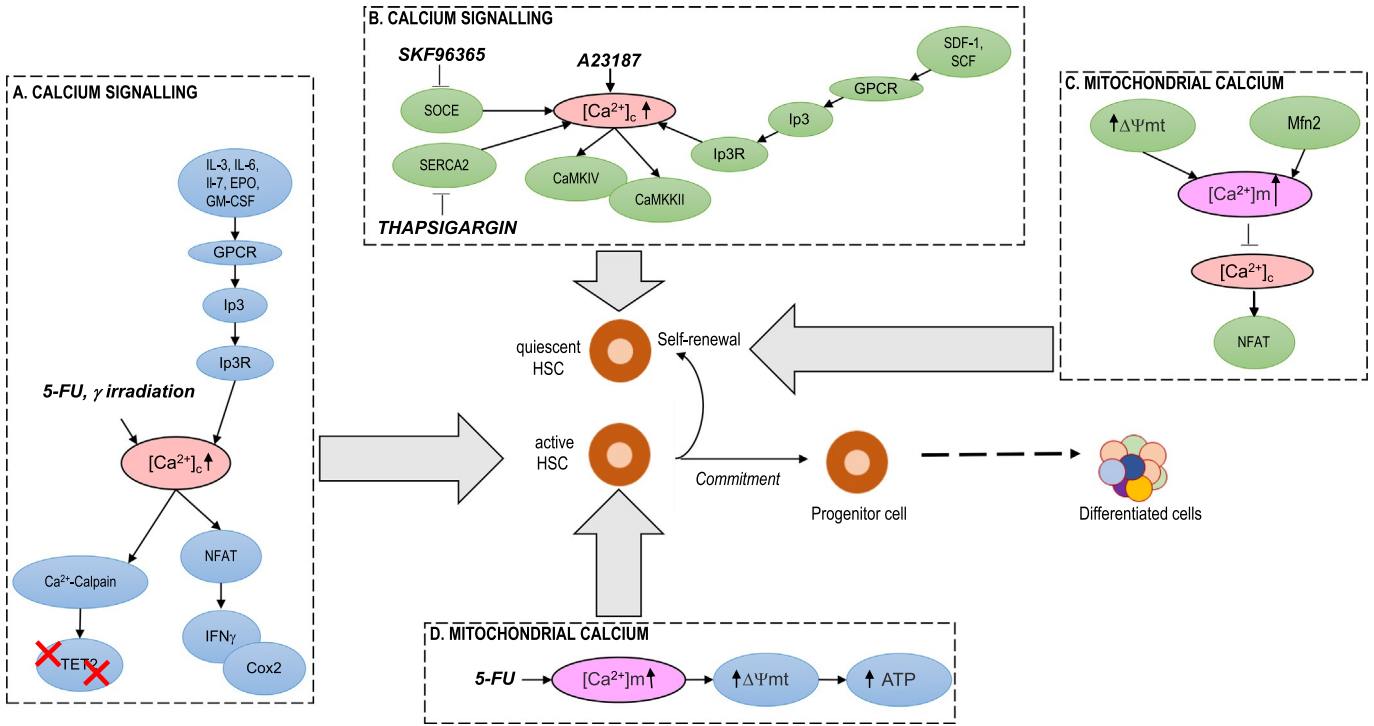

Fig. 2 Overview of Ca^{2+} involvement in HSC function. (A) Elevation of $[Ca^{2+}]_c$ by genotoxic stress or differentiation promoting cytokines are associated to HSC activation and commitment. In these conditions, Calpains induces degradation of the stimulator of self-renewal Tet2, while NFAT translocate to nucleus where can stimulate the transcription of factors promoting commitment. (B) High $[Ca^{2+}]_c$ was also reported in quiescent vs active HSC. Quiescence can be induced by stimulating with SDF-1 or SCF, as well as by the pharmacological elevation of $[Ca^{2+}]_c$ via SERCA-2 or SOCE inhibition and the use of ionophores. (C) HSC have high membrane potential which is expected to maintain high basal $[Ca^{2+}]_m$ in concert with Mfn2 activity. The resulting buffering activity on $[Ca^{2+}]_c$ blocks the NFAT nuclear translocation favoring HSC maintenance. (D) Stimulation with 5-FU induces the elevation of $[Ca^{2+}]_m$ that indirectly potentiates $\Delta\Psi m$ and ATP production, associated with HSC commitment.

This suggest that Ca^{2+} signals in HSC might be important for niche interaction. In agreement, one of the most characterized signaling molecule required for HSC homing is CXCR4. This receptor is highly expressed in HSC where mediates its interaction with multiple SDF-1 expressing cells in the vascular niche (Pinho and Frenette, 2019; Sugiyama et al., 2006). SDF-1 is chemoattractant for HSC and binding of its receptor is required for maintenance of quiescence (Nie et al., 2008). SDF-1 administration to cultured HSC inhibits the spontaneous entry into cell cycle. Interestingly, as previously described, SDF-1 elicit transient $[Ca^{2+}]_c$ elevation (Lam et al., 2011; Luchsinger et al., 2019). *In vivo* antagonization of the SDF-1-CXCR4 interaction through AMD3100 induces HSC mobilization and stimulation of hematopoiesis (Hübel et al., 2004). AMD3100 was demonstrated in fully differentiated hematopoietic cells, to potently inhibits Ca^{2+} mobilization via SDF-1 (Hatse et al., 2002).

Another ligand required for HSC interaction with its niche is the stem cell factor (SCF). This is released in the BM mainly (but not only) by arteriolar endothelial cells where bind the HSC marker c-kit (Asada et al., 2017).

In vivo studies have shown that deletion of SCF using endothelial cell-specific Cre strains, impairs HSC maintenance at steady state, and confirms the role of endothelial cells as niche regulators (Asada et al., 2017; Ding et al., 2012). In cultured HSC also SCF is able to elicit $[Ca^{2+}]_c$ transients (Paredes-Gamero et al., 2008).

If Ca^{2+} signaling is required for HSC installation and maintenance in their niche, there should be evidences that quiescent HSC have elevated $[Ca^{2+}]_c$. In a very recent report Fukushima et al. presented evidence in this favor (Fukushima et al., 2019). They developed a transgenic mouse stably expressing, in the hematopoietic system, a fluorescent marker for quiescent cells (mVenus-p27K$^-$) and compared basal $[Ca^{2+}]_c$ in dormant vs active HSC through the Ca^{2+} sensitive dye CaSiR-1. Interestingly they observed that quiescent HSC displayed higher $[Ca^{2+}]_c$ compared to cycling one, no significant alteration while compared to MPP, while a significant increase in $[Ca^{2+}]_c$ was detectable only in more CP. Also, quiescent cell with high $[Ca^{2+}]_c$ displayed full long term reconstitution capacity on opposite then low $[Ca^{2+}]_c$, in competitive BMT. They addressed the increased $[Ca^{2+}]_c$ to elevated expression of multiple channels involved in Ca^{2+} influx from plasma membrane, including L-type Ca^{2+} channels (LTCC) and transient receptor potential channel (TRPC). Additionally, they cultured HSC for 24 h with the Sarco/endoplasmic reticulum calcium-ATPase (SERCA)

inhibitor thapsigargin or the Ca^{2+} ionophore A23187. This treatment induces elevation of $[Ca^{2+}]_c$ (thought unexpected for a long thapsigargin exposure) and slightly promoted reconstitution capacity in BMT.

A partial confirmation comes from investigation of S100a6$^{-/-}$ animals. S100A6 protein is a member of the EF-hands S100 protein family, implicated in the regulation of several cellular functions, such as proliferation, apoptosis, cytoskeleton dynamics, and cellular response to stress factors. S100A6 is highly expressed in HSC and its genetic inactivation leads to major defects in HSC function. S100a6$^{-/-}$ mice have indeed significant BM hypocellularity, including reduction in the HSPC compartment. Also, HSC from S100a6$^{-/-}$ have limited reconstitution capacity in competitive BMT experiments, indicative of impaired HSC function. Interestingly, SCF induced $[Ca^{2+}]_c$ transient was ablated by S100A6 null cells, suggesting a that its regulation of HSC function is mediated by Ca^{2+}, but no overt alteration of quiescence were reported (Grahn et al., 2020).

Another study tested the effect of the store operated calcium entry (SOCE) inhibitor, SKF96365 on HSC function *in vitro* and *in vivo*. Isolated HSPC exposed to SKF96365 displayed reduced basal $[Ca^{2+}]_c$ and exit from quiescence causing expansion of HSC and committed progenitors. Accordingly, SKF96365-treated HSC transplanted into non-irradiated immune compromised mice displayed slightly better reconstitution capacity after 1 month, but not in the long term (4 month), suggesting that SOCE inhibition slightly promoted HSC commitment (Uslu et al., 2020).

In agreement for a role in Ca^{2+} signaling in regulation of HSC maintenance is the observation that Calmodulin-dependent Protein Kinase IV (CaMKIV) was demonstrated fundamental in the regulation of HSC quiescence. CaMKIV$^{-/-}$ animals have significant BM hypocellularity with reduced amount of HSPC. Reconstitution capacity of HSPC CaMKIV$^{-/-}$ was significantly altered, being able to provide hematopoiesis only for the first 6 weeks. Further, HSPC CaMKIV$^{-/-}$ were significantly faster cycling and failed to activated Bcl-2 expression, due to low phosphorylation status of CREB (Kitsos et al., 2005). Likewise, an impairment of the quiescence status was observed in Calcium/calmodulin-dependent kinase kinase 2 (CaMKK2)$^{-/-}$ HSC. Especially, HSCP CaMKK2$^{-/-}$ have increased proliferation and a transcriptional profile consistent with poor quiescence and increased differentiation. Should be noted that transplanted CaMKK2$^{-/-}$ progenitors have only slightly affected reconstitution capacity (at least after 4 months), but apparently are significantly resistant to irradiative stress

(Racioppi et al., 2017). These data indicate that CaMKIV and CaMKK2 can transduce Ca^{2+} signal of some sort to transcriptional control of quiescence (Fig. 2).

