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REVIEW ARTICLE

Metabolomics of healthy hematopoietic stem cells and leukemia stem cells

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Abstract

Background: Hematopoietic stem cells (HSCs) reside in the bone marrow (BM) and sustain life-long hematopoiesis by balancing quiescence, self-renewal, and differentiation. A key feature distinguishing quiescent HSCs from their activated counterparts is a shift in their metabolic profile, including changes in glycolytic flux and mitochondrial oxidative metabolism. Disruption of HSC homeostasis can lead to hematologic diseases such as BM failure, clonal hematopoiesis, or oncogenic transformation into leukemia stem cells (LSCs). Similar to HSCs, LSCs retain stem-like characteristics but acquire malignant features, including drug resistance and a reprogrammed metabolism, which results in distinct metabolic profiles that contribute to their pathogenesis. **Aim:** This review summarizes the key metabolic characteristics distinguishing healthy quiescent and active HSCs from oncogenic LSCs. In addition, we highlight modern tools for investigating the metabolome, which enable the identification of novel metabolites, metabolic interactions, pathways, and potential targets for diagnosis or therapeutic intervention in hematologic diseases. **Conclusion:** Metabolic regulation is essential for maintaining HSC quiescence, self-renewal, and lineage commitment, whereas its disruption often underlies oncogenic transformation into LSCs. Advances in metabolic profiling reveal key differences between healthy HSCs and LSCs and identify LSC vulnerabilities that sustain survival and therapeutic resistance. Targeting these hijacked metabolic pathways may facilitate the development of LSC-specific treatment while preserving normal hematopoiesis. Further investigation of stem cell metabolism will be critical for translating these insights into effective treatments for hematologic malignancies. **Relevance for patients:** Understanding the metabolic profiles of healthy HSCs and LSCs can facilitate the development of innovative techniques, technologies, and therapeutics. These advances can be applied to the identification, treatment, and prevention of hematologic disease. By elucidating the metabolome of LSCs, therapies can be designed to selectively target their unique metabolic pathways, dependencies, and resistance mechanisms.

Keywords: Hematopoietic stem cells; Leukemia stem cells; Metabolism; Metabolomics

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1. Introduction

Hematopoiesis is a tightly regulated process that produces differentiated blood cells to meet the physiological demands of the human body.[1,2] Located within the human bone marrow (BM) niche, hematopoietic stem cells (HSCs) play pivotal roles in sustaining these demands by intricately balancing self-renewal and regenerative proliferation with differentiation and lineage commitment at an average rate of 1×10^6 cells/s.[1-4] At homeostasis, HSCs in the BM are maintained in a highly quiescent state, the G_0 phase of the cell cycle, and only enter proliferation or differentiation upon stimulation.[1,4,5] When stimulated by extrinsic and intrinsic factors (e.g., colony-stimulating factors, growth factors, and cytokines),[6,7] HSCs either transition into a state of self-renewal or differentiate heterogeneously through a hierarchy of multipotent progenitors into fully differentiated myeloid and lymphoid cells to adapt to changing physiological needs.[1,4,5] The exit of HSCs from quiescence is an energy-intensive process that coincides with a rapid and critical metabolic shift from glycolysis to mitochondrial metabolism, including increased mitochondrial biogenesis to support oxidative phosphorylation (OXPHOS).[1,5,8-10] This metabolic shift is a cornerstone of the unique self-renewal and differentiation capabilities of HSCs.[5,8-10]

However, dysfunction in the metabolic processes that guide HSC exit from quiescence often leads to metabolic hijacking and ultimately the transformation of HSCs into leukemia stem cells (LSCs).[1,3,11-14] LSCs are found in the BM niche and are characterized by stemness features, including drug resistance, self-renewal, and lack of differentiation, as well as highly heterogeneous phenotypes, genetic mutations, and metabolic alterations.[3,12,13] While HSCs and LSCs share fundamental self-renewal and drug resistance capabilities (i.e., high expression of ATP-binding cassette [ABC] transporters for genotoxin efflux),[1] their metabolic programs differ significantly to support the altered biosynthetic and energetic demands associated with malignant transformation.[3,5,12-14] Understanding the metabolic divergence between HSCs and LSCs can provide insight into stem cell homeostasis, leukemogenesis, drug resistance, and therapeutic targeting.[11,13,14]