Many of the contrasting results described so far could be attributed to the differences on the experimental methods and to the complexity in properly measure Ca^{2+} in HSC (see Box 1). A better comprehension of the phenomenon could be achieved by the description of the Ca^{2+} dynamic rather than solely basal $[Ca^{2+}]_c$ as is clear that Ca^{2+} signals differing in frequency, amplitude or duration can lead to completely different outcome.

BOX 1 Challenges and pitfalls of Ca^{2+} measurements in HSC.

As for all primary cells, investigation of Ca^{2+} signaling in HSC is challenging. HSC investigation requires in first place their isolation from mouse BM in most cases, but also mouse FL as well as human BM. Though isolation procedures vary from the origin of the donor, these all share significant amount of time in Ca^{2+}-free PBS or PBS + 2% FBS (with expected $[Ca^{2+}]_o$ around 0.1 mM). Adult HSC resides in the BM where $[Ca^{2+}]$ have been recently estimated around 0.5 mM, though it could be higher in the arteriolar niche surrounding a significant proportion of quiescent HSC. These aspects represent a first significant limitation to investigation of Ca^{2+} signals, indeed the activity of PMCA usually outcompete the activity of SERCAs, especially when cellular samples are maintained in buffers with low $[Ca^{2+}]_o$. This cause the progressive depletion of Ca^{2+} from intracellular store and cytoplasm (which is poor in endogenous Ca^{2+} buffers). HSC isolation is then expected to significantly alter the intracellular distribution of Ca^{2+}, though the phenomenon should be limited by maintaining samples at 4 °C. This Ca^{2+} depletion artifact might be particularly significant when comparing HSC with committed progenitors, considering the high PMCA activity reported for this cell type, and could explain the dramatic low $[Ca^{2+}]_c$ observed.

As HSC are strongly affected by culturing, then Ca^{2+} recording is usually performed immediately at the end of the isolation procedure. Future investigation should then consider a Ca^{2+} repletion step (e.g., 30 min incubation at 37 °C in a buffer with $[Ca^{2+}]_o$ resembling BM milieu). Also, because of this limitation, Ca^{2+} investigation have been obtained so far only using fluorescent dyes. Fluorescent small molecules should be used carefully in HSCs, because of the high activity of the xenobiotic efflux pumps characterizing these cells and that can result in dye extrusion and uneven staining between HSC and committed progenitors (Morganti et al., 2019b). In the past, the extrusion of fluorescent dyes (e.g., Rhodamine 123 or Hoechst Blue vs Hoechst Red) was used as a technique to identify and isolate HSCs (Zhou et al., 2001) before of the characterization of CD150 as

Continued

BOX 1 Challenges and pitfalls of Ca^{2+} measurements in HSC.—cont'd

a marker of HSC (Oguro et al., 2013). This phenomenon should not impact the recording of steady state $[Ca^{2+}]_c$ using robust radiometric dyes as Indo-1, when a sufficient signal to noise ratio is achieved, but it will jeopardize recording obtained by non ratiometric dyes (e.g., Fluo-3, Fluo-4, or CaSir-1). Therefore, the correlation between dye intensity and quiescence should be considered carefully.

Another strong limitation in fluorescent dyes is poor targetability of fluorescent dyes to cellular district. All the AM dye indeed can virtually localize into all cellular compartments, but their K_D for Ca^{2+} usually allows the recording of Ca^{2+} in regions with low concentrations as cytoplasm. Some fluorescent dyes developed for Mg^{2+} investigation have been proven to report Ca^{2+} in intracellular stores (e.g., ER or Golgi) due to their low K_D (Rossi and Taylor, 2020).

A notable exception is represented by the rhodamine derivative Rhod-2 or X-Rhod-1. Thanks to the presence of one net positive charge, these compounds can easily accumulate the most electronegative intracellular compartments, mitochondria (the most represented) and peroxisomes. Rhod-2 was then considered the best option for $[Ca^{2+}]_m$ analysis in HSCs and provided so far valuable preliminary insights. Apart from being sensitive to xenobiotic fluxes as other dyes, these derivatives are nonetheless extremely sensitive to $\Delta\Psi$mt. Also, Rhod-2 have a high affinity for Ca^{2+} while mitochondria have been reporter that can accumulate $[Ca^{2+}]_m$ to concentration far above 0.1 mM. Therefore, Rhod-2 will easily saturate in cells with mitochondria very active in terms of Ca^{2+} signaling.

The ideal investigation of Ca^{2+} signaling in hematopoiesis should be obtained by the far superiors GECIs. A plethora of fluorescent or luminescent indicators have been developed so far, allowing Ca^{2+} recordings, virtually, in every cellular compartment (Bonora et al., 2013; Horikawa, 2015).

Isolated mouse HSCs can be transduced *in vitro* to allow GECIs expression then transplanted to investigate their Ca^{2+} homeostasis in a more native environment. Most intriguingly, BM structure and hematopoietic niche have been demonstrated to undergoes significant remodeling after γ-irradiation, a procedure usually required to allow proper HSC reconstitution during transplantation (Batsivari et al., 2020).

It could be then of extreme interest to compared Ca^{2+} homeostasis in HSC that undergoes to transplantation, compared to quiescent HSCs from untreated animals (e.g., from transgenic mice with stable expression of GECI), as it could provide useful insight in the participation of Ca^{2+} in the communications between HSCs and its niche as well as niche remodeling during stress.

3. Mitochondrial calcium in HSC regulation

The impact of mitochondria on HSC function has been intensively investigated in the last decade (Gurumurthy et al., 2010; Ito et al., 2019; Nakamura-Ishizu et al., 2020; Rimmelé et al., 2015). The BM niche which maintains HSC quiescent is hypoxic and allows stabilization of the Hypoxia-inducible factor 1-alpha. This is a transcription factor which configure HSC's metabolism so to sustain glucose consumption while limiting pyruvate entry into mitochondria (Takubo et al., 2010, 2013).

In agreement, HSC have been shown rely mostly on glycolysis rather than mitochondrial respiration for ATP recycling. Further, the activation of mitochondrial respiration is determinant in cell fate decision by means of the production of reactive oxygen species (ROS), which favors commitment against self-renewal (Chen et al., 2008; Ito et al., 2004, 2006).

The deeper investigation of HSC's mitochondria indicates that this organelle is also important in HSC maintenance. Indeed HSCs pharmacological inhibition of fatty acid oxidation (FAO)—the catabolism of fatty acid in the mitochondrial matrix that feeds the tricarboxylic acid cycle (TCA)—as well as deletion of FAO driver genes, strongly dampens self-renewal capacity (Ito et al., 2012). In agreement, genetic inactivation of electron transport chain (ETC) activity by deletion of genes coding for of an essential subunit of respiratory chain complex III (*Uqcrfs1*) or complex II (*SdhD*) were reported to cause severe pancytopenia and reduction in HSCs, respectively, indicating a meaning for ETC in HSC, regardless the low respiration that these cells can carry on (Ansó et al., 2017; Bejarano-García et al., 2016).

The use of common mitochondrial dyes (e.g., TMRM, Mitotrackers, Rhod 123) in combination with blockade of xenobiotic efflux (e.g., by exposure to Verapamil), revealed that mitochondrial content in HSC was largely underestimated and that display, *in vitro*, a higher mitochondrial membrane potential ($\Delta\Psi m$) compared to committed cells (Bonora et al., 2018; Mansell et al., 2021). HSC are characterized by a minimal configuration of ETC. Especially, we observed that HSC display low levels of respiratory complexes I and V, and that electrons enters ETC most entirely through the activity of respiratory complex II (Bonora et al., 2018; Morganti et al., 2019a). The elevated $\Delta\Psi mt$, despite low respiration rate, is most likely due to the ratio between respiratory complexes I–IV to complex V.

Being $\Delta\Psi m$ the driving force for mitochondrial Ca^{2+}, $[Ca^{2+}]_m$ (Fig. 1), it should be expected that HSC might have higher basal $[Ca^{2+}]_m$ compared

to the differentiating progeny. To date, no studies reported $[Ca^{2+}]_m$ in HSC compared to progenitors or fully differentiated cells. Still, Luchsinger et al. reported that isolated HSC cultured in low Ca^{2+} media significantly diminished the brightness of the Ca^{2+} sensitive rhodamine derivative Rhod-2. Also, they observed that respiration levels correlate with the Ca^{2+} availability in the media (Luchsinger et al., 2019). This was explained by the Ca^{2+} dependence of TCA cycle and F1/FO ATP synthase. The same study reported (as previously discussed) extremely low $[Ca^{2+}]_c$ (below 100 nM), considering that mitochondrial Ca^{2+}-dependent dehydrogenases have relatively low K_D for Ca^{2+}, it is plausible that HSC have a relatively high $[Ca^{2+}]_m$ at resting conditions, thanks to elevated $\Delta\Psi m$. In agreement, the same group reported evidence that mitofusin-2 (mfn2) is required for proper hematopoiesis. Mfn2 is a well-known regulator of mitochondrial dynamics, and the mfn2 KO HSC display a prototypical fragmentation phenotype. Also, loss of mfn2 leads to significant reduction of mitochondrial Ca^{2+} uptake capacity. In HSC, mfn2 KO displayed increased $[Ca^{2+}]_c$ elicited by SDF-1 and an elevation of the nuclear/cytoplasmic ratio of NFAT-C1 (Luchsinger et al., 2016).

Finally, mfn2 KO cells displayed impaired reconstitution capacity in competitive BMT experiments, though apparently biased for the lymphoid potential. In agreement, S100a6$^{-/-}$ HSC (which have defective HSC function) displayed blunted $[Ca^{2+}]_m$ uptake in response to SCF compared to wild-type as well as lower $\Delta\Psi m$ and maximal respiratory capacity (Grahn et al., 2020). This suggests that mitochondrial Ca^{2+} uptake (at least its buffer capacity) is instrumental for the maintenance of HSC function (Fig. 2).

This model is nonetheless not confirmed by investigation of HSC function in Drp1 KO cells. Drp1 is the main executor of the mitochondrial fission. Its regulation of mitochondrial network dynamics have been reported to impact $[Ca^{2+}]_m$ in multiple cellular (Favaro et al., 2019; Szabadkai et al., 2004). Especially, overexpression of Drp1 induces hyper-fragmentation, causing isolation of mitochondrial particles which do not have a direct contact with ER and, therefore, reduces mitochondrial Ca^{2+} uptake. In parallel Drp1 inactivation, generates an hyperfused mitochondrial network, favoring the diffusion of Ca^{2+} within mitochondrial matrix. While $[Ca^{2+}]_m$ in HSC was never reported during Drp1 modulation, it is expected that its genetic inactivation should lead to increased buffering capacity, therefore favoring HSC maintenance. A recent report displayed instead that Drp1$^{-/-}$ HSC have reduced reconstitution capacity, thought it was explained by the inability of mitochondria to properly segregate during HSC division (Hinge et al., 2020).

A more recent report investigated basal $[Ca^{2+}]_m$ in HSCs during stress hematopoiesis. Umemoto et al. reported that HSC activation by intraperitoneal injection of 5-FU elevates basal $[Ca^{2+}]_c$, $\Delta\Psi m$ and basal $[Ca^{2+}]_m$. Also, exposure to Nifedipine, a LTCC blocker, inhibited mitochondrial functions and prolonged the cell cycle phase of HSCs which resulted in slower recovery of stress hematopoiesis (Umemoto et al., 2018).

In agreement, a RNA sequencing study from mouse HSC display that some members of the mitochondrial calcium uniporter complex (Micu1, Micu2 and Smdt1), have a tendency in increase their expression from quiescent HSC to active to MPP (Cabezas-Wallscheid et al., 2017).

Finally, mitochondrial Ca^{2+} transfer from ER to mitochondria regulate autophagic flux and mitochondrial degradation (Calì et al., 2013; Cárdenas and Foskett, 2012; Gomez-Suaga et al., 2017). HSC have been shown strong dependency on autophagy and its subroutine mitophagy. Especially, it was demonstrated that genetic inactivation of autophagy is detrimental for HSC maintenance, leading to premature aging (Ho et al., 2017) and that HSC maintain a high rate of mitophagy to ensure mitochondrial clearance (Ito et al., 2016; Jin et al., 2018) which results in favored HSC self-renewal capacity (Fig. 2).

4. Calcium homeostasis deregulation in malignant hematopoiesis

Alterations in the control of HSC quiescence as well as their division balance causes hematological malignancies (Heidel et al., 2011; Park et al., 2019) such as chronic leukemia (CL) or acute myelogenous leukemia (AML). A panel of recurrent mutations have been described in these conditions, offering insights on the molecular mechanism underlining their insurgence and development (Kim et al., 2017; Lindsley et al., 2015; Stevens et al., 2018; Thol et al., 2017). In CL or *de novo* AML the earliest mutation is the trigger event for the development of the disease. On opposite, a subgroup of AML (secondary AML) develop from other hematological conditions, often generalized as pre-leukemia (e.g., clonal hematopoiesis of indeterminate potential, CHIP or myelodysplastic syndromes, MDS). The transition from pre-leukemia to secondary AML is driven by the acquisition of additional mutations, conferring the neoplastic phenotype (Chen et al., 2019; Corces-Zimmerman et al., 2014; Koeffler and Leong, 2017; Makishima et al., 2017; Nagata and Maciejewski, 2019; Saeed et al., 2021; Shlush et al., 2014).

To date, no direct measurement reports $[Ca^{2+}]_m$ handling in pre-leukemic HSC or malignant stem cell (SC) compared to their normal counterparts. Still, many observations could help us depicting model for $[Ca^{2+}]_m$ in malignant hematopoiesis. As for each malignant condition also preleukemic-HSC or malignant-SC have been reported as resistant to apoptosis (Jilg et al., 2016; Pandolfi et al., 2013; Xu et al., 2012).

Mitochondria participates to regulated cell death (RCD) via at least two pathways—the mitochondrial outer membrane permeabilization (MOMP) and the mitochondrial permeability transition (MPT)—both of which can be triggered by transfer of Ca^{2+} from ER to mitochondria characterized by long duration but low amplitude (Adachi et al., 1997; Bonora et al., 2015; Marchetti et al., 1996). Not surprisingly, multiple reports indicate that this kind of Ca^{2+} signals can be elicited in leukemic cells by a variety of compounds, often favoring RCD engagement in the malignant cells compared to HSPCs (Bouchet et al., 2016; Ge et al., 2019; Metts et al., 2017; Pinton et al., 2011).

Most significantly, several genes involved in the emergence of malignant hematopoiesis have been related to the control of ER to mitochondria Ca^{2+} transfer. Bcl-2 is one of the first oncogene ever discovered and it was isolated from a B-cell lymphoma. Bcl-2 is well characterized as a master suppressor of RCD in almost all its sub-routines. It is also capable of suppressing mitochondrial Ca^{2+} uptake by either lowering $[Ca^{2+}]_{er}$ at steady state and inhibiting the activity of the Ip3Rs (Bittremieux et al., 2016; Pinton et al., 2001). A comparable phenotype is also attributed to another anti-apoptotic member of the Bcl-2 family, Mcl-1 (Bittremieux et al., 2016; Eckenrode et al., 2010). Bcl-2 is expressed in hematopoietic system, though is not enriched in HSC, but rather in lymphoid progenitors. Interestingly, its overexpression in HSC do not cause spontaneous development of a malignant phenotype but suppresses spontaneous apoptosis and support the quiescent state (Domen et al., 2000; Kollek et al., 2016). In contrast, *Mcl-1* is highly expressed in the stem/progenitor's compartment and critically required for the maintenance of HSCs. *Mcl-1* conditional deletion induces depletion of HSPC due to increased apoptotic rates, leading to bone marrow failure (Opferman et al., 2005). Also its pharmacological inhibition support a role for Mcl-1 in the control of HSC function (Campbell et al., 2010). Same line of evidence is regarding the promyelocytic leukemia (Pml) and phosphatase and tensin homolog (Pten) (Morotti et al., 2015; Testa and Lo-Coco, 2016). Both genes are affected by loss of function mutations frequently associated to myeloid malignancies (Ito et al., 2008;

Zhang et al., 2006). Interestingly, both proteins can localize at the contact sites between ER and mitochondria, where sustains the activity of Ip3Rs and therefore mitochondrial Ca^{2+} uptake to control both metabolic demands and RCD engagement (Bononi et al., 2013; Giorgi et al., 2010; Missiroli et al., 2016). In terms of HSC function both genes are required for stem cell maintenance. Indeed, genetic inactivation of both Pten and Pml lead to excess of HSC proliferation which can easily repopulate BM during competitive transplantation only in the short term, while failing in the long term, indicative of exhaustion of HSC capacity (Ito et al., 2008; Zhang et al., 2006). Also, for Pten was reported spontaneous development of myeloid malignancy.

The tumor suppressor p53 is non-surprisingly another fundamental regulator of hematopoiesis. Data from $tp53^{-/-}$ mice indicates that in steady state conditions, p53 is required to positively regulate HSC quiescence, apparently via a GFI-1/NECDIN pathway rather than via p21 (Liu et al., 2009; Yamashita et al., 2016). At the mitochondrial level, p53 is reported to have multiple functions. It transcriptionally modulate the expression of assembly factors that favors respiratory chain (Matoba et al., 2006). During stress conditions, it can translocate to outer mitochondrial membrane to directly activated MOMP and MPT (Talos et al., 2005; Vaseva et al., 2012). Most interestingly, at steady state p53 localize to ER and favor SERCA2 activity via direct binding (Giorgi et al., 2015a). This elevates $[Ca^{2+}]_{er}$ then Ca^{2+} transfer to mitochondria (Giorgi et al., 2015b).

These pieces of evidence suggest that deregulation of $[Ca^{2+}]_m$ could be involved in the development of HSC-related leukemic condition. Surprisingly, the described conditions, though all sharing alterations in $[Ca^{2+}]_m$, only in a minor of cases results in the spontaneous development of a malignancy, while the most affected HSC property appears to be quiescence. This suggest that the relevance for $[Ca^{2+}]_m$ in this group of malignancies might require the collaboration of additional mutations.

Of the many genes identified so far that are target of preleukemic mutation, none was related to $[Ca^{2+}]_m$ homeostasis, with exception of enhancer of zeste homolog 2 (EZH2) (Bowman et al., 2018; Saeed et al., 2021; Thol et al., 2017). This is a histone methyltransferase part of the polycomb repressive complex 2. Its frequently altered in hematological disorders, spanning from CHIP to MDS and sAML. Its activity in HSC function is still controversial though most evidence indicates that is required for the control of HSC commitment (Herviou et al., 2016). Its genetic manipulation appears to be significantly more efficient in FL-HSC rather than BM-HSC, most

likely because of a compensation by EZH1. In cancer cells of epithelial origin, pharmacological inhibition, or shRNA inactivation of EZH2 significantly impairs the expression of mitochondrial calcium uptake protein 1 (MICU1) (Zhou et al., 2015). This is the gatekeeper of the mitochondrial calcium uniporter (MCU), that confers to the channel low affinity for Ca^{2+} (Marchi et al., 2020). MICU1 down-regulation, increases channel permeability, favoring elevation of basal $[Ca^{2+}]_m$. This also often associated with increased ROS production and predisposed cells to succumb RCD.

5. Conclusion

HSC function is of vital importance for a physiological hematopoiesis throughout all life and is therefore the results of multiple, balanced signals that regulates quiescence and self/renewal vs commitment. Considering the emerged meaning of mitochondrial physiology in the regulation of these function and the well-established role of intracellular Ca^{2+} as signaling molecules, it was just expectable that investigation of mitochondrial Ca^{2+} homeostasis would also reach the hematopoietic field.