Advances in metabolic profiling have enabled researchers to dissect these metabolic states and provide insight into what drives the pathological shift from HSCs to LSCs.[15,16] Through metabolic profiling, recent studies have confirmed that profound alterations in cellular metabolism are a key determinant of ultimate stem cell behavior. Meanwhile, several studies have developed emerging metabolic tools to shed light on the biochemical pathways and dynamic metabolic characteristics that guide the transition between quiescent and active HSCs and initiate oncogenic transformation into LSCs.[15-17] In this review, we summarize the key metabolic features that guide HSC activation and distinguish healthy HSCs from LSCs. Furthermore, we highlight how advances in metabolomic technologies can decode the metabolic framework and uncover novel biochemical pathways and therapeutic strategies for regulating and targeting HSC and LSC metabolism, respectively.

2. Metabolic landscape of healthy HSCs

2.1. Metabolic profile of HSCs within the BM microenvironment

Under normal homeostatic conditions, HSCs are preserved in extended periods of quiescence through a tightly regulated balance between glycolysis and OXPHOS dependence[1,4,5,9,10,14] (Figure 1). Quiescent HSCs primarily rely on glycolysis for the minimal energetic demands of stem cell maintenance, as previously stated, and allow for the minimization of reactive oxygen species (ROS) production commonly associated with fatty acid oxidation (FAO), such that quiescent HSCs can preserve their genomic integrity and protect themselves against metabolic stress and functional decline.[1,4,5,9,10,14] This level of sustained quiescence is largely due to the hypoxic microenvironment of the BM niche and provides protection against genomic instability, metabolic stress, and functional decline induced by ROS production.[1,8,14]

The BM niche provides HSCs with a stabilized and specialized microenvironment comprised of support cells and extracellular components. These components include a heterogenous array of osteoblasts, endothelial cells, mesenchymal stem cells, adipocytes, fibroblasts, macrophages, and extracellular matrix proteins that support the hypoxic microenvironment and provide favorable conditions to maintain HSC quiescence.[1,8,18] Furthermore, the BM niche provides HSCs with several critical niche factors, modulators of cell cycle progression, and developmental signaling pathways that work independently and often redundantly to maintain quiescence and stemness (Table 1).[1,19,20]

Niche factors, including transforming growth factor beta 1 (TGF-β1),[29-32] angiopoietin-1 (Ang-1),[33] stromal cell-derived factor-1α (CXCL12),[34,35] stem cell factor (SCF),[36-38] thrombopoietin (TPO),[39] and osteopontin (OPN),[40,41] work to enforce HSC quiescence by influencing the regulators of the cell cycle or acting as negative regulators of proliferation and differentiation. The TGF-β1 niche factor promotes HSC dormancy through the activation of the Smad signaling pathway, which ultimately inhibits HSC proliferation and differentiation.[29-32] Ang-1 is secreted