To date seminal investigation are starting to depict a model of this topic. We now know that (i) there is a remodeling of all intracellular (including mitochondrial) Ca^{2+} homeostasis in HSC exiting quiescence and undergoing commitment, (ii) HSC can sense $[Ca^{2+}]_o$, (iii) pharmacological or genetic regulation of Ca^{2+} homeostasis can alter HSC quiescence status as well as *in vitro* and *in vivo* function, (iv) there are Ca^{2+} sensitive molecular participant involved in direct regulation of HSC quiescence or commitment programs.

These are all important information that prove the participation in Ca^{2+} signaling in hematopoiesis. Still, information is currently somewhat fragmentary and sometimes contradictory. We can currently propose at least two models, in the first Ca^{2+} signals are resting in the quiescent HSC, while activating when commitment is needed (e.g., during 5-FU or adenine nucleotide stimulation). Activation of a "pro-commitment" Ca^{2+} signal favors nuclear translocation of the commitment promoter NFAT and degradation of the self-renewal promoter Tet2. On the mitochondrial side, quiescent cells mitochondria actively participate to clear $[Ca^{2+}]_c$ via buffering while in active HSC of $[Ca^{2+}]_m$ activates respiration.

On the other side, a pro-quiescence model indicates that quiescent cells have higher Ca^{2+} and that niche factors favor $[Ca^{2+}]_c$ elevation to sustain quiescence (possibly via Calmodulin/CaMKs pathway).

As we still do not know amplitude, frequency, and duration of these Ca^{2+} signals or if these are different from apparent steady state conditions, the two model might be coexisting.

Two major lines of evidence are now required: (1) direct measurements of Ca^{2+} dynamics via the use of high performance genetically encoded calcium indicators (GECI) in cells from different stages of hematopoiesis and (2) investigate HSC function in models that specifically ablate fundamental members of the Ca^{2+} toolkit.

Indeed, many evidence are collected by investigating molecular actors which participates in multiple signaling pathways other than Ca^{2+}. Of extreme interest will be to understand the HSC function in transgenic animals which inactivates MCU, the mitochondrial Ca^{2+} exchanger NCLX (involved in the off-phase of mitochondrial Ca^{2+} signals, Fig. 1) or the ER channels Ip3Rs and ryanodine receptors (Fig. 1).

This information will allow to better define the mechanism of HSC regulation, especially in the definition of therapeutic regiment for hematological disease. Indeed, we have now available multiple compounds that can selectively inactivate the MCU or NCLX which should reduce or elevate $[Ca^{2+}]_m$, respectively. Of interest, Mitoxantrone, an anthracenedione structurally related to the anthracycline antibiotics, is recognized as a useful drug in first line therapy against AML (Patzke and Emadi, 2020) and have been recently reported as a potent inhibitor of MCU (Arduino et al., 2017).

Future investigation then might expose unexpected therapeutic strategies, based on the manipulation of mitochondrial Ca^{2+} signaling, for the treatment of hematological malignancies as well as improving the success of BM or cord blood transplantation.

Author contributions

M.B. and P.P. conceived the article; M.B. and A.K. wrote the first version of the manuscript with constructive input from P.P.; A.K. prepared display items under the supervision of M.B. and P.P. Figures are original and have not been published before. All authors reviewed and edited the manuscript before submission. All authors have read and agreed to the published version of the manuscript.

Funding

P.P. is grateful to Camilla degli Scrovegni for continuous support. The Signal Transduction Laboratory is supported by the Italian Association for Cancer Research Grant IG-23670 (to P.P.) and Grant IG-19803 (to C.G.), A-ROSE, Telethon Grant GGP11139B (to P.P.); Progetti di Rilevante Interesse Nazionale Grants PRIN2017E5L5P3 (to P.P.) and PRIN20177E9EPY (to C.G.); Italian Ministry of Health Grant GR-2013-02356747 (to C.G.); local funds from the University of Ferrara (to P.P. and M.B.).

Conflicts of interest

The authors declare no conflict of interest.

References

Abkowitz, J.L., Robinson, A.E., Kale, S., Long, M.W., Chen, J., 2003. Mobilization of hematopoietic stem cells during homeostasis and after cytokine exposure. Blood 102, 1249–1253. https://doi.org/10.1182/blood-2003-01-0318.

Adachi, S., Cross, A.R., Babior, B.M., Gottlieb, R.A., 1997. Bcl-2 and the outer mitochondrial membrane in the inactivation of cytochrome c during Fas-mediated apoptosis. J. Biol. Chem. 272, 21878–21882. https://doi.org/10.1074/jbc.272.35.21878.

Adamiak, M., Bujko, K., Cymer, M., Plonka, M., Glaser, T., Kucia, M., Ratajczak, J., Ulrich, H., Abdel-Latif, A., Ratajczak, M.Z., 2018. Novel evidence that extracellular nucleotides and purinergic signaling induce innate immunity-mediated mobilization of hematopoietic stem/progenitor cells. Leukemia 32, 1920–1931. https://doi.org/10.1038/s41375-018-0122-0.

Adams, G.B., Chabner, K.T., Alley, I.R., Olson, D.P., Szczepiorkowski, Z.M., Poznansky, M.C., Kos, C.H., Pollak, M.R., Brown, E.M., Scadden, D.T., 2006. Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. Nature 439, 599–603. https://doi.org/10.1038/nature04247.

Ansó, E., Weinberg, S.E., Diebold, L.P., Thompson, B.J., Malinge, S., Schumacker, P.T., Liu, X., Zhang, Y., Shao, Z., Steadman, M., Marsh, K.M., Xu, J., Crispino, J.D., Chandel, N.S., 2017. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. Nat. Cell Biol. 19, 614–625. https://doi.org/10.1038/ncb3529.

Arai, F., Hirao, A., Ohmura, M., Sato, H., Matsuoka, S., Takubo, K., Ito, K., Koh, G.Y., Suda, T., 2004. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 118, 149–161. https://doi.org/10.1016/j.cell.2004.07.004.

Arai, F., Hirao, A., Suda, T., 2005. Regulation of hematopoietic stem cells by the niche. Trends Cardiovasc. Med. 15, 75–79. https://doi.org/10.1016/j.tcm.2005.03.002.

Arduino, D.M., Wettmarshausen, J., Vais, H., Navas-Navarro, P., Cheng, Y., Leimpek, A., Ma, Z., Delrio-Lorenzo, A., Giordano, A., Garcia-Perez, C., Médard, G., Kuster, B., García-Sancho, J., Mokranjac, D., Foskett, J.K., Alonso, M.T., Perocchi, F., 2017. Systematic identification of MCU modulators by orthogonal interspecies chemical screening. Mol. Cell 67, 711–723.e7. https://doi.org/10.1016/j.molcel.2017.07.019.

Asada, N., Kunisaki, Y., Pierce, H., Wang, Z., Fernandez, N.F., Birbrair, A., Ma'ayan, A., Frenette, P.S., 2017. Differential cytokine contributions of perivascular haematopoietic stem cell niches. Nat. Cell Biol. 19, 214–223. https://doi.org/10.1038/ncb3475.

Baccin, C., Al-Sabah, J., Velten, L., Helbling, P.M., Grünschläger, F., Hernández-Malmierca, P., Nombela-Arrieta, C., Steinmetz, L.M., Trumpp, A., Haas, S., 2020. Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization. Nat. Cell Biol. 22, 38–48. https://doi.org/10.1038/s41556-019-0439-6.

Baldridge, M.T., King, K.Y., Boles, N.C., Weksberg, D.C., Goodell, M.A., 2010. Quiescent haematopoietic stem cells are activated by IFN-γ in response to chronic infection. Nature 465, 793–797. https://doi.org/10.1038/nature09135.

Barbosa, C.M., Leon, C.M., Nogueira-Pedro, A., Wasinsk, F., Araujo, R.C., Miranda, A., Ferreira, A.T., Paredes-Gamero, E.J., 2011. Differentiation of hematopoietic stem cell and myeloid populations by ATP is modulated by cytokines. Cell Death Dis. 2, e165. https://doi.org/10.1038/cddis.2011.49.

Batsivari, A., Haltalli, M.L.R., Passaro, D., Pospori, C., Lo Celso, C., Bonnet, D., 2020. Dynamic responses of the haematopoietic stem cell niche to diverse stresses. Nat. Cell Biol. 22, 7–17. https://doi.org/10.1038/s41556-019-0444-9.

Beals, C.R., Clipstone, N.A., Ho, S.N., Crabtree, G.R., 1997. Nuclear localization of NF-ATc by a calcineurin-dependent, cyclosporin-sensitive intramolecular interaction. Genes Dev. 11, 824–834. https://doi.org/10.1101/gad.11.7.824.

Bejarano-García, J.A., Millán-Uclés, Á., Rosado, I.V., Sánchez-Abarca, L.I., Caballero-Velázquez, T., Durán-Galván, M.J., Pérez-Simón, J.A., Piruat, J.I., 2016. Sensitivity of hematopoietic stem cells to mitochondrial dysfunction by SdhD gene deletion. Cell Death Dis. 7, e2516. https://doi.org/10.1038/cddis.2016.411.

Bittremieux, M., Parys, J.B., Pinton, P., Bultynck, G., 2016. ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca2+ signaling. Biochim. Biophys. Acta 1863, 1364–1378. https://doi.org/10.1016/j.bbamcr.2016.01.002.

Bononi, A., Bonora, M., Marchi, S., Missiroli, S., Poletti, F., Giorgi, C., Pandolfi, P.P.P., Pinton, P., 2013. Identification of PTEN at the ER and MAMs and its regulation of Ca(2+) signaling and apoptosis in a protein phosphatase-dependent manner. Cell Death Differ. 20, 1631–1643. https://doi.org/10.1038/cdd.2013.77.

Bonora, M., Giorgi, C., Bononi, A., Marchi, S., Patergnani, S., Rimessi, A., Rizzuto, R., Pinton, P., 2013. Subcellular calcium measurements in mammalian cells using jellyfish photoprotein aequorin-based probes. Nat. Protoc. 8, 2105–2118. https://doi.org/10.1038/nprot.2013.127.

Bonora, M., Wieckowski, M.R.R., Chinopoulos, C., Kepp, O., Kroemer, G., Galluzzi, L., Pinton, P., 2015. Molecular mechanisms of cell death: central implication of ATP synthase in mitochondrial permeability transition. Oncogene 34, 1475–1486. https://doi.org/10.1038/onc.2014.96.

Bonora, M., Ito, K.K., Morganti, C., Pinton, P., Ito, K.K., 2018. Membrane-potential compensation reveals mitochondrial volume expansion during HSC commitment. Exp. Hematol. 68, 30–37.e1. https://doi.org/10.1016/j.exphem.2018.10.012.

Bouchet, S., Tang, R., Fava, F., Legrand, O., Bauvois, B., 2016. The CNGRC-GG-D (KLAKLAK)2 peptide induces a caspase-independent, Ca2+-dependent death in human leukemic myeloid cells by targeting surface aminopeptidase N/CD13. Oncotarget 7, 19445–19467. https://doi.org/10.18632/oncotarget.6523.

Bowie, M.B., McKnight, K.D., Kent, D.G., McCaffrey, L., Hoodless, P.A., Eaves, C.J., 2006. Hematopoietic stem cells proliferate until after birth and show a reversible phase-specific engraftment defect. J. Clin. Invest. 116, 2808–2816. https://doi.org/10.1172/JCI28310.

Bowman, R.L., Busque, L., Levine, R.L., 2018. Clonal hematopoiesis and evolution to hematopoietic malignancies. Cell Stem Cell 22, 157–170. https://doi.org/10.1016/j.stem.2018.01.011.

Cabezas-Wallscheid, N., Buettner, F., Sommerkamp, P., Klimmeck, D., Ladel, L., Thalheimer, F.B., Pastor-Flores, D., Roma, L.P., Renders, S., Zeisberger, P., Przybylla, A., Schönberger, K., Scognamiglio, R., Altamura, S., Florian, C.M., Fawaz, M., Vonficht, D., Tesio, M., Collier, P., Pavlinic, D., Geiger, H., Schroeder, T., Benes, V., Dick, T.P., Rieger, M.A., Stegle, O., Trumpp, A., 2017. Vitamin A-retinoic acid signaling regulates hematopoietic stem cell dormancy. Cell 169, 807–823.e19. https://doi.org/10.1016/j.cell.2017.04.018.

Calì, T., Ottolini, D., Negro, A., Brini, M., 2013. Enhanced parkin levels favor ER-mitochondria crosstalk and guarantee Ca2+ transfer to sustain cell bioenergetics. Biochim. Biophys. Acta, Mol. Basis Dis. 1832, 495–508. https://doi.org/10.1016/j.bbadis.2013.01.004.

Campbell, C.J.V., Lee, J.B., Levadoux-Martin, M., Wynder, T., Xenocostas, A., Leber, B., Bhatia, M., 2010. The human stem cell hierarchy is defined by a functional dependence on Mcl-1 for self-renewal capacity. Blood 116, 1433–1442. https://doi.org/10.1182/blood-2009-12-258095.

Cancilla, D., Rettig, M.P., DiPersio, J.F., 2020. Targeting CXCR4 in AML and ALL. Front. Oncol. 10, 1672. https://doi.org/10.3389/fonc.2020.01672.

Cárdenas, C., Foskett, J.K., 2012. Mitochondrial Ca(2+) signals in autophagy. Cell Calcium 52, 44–51. https://doi.org/10.1016/j.ceca.2012.03.001.

Chavez-Abiega, S., Mos, I., Centeno, P.P., Elajnaf, T., Schlattl, W., Ward, D.T., Goedhart, J., Kallay, E., 2020. Sensing extracellular calcium—an insight into the structure and function of the calcium-sensing receptor (CaSR). In: Advances in Experimental Medicine and Biology. Springer, New York LLC, pp. 1031–1063. https://doi.org/10.1007/978-3-030-12457-1_41.

Chen, C., Liu, Y., Liu, R., Ikenoue, T., Guan, K.-L., Liu, Y., Zheng, P., 2008. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J. Exp. Med. 205, 2397–2408. https://doi.org/10.1084/jem.20081297.

Chen, J., Kao, Y.-R., Sun, D., Todorova, T.I., Reynolds, D., Narayanagari, S.-R., Montagna, C., Will, B., Verma, A., Steidl, U., 2019. Myelodysplastic syndrome progression to acute myeloid leukemia at the stem cell level. Nat. Med. 25, 103–110. https://doi.org/10.1038/s41591-018-0267-4.

Cimmino, L., Aifantis, I., 2017. Alternative roles for oxidized mCs and TETs. Curr. Opin. Genet. Dev. 42, 1–7. https://doi.org/10.1016/j.gde.2016.11.003.

Corces-Zimmerman, M.R., Hong, W.J., Weissman, I.L., Medeiros, B.C., Majeti, R., 2014. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc. Natl. Acad. Sci. U. S. A. 111, 2548–2553. https://doi.org/10.1073/pnas.1324297111.

Coutu, D.L., Kokkaliaris, K.D., Kunz, L., Schroeder, T., 2017. Three-dimensional map of nonhematopoietic bone and bone-marrow cells and molecules. Nat. Biotechnol. 35, 1202–1210. https://doi.org/10.1038/nbt.4006.

Ding, L., Saunders, T.L., Enikolopov, G., Morrison, S.J., 2012. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature 481, 457–462. https://doi.org/10.1038/nature10783.

Domen, J., Cheshier, S.H., Weissman, I.L., 2000. The role of apoptosis in the regulation of hematopoietic stem cells: overexpression of Bcl-2 increases both their number and repopulation potential. J. Exp. Med. 191, 253–264. https://doi.org/10.1084/jem.191.2.253.

Eckenrode, E.F., Yang, J., Velmurugan, G.V., Kevin Foskett, J., White, C., 2010. Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca2+ signaling. J. Biol. Chem. 285, 13678–13684. https://doi.org/10.1074/jbc.M109.096040.

Ellis, S.L., Grassinger, J., Jones, A., Borg, J., Camenisch, T., Haylock, D., Bertoncello, I., Nilsson, S.K., 2011. The relationship between bone, hemopoietic stem cells, and vasculature. Blood 118, 1516–1524. https://doi.org/10.1182/blood-2010-08-303800.

Favaro, G., Romanello, V., Varanita, T., Andrea Desbats, M., Morbidoni, V., Tezze, C., Albiero, M., Canato, M., Gherardi, G., De Stefani, D., Mammucari, C., Blaauw, B., Boncompagni, S., Protasi, F., Reggiani, C., Scorrano, L., Salviati, L., Sandri, M., 2019. DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Nat. Commun. 10, 2576. https://doi.org/10.1038/s41467-019-10226-9.

Fukushima, T., Tanaka, Y., Hamey, F.K., Chang, C.H., Oki, T., Asada, S., Hayashi, Y., Fujino, T., Yonezawa, T., Takeda, R., Kawabata, K.C., Fukuyama, T., Umemoto, T., Takubo, K., Takizawa, H., Goyama, S., Ishihama, Y., Honda, H., Göttgens, B., Kitamura, T., 2019. Discrimination of dormant and active hematopoietic stem cells by G0 marker reveals dormancy regulation by cytoplasmic calcium. Cell Rep. 29, 4144–4158.e7. https://doi.org/10.1016/j.celrep.2019.11.061.

Ge, C., Huang, H., Huang, F., Yang, T., Zhang, T., Wu, H., Zhou, H., Chen, Q., Shi, Y., Sun, Y., Liu, L., Wang, X., Pearson, R.B., Cao, Y., Kang, J., Fu, C., 2019.

Neurokinin-1 receptor is an effective target for treating leukemia by inducing oxidative stress through mitochondrial calcium overload. Proc. Natl. Acad. Sci. U. S. A. 116, 19635–19645. https://doi.org/10.1073/pnas.1908998116.

Giorgi, C., Ito, K., Lin, H.K., Santangelo, C., Wieckowski, M.R., Lebiedzinska, M., Bononi, A., Bonora, M., Duszynski, J., Bernardi, R., Rizzuto, R., Tacchetti, C., Pinton, P., Pandolfi, P.P., 2010. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. Science 330, 1247–1251. https://doi.org/10.1126/science.1189157.

Giorgi, C., Bonora, M., Sorrentino, G., Missiroli, S., Poletti, F., Suski, J.M., Ramirez, F.G., Rizzuto, R., Di Virgilio, F., Zito, E., Pandolfi, P.P., Wieckowski, M.R., Mammano, F., Del Sal, G., Pinton, P., 2015a. P53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner. Proc. Natl. Acad. Sci. U. S. A. 112, 1779–1784. https://doi.org/10.1073/pnas.1410723112.

Giorgi, C., Bonora, M., Missiroli, S., Poletti, F., Ramirez, F.G.F.G., Morciano, G., Morganti, C., Pandolfi, P.P.P.P., Mammano, F., Pinton, P., 2015b. Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling. Oncotarget 6, 1435–1445. https://doi.org/10.18632/oncotarget.2935.

Gomez-Suaga, P., Paillusson, S., Stoica, R., Noble, W., Hanger, D.P., Miller, C.C.J., 2017. The ER-mitochondria tethering complex VAPB-PTPIP51 regulates autophagy. Curr. Biol. 27, 371–385. https://doi.org/10.1016/j.cub.2016.12.038.

Graf, T., 2002. Differentiation plasticity of hematopoietic cells. Blood 99, 3089–3101. https://doi.org/10.1182/blood.V99.9.3089.

Grahn, T.H.M., Niroula, A., Végvári, Á., Oburoglu, L., Pertesi, M., Warsi, S., Safi, F., Miharada, N., Garcia, S.C., Siva, K., Liu, Y., Rörby, E., Nilsson, B., Zubarev, R.A., Karlsson, S., 2020. S100A6 is a critical regulator of hematopoietic stem cells. Leukemia 34, 3323–3337. https://doi.org/10.1038/s41375-020-0901-2.