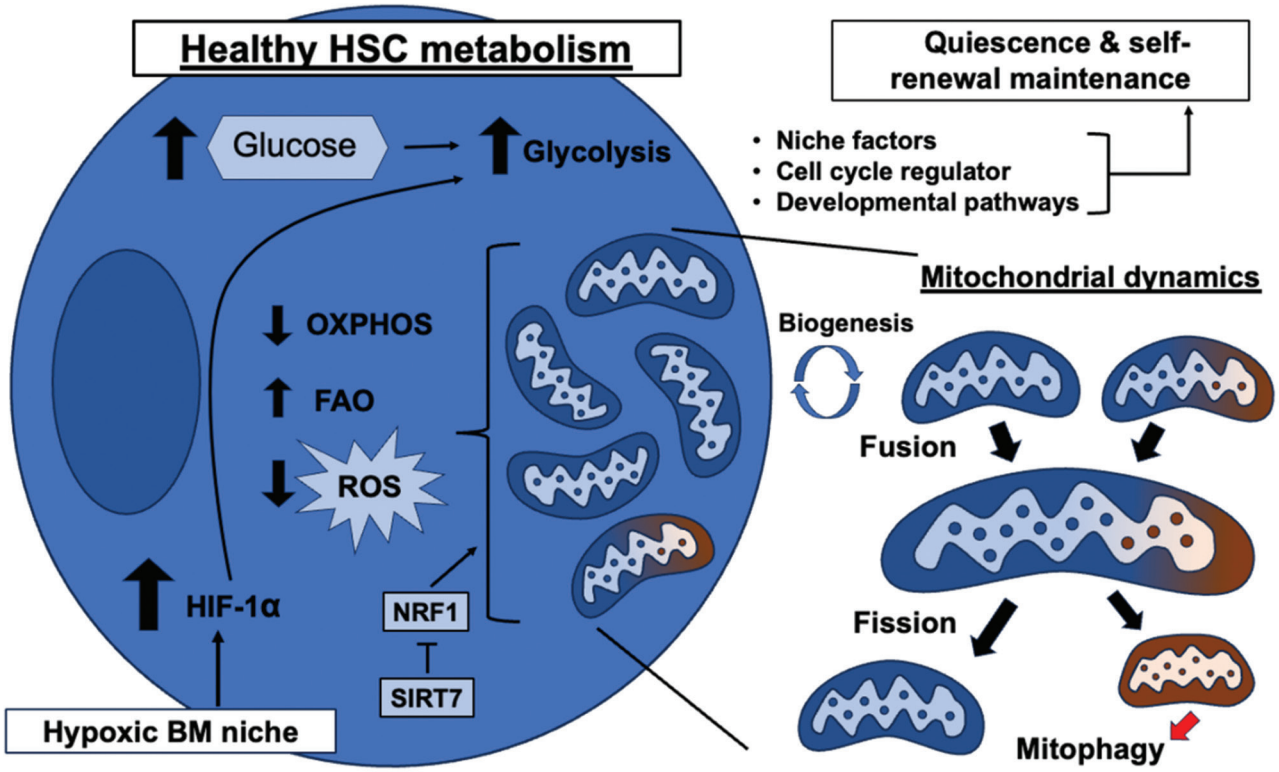

Figure 1. Healthy HSC metabolism. A graphical depiction of a healthy HSC located within the hypoxic BM niche, which creates an ideal environment for stabilizing HIF-1α.[1,2,21] The increased expression of HIF-1α reinforces the preference of quiescent HSCs for anaerobic glycolysis and minimizes the use of mitochondrial OXPHOS and the production of destructive ROS.[1,2,21] Decreased ROS production provides protection from genetic instability, metabolic stress, and functional decline of the HSC induced by ROS, while helping maintain cell quiescence.[1,2] Alongside increased glucose uptake for anaerobic glycolysis, quiescent HSCs also rely on FAO, thereby reducing reliance on OXPHOS.[5,22] However, OXPHOS is not eliminated but instead maintained at low levels with the help of SIRT7-mediated inhibition of NRF1, which suppresses mitochondrial biogenesis, function, and OXPHOS to reduce ROS accumulation.[1,23,24] Although OXPHOS is largely suppressed, mitochondrial homeostasis in healthy quiescent HSCs is maintained through the balance of mitochondrial biogenesis, fusion, fission, and mitophagy, collectively termed "mitochondrial dynamics."[5,9,10]
Abbreviations: BM: Bone marrow; FAO: Fatty acid oxidation; HIF-1α: Hypoxia-inducible factor 1α; HSC: Hematopoietic stem cell; NRF1: Nuclear regulatory factor 1; OXPHOS: Oxidative phosphorylation; ROS: Reactive oxygen species; SIRT7: Sirtuin 7.