Gurumurthy, S., Xie, S.Z., Alagesan, B., Kim, J., Yusuf, R.Z., Saez, B., Tzatsos, A., Ozsolak, F., Milos, P., Ferrari, F., Park, P.J., Shirihai, O.S., Scadden, D.T., Bardeesy, N., 2010. The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. Nature 468, 659–663. https://doi.org/10.1038/nature09572.

Hadad, I., Veithen, A., Springael, J.Y., Sotiropoulou, P.A., Mendes Da Costa, A., Miot, F., Naeije, R., De Deken, X., Entee, K.M., 2013. Stroma cell-derived factor-1α signaling enhances calcium transients and beating frequency in rat neonatal cardiomyocytes. PLoS One 8, e56007. https://doi.org/10.1371/journal.pone.0056007.

Hatse, S., Princen, K., Bridger, G., De Clercq, E., Schols, D., 2002. Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. FEBS Lett. 527, 255–262. https://doi.org/10.1016/S0014-5793(02)03143-5.

Heidel, F.H., Mar, B.G., Armstrong, S.A., 2011. Self-renewal related signaling in myeloid leukemia stem cells. Int. J. Hematol. 94, 109–117. https://doi.org/10.1007/s12185-011-0901-0.

Herviou, L., Cavalli, G., Cartron, G., Klein, B., Moreaux, J., 2016. EZH2 in normal hematopoiesis and hematological malignancies. Oncotarget 7, 2284–2296. https://doi.org/10.18632/oncotarget.6198.

Hinge, A., He, J., Bartram, J., Javier, J., Xu, J., Fjellman, E., Sesaki, H., Li, T., Yu, J., Wunderlich, M., Mulloy, J., Kofron, M., Salomonis, N., Grimes, H.L., Filippi, M.D., 2020. Asymmetrically segregated mitochondria provide cellular memory of hematopoietic stem cell replicative history and drive HSC attrition. Cell Stem Cell 26, 420–430.e6. https://doi.org/10.1016/j.stem.2020.01.016.

Ho, T.T., Warr, M.R., Adelman, E.R., Lansinger, O.M., Flach, J., Verovskaya, E.V., Figueroa, M.E., Passegué, E., 2017. Autophagy maintains the metabolism and function of young and old stem cells. Nature 543, 205–210. https://doi.org/10.1038/nature21388.

Hogan, P.G., Chen, L., Nardone, J., Rao, A., 2003. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev. 17, 2205–2232. https://doi.org/10.1101/gad.1102703.

Horikawa, K., 2015. Recent progress in the development of genetically encoded Ca2+ indicators. J. Med. Invest. 62, 24–28. https://doi.org/10.2152/jmi.62.24.

Høyer, M., Nielsen, O.S., 1992. Influence of dose on regeneration of murine hematopoietic stem cells after total body irradiation and 5-fluorouracil. Oncologia 49, 166–172. https://doi.org/10.1159/000227033.

Hübel, K., Liles, W.C., Broxmeyer, H.E., Rodger, E., Wood, B., Cooper, S., Hangoc, G., MacFarland, R., Bridger, G.J., Henson, G.W., Calandra, G., Dale, D.C., 2004. Leukocytosis and mobilization of CD34+ hematopoietic progenitor cells by AMD3100, a CXCR4 antagonist. Support. Cancer Ther. 1, 165–172. https://doi.org/10.3816/sct.2004.n.008.

Ito, K., Ito, K., 2018. Hematopoietic stem cell fate through metabolic control. Exp. Hematol. 64, 1–11. https://doi.org/10.1016/j.exphem.2018.05.005.

Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., Ikeda, Y., Mak, T.W., Suda, T., 2004. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 431, 997–1002. https://doi.org/10.1038/nature02989.

Ito, K., Hirao, A., Arai, F., Takubo, K., Matsuoka, S., Miyamoto, K., Ohmura, M., Naka, K., Hosokawa, K., Ikeda, Y., Suda, T., 2006. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat. Med. 12, 446–451. https://doi.org/10.1038/nm1388.

Ito, K., Bernardi, R., Morotti, A., Matsuoka, S., Saglio, G., Ikeda, Y., Rosenblatt, J., Avigan, D.E., Teruya-Feldstein, J., Pandolfi, P.P., 2008. PML targeting eradicates quiescent leukaemia-initiating cells. Nature 453, 1072–1078. https://doi.org/10.1038/nature07016.

Ito, K., Carracedo, A., Weiss, D., Arai, F., Ala, U., Avigan, D.E., Schafer, Z.T., Evans, R.M., Suda, T., Lee, C.-H., Pandolfi, P.P., 2012. A PML–PPAR-δ pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat. Med. 18, 1350–1358. https://doi.org/10.1038/nm.2882.

Ito, K., Turcotte, R., Cui, J., Zimmerman, S.E., Pinho, S., Mizoguchi, T., Arai, F., Runnels, J.M., Alt, C., Teruya-Feldstein, J., Mar, J.C., Singh, R., Suda, T., Lin, C.P., Frenette, P.S., Ito, K., 2016. Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. Science 354, 1156–1160. https://doi.org/10.1126/science.aaf5530.

Ito, K., Bonora, M., Ito, K., 2019. Metabolism as master of hematopoietic stem cell fate. Int. J. Hematol. 109, 18–27. https://doi.org/10.1007/s12185-018-2534-z.

Jilg, S., Reidel, V., Müller-Thomas, C., König, J., Schauwecker, J., Höckendorf, U., Huberle, C., Gorka, O., Schmidt, B., Burgkart, R., Ruland, J., Kolb, H.J., Peschel, C., Oostendorp, R.A.J., Götze, K.S., Jost, P.J., 2016. Blockade of BCL-2 proteins efficiently induces apoptosis in progenitor cells of high-risk myelodysplastic syndromes patients. Leukemia 30, 112–123. https://doi.org/10.1038/leu.2015.179.

Jin, G., Xu, C., Zhang, X., Long, J., Rezaeian, A.H., Liu, C., Furth, M.E., Kridel, S., Pasche, B., Bian, X.W., Lin, H.K., 2018. Atad3a suppresses Pink1-dependent mitophagy to maintain homeostasis of hematopoietic progenitor cells article. Nat. Immunol. 19, 29–40. https://doi.org/10.1038/s41590-017-0002-1.

Katayama, Y., Battista, M., Kao, W.M., Hidalgo, A., Peired, A.J., Thomas, S.A., Frenette, P.S., 2006. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 124, 407–421. https://doi.org/10.1016/j.cell.2005.10.041.

Kim, T., Tyndel, M.S., Kim, H.J., Ahn, J.S., Choi, S.H., Park, H.J., Kim, Y.K., Yang, D.H., Lee, J.J., Jung, S.H., Kim, S.Y., Min, Y.H., Cheong, J.W., Sohn, S.K., Moon, J.H., Choi, M., Lee, M., Zhang, Z., Kim, D.D.H., 2017. The clonal origins of leukemic progression of myelodysplasia. Leukemia 31, 1928–1935. https://doi.org/10.1038/leu.2017.17.

Kitsos, C.M., Sankar, U., Illario, M., Colomer-Font, J.M., Duncan, A.W., Ribar, T.J., Reya, T., Means, A.R., 2005. Calmodulin-dependent protein kinase IV regulates hematopoietic stem cell maintenance. J. Biol. Chem. 280, 33101–33108. https://doi.org/10.1074/jbc.M505208200.

Ko, M., Bandukwala, H.S., An, J., Lamperti, E.D., Thompson, E.C., Hastie, R., Tsangaratou, A., Rajewsky, K., Koralov, S.B., Rao, A., 2011. Ten-eleven-translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. Proc. Natl. Acad. Sci. U. S. A. 108, 14566–14571. https://doi.org/10.1073/pnas.1112317108.

Koeffler, H.P., Leong, G., 2017. Preleukemia: one name, many meanings. Leukemia 31, 534–542. https://doi.org/10.1038/leu.2016.364.

Kollek, M., Müller, A., Egle, A., Erlacher, M., 2016. Bcl-2 proteins in development, health, and disease of the hematopoietic system. FEBS J. 283, 2779–2810. https://doi.org/10.1111/febs.13683.

Lam, B.S., Cunningham, C., Adams, G.B., 2011. Pharmacologic modulation of the calcium-sensing receptor enhances hematopoietic stem cell lodgment in the adult bone marrow. Blood 117, 1167–1175. https://doi.org/10.1182/blood-2010-05-286294.

Li, Z., Wang, J., Xu, M., Cai, X., Cai, C.-L., Zhang, W., Petersen, B.E., Yang, F.-C., 2011. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood 118, 4509–4518. https://doi.org/10.1182/blood-2010-12-325241.

Lindsley, R.C., Mar, B.G., Mazzola, E., Grauman, P.V., Shareef, S., Allen, S.L., Pigneux, A., Wetzler, M., Stuart, R.K., Erba, H.P., Damon, L.E., Powell, B.L., Lindeman, N., Steensma, D.P., Wadleigh, M., DeAngelo, D.J., Neuberg, D., Stone, R.M., Ebert, B.L., 2015. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood 125, 1367–1376. https://doi.org/10.1182/blood-2014-11-610543.

Liu, Y., Elf, S.E., Miyata, Y., Sashida, G., Liu, Y., Huang, G., Di Giandomenico, S., Lee, J.M., Deblasio, A., Menendez, S., Antipin, J., Reva, B., Koff, A., Nimer, S.D., 2009. p53 regulates hematopoietic stem cell quiescence. Cell Stem Cell 4, 37–48. https://doi.org/10.1016/j.stem.2008.11.006.

Luchsinger, L.L., de Almeida, M.J., Corrigan, D.J., Mumau, M., Snoeck, H.-W., 2016. Mitofusin 2 maintains haematopoietic stem cells with extensive lymphoid potential. Nature 529, 528–531. https://doi.org/10.1038/nature16500.

Luchsinger, L.L., Strikoudis, A., Danzl, N.M., Bush, E.C., Finlayson, M.O., Satwani, P., Sykes, M., Yazawa, M., Snoeck, H.W., 2019. Harnessing hematopoietic stem cell low intracellular calcium improves their maintenance in vitro. Cell Stem Cell 25, 225–240.e7. https://doi.org/10.1016/j.stem.2019.05.002.

Makishima, H., Yoshizato, T., Yoshida, K., Sekeres, M.A., Radivoyevitch, T., Suzuki, H., Przychodzen, B.J., Nagata, Y., Meggendorfer, M., Sanada, M., Okuno, Y., Hirsch, C., Kuzmanovic, T., Sato, Y., Sato-Otsubo, A., Laframboise, T., Hosono, N., Shiraishi, Y., Chiba, K., Haferlach, C., Kern, W., Tanaka, H., Shiozawa, Y., Gómez-Seguí, I., Husseinzadeh, H.D., Thota, S., Guinta, K.M., Dienes, B., Nakamaki, T., Miyawaki, S., Sauntharajah, Y., Chiba, S., Miyano, S., Shih, L.Y., Haferlach, T., Ogawa, S., MacIejewski, J.P., 2017. Dynamics of clonal evolution in myelodysplastic syndromes. Nat. Genet. 49, 204–212. https://doi.org/10.1038/ng.3742.

Mansell, E., Sigurdsson, V., Deltcheva, E., Brown, J., James, C., Miharada, K., Soneji, S., Larsson, J., Enver, T., 2021. Mitochondrial potentiation ameliorates age-related heterogeneity in hematopoietic stem cell function. Cell Stem Cell 28, 241–256.e6. https://doi.org/10.1016/j.stem.2020.09.018.

Marchetti, P., Castedo, M., Susin, S.A., Zamzami, N., Hirsch, T., Macho, A., Haeffner, A., Hirsch, F., Geuskens, M., Kroemer, G., 1996. Mitochondrial permeability transition is a central coordinating event of apoptosis. J. Exp. Med. 184, 1155–1160. https://doi.org/10.1084/jem.184.3.1155.

Marchi, S., Giorgi, C., Galluzzi, L., Pinton, P., 2020. Ca2+ fluxes and cancer. Mol. Cell 78, 1055–1069. https://doi.org/10.1016/j.molcel.2020.04.017.

Matoba, S., Kang, J.-G.G., Patino, W.D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P.J., Bunz, F., Hwang, P.M., 2006. p53 regulates mitochondrial respiration. Science 312, 1650–1653. https://doi.org/10.1126/science.1126863.

Metts, J., Bradley, H.L., Wang, Z., Shah, N.P., Kapur, R., Arbiser, J.L., Bunting, K.D., 2017. Imipramine blue sensitively and selectively targets FLT3-ITD positive acute myeloid leukemia cells. Sci. Rep. 7, 1–10. https://doi.org/10.1038/s41598-017-04796-1.

Missiroli, S., Bonora, M., Patergnani, S., Poletti, F., Perrone, M., Gafà, R., Magri, E., Raimondi, A., Lanza, G., Tacchetti, C., Kroemer, G., Pandolfi, P.P., Pinton, P., Giorgi, C., 2016. PML at mitochondria-associated membranes is critical for the repression of autophagy and cancer development. Cell Rep. 16, 2415–2427. https://doi.org/10.1016/j.celrep.2016.07.082.

Moignard, V., Macaulay, I.C., Swiers, G., Buettner, F., Schütte, J., Calero-Nieto, F.J., Kinston, S., Joshi, A., Hannah, R., Theis, F.J., Jacobsen, S.E., de Bruijn, M.F., Göttgens, B., 2013. Characterization of transcriptional networks in blood stem and progenitor cells using high-throughput single-cell gene expression analysis. Nat. Cell Biol. 15, 363–372. https://doi.org/10.1038/ncb2709.

Morganti, C., Bonora, M., Ito, K., Ito, K., 2019a. Electron transport chain complex II sustains high mitochondrial membrane potential in hematopoietic stem and progenitor cells. Stem Cell Res. 40, 101573. https://doi.org/10.1016/j.scr.2019.101573.

Morganti, C., Bonora, M., Ito, K., 2019b. Improving the accuracy of flow cytometric assessment of mitochondrial membrane potential in hematopoietic stem and progenitor cells through the inhibition of efflux pumps. J. Vis. Exp. 149. https://doi.org/10.3791/60057.

Morita, Y., Ema, H., Yamazaki, S., Nakauch, H., 2006. Non-side-population hematopoietic stem cells in mouse bone marrow. Blood 108, 2850–2856. https://doi.org/10.1182/blood-2006-03-010207.

Morotti, A., Panuzzo, C., Crivellaro, S., Carrà, G., Torti, D., Guerrasio, A., Saglio, G., 2015. The role of PTEN in myeloid malignancies. Hematol. Rep. 7, 5844. https://doi.org/10.4081/hr.2015.6027.

Morrison, S.J., Scadden, D.T., 2014. The bone marrow niche for haematopoietic stem cells. Nature 505, 327–334. https://doi.org/10.1038/nature12984.

Nagata, Y., Maciejewski, J.P., 2019. The functional mechanisms of mutations in myelodysplastic syndrome. Leukemia 33, 2779–2794. https://doi.org/10.1038/s41375-019-0617-3.

Nakamura-Ishizu, A., Ito, K., Suda, T., 2020. Hematopoietic stem cell metabolism during development and aging. Dev. Cell 54, 239–255. https://doi.org/10.1016/j.devcel.2020.06.029.

Nie, Y., Han, Y.C., Zou, Y.R., 2008. CXCR4 is required for the quiescence of primitive hematopoietic cells. J. Exp. Med. 205, 777–783. https://doi.org/10.1084/jem.20072513.

Ogawa, M., 1993. Differentiation and proliferation of hematopoietic stem cells. Blood 81, 2844–2853. https://doi.org/10.1182/blood.v81.11.2844.2844.

Oguro, H., Ding, L., Morrison, S.J., 2013. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell 13, 102–116. https://doi.org/10.1016/j.stem.2013.05.014.

Opferman, J.T., Iwasaki, H., Ong, C.C., Suh, H., Mizuno, S.I., Akashi, K., Korsmeyer, S.J., 2005. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science 307, 1101–1104. https://doi.org/10.1126/science.1106114.

Pandolfi, A., Barreyro, L., Steidl, U., 2013. Concise review: preleukemic stem cells: molecular biology and clinical implications of the precursors to Leukemia stem cells. Stem Cells Transl. Med. 2, 143–150. https://doi.org/10.5966/sctm.2012-0109.

Paredes-Gamero, E.J., Leon, C.M.M.P., Borojevic, R., Oshiro, M.E.M., Ferreira, A.T., 2008. Changes in intracellular Ca2+ levels induced by cytokines and P2 agonists differentially modulate proliferation or commitment with macrophage differentiation in murine hematopoietic cells. J. Biol. Chem. 283, 31909–31919. https://doi.org/10.1074/jbc.M801990200.

Park, C.S., Lewis, A., Chen, T., Lacorazza, D., 2019. Concise review: regulation of self-renewal in normal and malignant hematopoietic stem cells by Krüppel-like factor 4. Stem Cells Transl. Med. 8, 568–574. https://doi.org/10.1002/sctm.18-0249.

Patzke, C.L., Emadi, A., 2020. High dose cytarabine, mitoxantrone, pegasapargase (HAM-pegA) in combination with dasatinib for the first-line treatment of Philadelphia chromosome positive mixed phenotype acute leukemia. Am. J. Leuk. Res. 4.

Petit, I., Ponomaryov, T., Zipori, D., Tsvee, L., 2002. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat. Immunol. 3, 687–694. https://doi.org/10.1038/ni813.

Pinho, S., Frenette, P.S., 2019. Haematopoietic stem cell activity and interactions with the niche. Nat. Rev. Mol. Cell Biol. 20, 303–320. https://doi.org/10.1038/s41580-019-0103-9.

Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F., Pozzan, T., Rizzuto, R., 2001. The Ca2+ concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. EMBO J. 20, 2690–2701. https://doi.org/10.1093/emboj/20.11.2690.

Pinton, P., Giorgi, C., Pandolfi, P.P., 2011. The role of PML in the control of apoptotic cell fate: a new key player at ER-mitochondria sites. Cell Death Differ. 18, 1450–1456. https://doi.org/10.1038/cdd.2011.31.

Racioppi, L., Lento, W., Huang, W., Arvai, S., Doan, P.L., Harris, J.R., Marcon, F., Nakaya, H.I., Liu, Y., Chao, N., 2017. Calcium/calmodulin-dependent kinase kinase 2 regulates hematopoietic stem and progenitor cell regeneration. Cell Death Dis. 8, e3076. https://doi.org/10.1038/cddis.2017.474.

Rasmussen, K.D., Helin, K., 2016. Role of TET enzymes in DNA methylation, development, and cancer. Genes Dev. 30, 733–750. https://doi.org/10.1101/gad.276568.115.

Ratajczak, M.Z., Adamiak, M., Plonka, M., Abdel-Latif, A., Ratajczak, J., 2018. Mobilization of hematopoietic stem cells as a result of innate immunity-mediated sterile inflammation in the bone marrow microenvironment—the involvement of extracellular nucleotides and purinergic signaling. Leukemia 32, 1116–1123. https://doi.org/10.1038/s41375-018-0087-z.

Rimmelé, P., Liang, R., Bigarella, C.L., Kocabas, F., Xie, J., Serasinghe, M.N., Chipuk, J., Sadek, H., Zhang, C.C., Ghaffari, S., 2015. Mitochondrial metabolism in hematopoietic stem cells requires functional FOXO3. EMBO Rep. 16, 1164–1176. https://doi.org/10.15252/embr.201439704.

Rossi, A.M., Taylor, C.W., 2020. Reliable measurement of free Ca2+ concentrations in the ER lumen using Mag-Fluo-4. Cell Calcium 87, 102188. https://doi.org/10.1016/j.ceca.2020.102188.