Table 1. Key metabolic themes across normal hematopoietic stem cells and leukemia stem cells

Metabolic theme	Quiescent normal HSCs	Active normal HSCs	LSCs	References
Glycolysis	Increased glycolysis	Moderate glycolysis to support OXPHOS	Aerobic glycolysis (i.e., Warburg effect), in oxygen-rich conditions	5,14
Mitochondrial respiration	Low respiratory flux, minimal OXPHOS	Increased respiratory flux, enhanced OXPHOS to support proliferation	Enlarged mitochondrial mass, respiratory flux, and OXPHOS; reliance on mitochondrial metabolism for survival	5,14,25
FAO	Enhanced FAO to support energy demand and maintain stemness	Sustained FAO to meet energy demands during activation	Increased FAO utilization, crucial for survival and proliferation	5,22,26
ROS levels and redox state	Low ROS levels, robust antioxidant systems (enzymatic/non-enzymatic)	Increased mitochondrial-derived ROS levels, balanced by antioxidant responses	Elevated ROS levels, dependence on antioxidant systems for survival (e.g., glutathione pathway)	5,6,10
Hypoxia and HIF-1α pathway	Hypoxic niche preference, stabilized HIF-1α to maintain quiescence	Reduced HIF-1α stabilization upon exiting the hypoxic niche and proliferation/differentiation	Increased stabilization of HIF-1α and downstream pathways supporting survival, drug resistance, and leukemia progression	6,21,27,28

Abbreviations: FAO: Fatty acid oxidation; HIF-1α: Hypoxia-inducible factor 1α; HSC: Hematopoietic stem cell; LSC: Leukemia stem cell; OXPHOS: Oxidative phosphorylation; ROS: Reactive oxygen species.

primarily by osteoblasts and enhances HSC quiescence by stabilizing stem cell interactions through the Tie2 and phosphatidylinositol 3-kinase/protein kinase B pathways.[33] The niche factor CXCL12 is produced by BM stromal and endothelial cells and binds the C-X-C motif chemokine receptor type 4 on HSCs to reinforce dormancy.[34,35] Secreted by osteoblasts and adipocytes, SCF supports HSC quiescence by regulating metabolic homeostasis and promoting survival.[36-38] Alternatively, TPO limits cell cycle entry by stimulating the expression of Tie2 on HSCs[42] and preserves stemness.[39,42] OPN, secreted by osteoblasts and stromal cells, interacts with integrin receptors to suppress cell cycle progression and enhance HSC anchoring in the BM niche.[40,41]

Moreover, cell cycle regulatory components are equally essential for governing HSC dormancy and quiescence by tightly controlling cell cycle entry and progression.[1,19,20] These include members of the retinoblastoma (Rb) family[43-45] and the Forkhead box class O proteins,[46,47] as well as the interaction between cyclin D and cyclin-dependent kinase (CDK) 4/6,[48,49] and the CDK interacting protein/kinase inhibitory protein family (e.g., p21, p27, p57, and p53).[50-52] In HSCs, the Rb family restricts the transition from G_0/G_1 into S phase by suppressing DNA replication and cell proliferation through regulation of TPO-mediated signaling, thus reinforcing a quiescent state.[43-45] The Forkhead box class O transcription factor proteins suppress HSC cell cycle progression and are involved in ROS resistance, further promoting HSC quiescence.[46,47] Alternatively, cyclin D and CDK4/6 form a complex as a fundamental step controlling progression of the cell cycle out of quiescence by inhibiting Rb and promoting transition from G_1 into S phase.[48,49] The CDK-interacting protein/kinase inhibitory protein family functions to suppress the activity of the cyclin D–CDK4/6 complex, thereby preserving HSC dormancy and quiescence.[50-52]