Saeed, B.R., Manta, L., Raffel, S., Pyl, P.T., Buss, E.C., Wang, W., Eckstein, V., Jauch, A., Trumpp, A., Huber, W., Ho, A.D., Lutz, C., 2021. Analysis of nonleukemic cellular subcompartments reconstructs clonal evolution of acute myeloid leukemia and identifies therapy-resistant preleukemic clones. Int. J. Cancer 148, 2825–2838. https://doi.org/10.1002/ijc.33461.

Schoedel, K.B., Morcos, M.N.F., Zerjatke, T., Roeder, I., Grinenko, T., Voehringer, D., Göthert, J.R., Waskow, C., Roers, A., Gerbaulet, A., 2016. The bulk of the hematopoietic stem cell population is dispensable for murine steady-state and stress hematopoiesis. Blood 128, 2285–2296. https://doi.org/10.1182/blood-2016-03-706010.

Seita, J., Weissman, I.L., 2010. Hematopoietic stem cell: self-renewal versus differentiation. Wiley Interdiscip. Rev. Syst. Biol. Med. 2, 640–653. https://doi.org/10.1002/wsbm.86.

Shlush, L.I., Zandi, S., Mitchell, A., Chen, W.C., Brandwein, J.M., Gupta, V., Kennedy, J.A., Schimmer, A.D., Schuh, A.C., Yee, K.W., McLeod, J.L., Doedens, M., Medeiros, J.J.F., Marke, R., Kim, H.J., Lee, K., McPherson, J.D., Hudson, T.J., Pan-Leukemia Gene Panel Consortium, H.A.L.T., Brown, A.M.K., Yousif, F., Trinh, Q.M., Stein, L.D., Minden, M.D., Wang, J.C.Y., Dick, J.E., 2014. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 506, 328–333. https://doi.org/10.1038/nature13038.

Song, J., Kiel, M.J., Wang, Z., Wang, J., Taichman, R.S., Morrison, S.J., Krebsbach, P.H., 2010. An in vivo model to study and manipulate the hematopoietic stem cell niche. Blood 115, 2592–2600. https://doi.org/10.1182/blood-2009-01-200071.

Stevens, B.M., Khan, N., D'Alessandro, A., Nemkov, T., Winters, A., Jones, C.L., Zhang, W., Pollyea, D.A., Jordan, C.T., 2018. Characterization and targeting of malignant stem cells in patients with advanced myelodysplastic syndromes. Nat. Commun. 9, 1–14. https://doi.org/10.1038/s41467-018-05984-x.

Sugimura, R., He, X.C., Venkatraman, A., Arai, F., Box, A., Semerad, C., Haug, J.S., Peng, L., Zhong, X.B., Suda, T., Li, L., 2012. Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. Cell 150, 351–365. https://doi.org/10.1016/j.cell.2012.05.041.

Sugiyama, T., Kohara, H., Noda, M., Nagasawa, T., 2006. Maintenance of the hematopoietic stem cell Pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 25, 977–988. https://doi.org/10.1016/j.immuni.2006.10.016.

Szabadkai, G., Simoni, A.M., Chami, M., Wieckowski, M.R., Youle, R.J., Rizzuto, R., 2004. Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+-mediated apoptosis. Mol. Cell 16, 59–68. https://doi.org/10.1016/j.molcel.2004.09.026.

Tai-Nagara, I., Matsuoka, S., Ariga, H., Suda, T., 2014. Mortalin and DJ-1 coordinately regulate hematopoietic stem cell function through the control of oxidative stress. Blood 123, 41–50. https://doi.org/10.1182/blood-2013-06-508333.

Takubo, K., Goda, N., Yamada, W., Iriuchishima, H., Ikeda, E., Kubota, Y., Shima, H., Johnson, R.S., Hirao, A., Suematsu, M., Suda, T., 2010. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell 7, 391–402. https://doi.org/10.1016/j.stem.2010.06.020.

Takubo, K., Nagamatsu, G., Kobayashi, C.I., Nakamura-Ishizu, A., Kobayashi, H., Ikeda, E., Goda, N., Rahimi, Y., Johnson, R.S., Soga, T., Hirao, A., Suematsu, M., Suda, T., 2013. Regulation of glycolysis by Pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. Cell Stem Cell 12, 49–61. https://doi.org/10.1016/j.stem.2012.10.011.

Talos, F., Petrenko, O., Mena, P., Moll, U.M., 2005. Mitochondrially targeted p53 has tumor suppressor activities in vivo. Cancer Res. 65, 9971–9981. https://doi.org/10.1158/0008-5472.CAN-05-1084.

Testa, U., Lo-Coco, F., 2016. Prognostic factors in acute promyelocytic leukemia: strategies to define high-risk patients. Ann. Hematol. 95, 673–680. https://doi.org/10.1007/s00277-016-2622-1.

Thol, F., Klesse, S., Köhler, L., Gabdoulline, R., Kloos, A., Liebich, A., Wichmann, M., Chaturvedi, A., Fabisch, J., Gaidzik, V.I., Paschka, P., Bullinger, L., Bug, G., Serve, H., Göhring, G., Schlegelberger, B., Lübbert, M., Kirchner, H., Wattad, M., Kraemer, D., Hertenstein, B., Heil, G., Fiedler, W., Krauter, J., Schlenk, R.F., Döhner, K., Döhner, H., Ganser, A., Heuser, M., 2017. Acute myeloid leukemia derived from lympho-myeloid clonal hematopoiesis. Leukemia 31, 1286–1295. https://doi.org/10.1038/leu.2016.345.

Umemoto, T., Hashimoto, M., Matsumura, T., Nakamura-Ishizu, A., Suda, T., 2018. Ca2+-mitochondria axis drives cell division in hematopoietic stem cells. J. Exp. Med. 215, 2097–2113. https://doi.org/10.1084/jem.20180421.

Uslu, M., Albayrak, E., Kocabaş, F., 2020. Temporal modulation of calcium sensing in hematopoietic stem cells is crucial for proper stem cell expansion and engraftment. J. Cell. Physiol. 235, 9644–9666. https://doi.org/10.1002/jcp.29777.

Vaseva, A.V., Marchenko, N.D., Ji, K., Tsirka, S.E., Holzmann, S., Moll, U.M., 2012. p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell 149, 1536–1548. https://doi.org/10.1016/j.cell.2012.05.014.

Wang, Z., Ema, H., 2016. Mechanisms of self-renewal in hematopoietic stem cells. Int. J. Hematol. 103, 498–509. https://doi.org/10.1007/s12185-015-1919-5.

Xiang, Y., Li, Y., Zhang, Z., Cui, K., Wang, S., Yuan, X.B., Wu, C.P., Poo, M.M., Duan, S., 2002. Nerve growth cone guidance mediated by G protein-coupled receptors. Nat. Neurosci. 5, 843–848. https://doi.org/10.1038/nn899.

Xu, F., Yang, R., Wu, L., He, Q., Zhang, Z., Zhang, Q., Yang, Y., Guo, J., Chang, C., Li, X., 2012. Overexpression of BMI1 confers clonal cells resistance to apoptosis and contributes to adverse prognosis in myelodysplastic syndrome. Cancer Lett. 317, 33–40. https://doi.org/10.1016/j.canlet.2011.11.012.

Yamashita, M., Nitta, E., Suda, T., 2016. Regulation of hematopoietic stem cell integrity through p53 and its related factors. Ann. N. Y. Acad. Sci. 1370, 45–54. https://doi.org/10.1111/nyas.12986.

Yang, L., Wang, L., Geiger, H., Cancelas, J.A., Mo, J., Zheng, Y., 2007. Rho GTPase Cdc42 coordinates hematopoietic stem cell quiescence and niche interaction in the bone marrow. Proc. Natl. Acad. Sci. U. S. A. 104, 5091–5096. https://doi.org/10.1073/pnas.0610819104.

Zhang, J., Grindley, J.C., Yin, T., Jayasinghe, S., He, X.C., Ross, J.T., Haug, J.S., Rupp, D., Porter-Westpfahl, K.S., Wiedemann, L.M., Wu, H., Li, L., 2006. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature 441, 518–522. https://doi.org/10.1038/nature04747.

Zhang, X., Su, J., Jeong, M., Ko, M., Huang, Y., Park, H.J., Guzman, A., Lei, Y., Huang, Y.H., Rao, A., Li, W., Goodell, M.A., 2016. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. Nat. Genet. 48, 1014–1023. https://doi.org/10.1038/ng.3610.

Zhang, J., Wu, Q., Johnson, C.B., Pham, G., Kinder, J.M., Olsson, A., Slaughter, A., May, M., Weinhaus, B., D'Alessandro, A., Engel, J.D., Jiang, J.X., Kofron, J.M., Huang, L.F., Prasath, V.B.S., Way, S.S., Salomonis, N., Grimes, H.L., Lucas, D., 2021. In situ mapping identifies distinct vascular niches for myelopoiesis. Nature 590, 457–462. https://doi.org/10.1038/s41586-021-03201-2.

Zhao, J.L., Ma, C., O'Connell, R.M., Mehta, A., Diloreto, R., Heath, J.R., Baltimore, D., 2014. Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell Stem Cell 14, 445–459. https://doi.org/10.1016/j.stem.2014.01.007.

Zhou, S., Schuetz, J.D., Bunting, K.D., Colapietro, A.M., Sampath, J., Morris, J.J., Lagutina, I., Grosveld, G.C., Osawa, M., Nakauchi, H., Sorrentino, B.P., 2001. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat. Med. 7, 1028–1034.

Zhou, X., Ren, Y., Kong, L., Cai, G., Sun, S., Song, W., Wang, Y., Jin, R., Qi, L., Mei, M., Wang, X., Kang, C., Li, M., Zhang, L., 2015. Targeting EZH2 regulates tumor growth and apoptosis through modulating mitochondria dependent cell-death pathway in HNSCC. Oncotarget 6, 33720–33732. https://doi.org/10.18632/oncotarget.5606.