In addition, evolutionarily conserved pathways, including the Wnt,[53,54] Notch,[53,55,56] and Hedgehog (HH)[57] developmental pathways, play supportive roles in the maintenance of HSC quiescence, self-renewal, and inhibition of differentiation.[1,20,37] Wnt signaling promotes HSC self-renewal through downstream activation of β-catenin and transcriptional genes governing the cell cycle, while also suppressing lineage-specific transcription factors.[53,54] Interestingly, overexpression of β-catenin in HSCs increases HSC expansion and inhibits differentiation both *in vitro* and *in vivo*.[58] Alternatively, Notch signaling reinforces HSC quiescence and self-renewal in an undifferentiated state by upregulating transcriptional repressors that inhibit differentiation and enhance cell cycle repression.[53,55,56] Through the activation of the Gli transcription factor, HH signaling similarly supports both quiescence and repression of lineage commitment in HSCs.[57] Of interest, mice deficient in *Gli1* exhibit decreased HSC proliferation and enhanced short- and long-term HSC engraftment, supporting the role of HH signaling in HSC maintenance and quiescence.[59]

Collectively, these findings demonstrate the tight regulation of HSC quiescence and how the niche-derived factors, cell cycle regulatory components, and developmental pathways work independently or cooperatively to ensure long-term hematopoietic homeostasis and regenerative capacity.

2.2. Glycolysis versus OXPHOS in quiescent and active HSCs

Sustained quiescence in HSCs depends on maintaining low metabolic rates and minimal mitochondrial OXPHOS.[1,5,14] However, the transition out of dormancy involves rapid changes in cellular metabolism.[5,14] This transition from highly glycolytic metabolism to OXPHOS supports the heightened energetic demands required for rapid proliferation and differentiation.[5,14] Glycolysis is a central metabolic pathway through which cells can derive critical metabolites and metabolic precursors from a single glucose molecule.[60] In the quiescent state, HSCs rely on glycolysis independent of the presence of oxygen. This is important, since a primary characteristic of the BM niche is a highly hypoxic microenvironment.[1,6,21] In fact, *in vivo* measurements in the hypoxic BM niche showed a local oxygen tension (pO_2) of <32 mm Hg[61] compared with an average atrial pO_2 of 90 mmHg.[62] Such a hypoxic microenvironment creates ideal conditions to stabilize a key transcription factor and mediator of cellular hypoxia, hypoxia-inducible factor (HIF)-1α.[21,63] HSCs are known to express HIF-1α at high levels.[21] Previous studies have shown that HIF-1α–deficient mice exhibit a loss of HSC quiescence and decreased HSC abundance, highlighting the importance of HIF-1α stabilization in HSCs.[64] Alternatively, overexpression of HIF-1α maintains quiescence but decreases transplantation capacity, suggesting an intricate and tightly regulated balance of HIF-1α expression in the maintenance of HSC quiescence.[64] Nevertheless, the stabilization of HIF-1α reinforces the prioritization of glycolytic metabolism, thereby providing long-term protection of HSCs from oxidative damage by OXPHOS-derived ROS.[1,5,6,21]

Upon activation, HSCs increase glycolytic influx to meet the rising metabolic and biosynthetic demands required for proliferation and differentiation.[5,14,19] This compels HSCs to increase expression of glycolytic transporters (e.g., glucose transporter 1 [GLUT1]) downstream of TGF-β1 stimulation, thereby enhancing

glucose intake and providing the necessary metabolites for rapid expansion.[5,14,65] These include pyruvate, required for mitochondrial OXPHOS,[60] and other precursors required for the biosynthesis of nucleotides,[66] lipids,[60,67] proteins,[60] and carbohydrates.[52,60,68]

In quiescent HSCs, mitochondrial OXPHOS is active but maintained at relatively low levels, potentially to limit ROS generation, protect cells from oxidative damage, and preserve their long-term self-renewal capacity.[1,4,5] This suppression is maintained largely by the BM niche factors, cell dormancy, and several developmental pathways. Interestingly, quiescent HSC mitochondria are further repressed through the suppression of nuclear regulatory factor 1 (NRF1) by sirtuin 7 (SIRT7).[1,24,69] NRF1 is a transcription factor that regulates mitochondrial biogenesis and function, enhancing OXPHOS and ROS accumulation.[23] In contrast, SIRT7 acts as a metabolic checkpoint that maintains HSC quiescence through the inhibition of NRF1.[70,71] Several studies suggest that this interaction also suppresses the mitochondrial unfolded protein response and metabolic activation, thereby enabling HSCs to maintain high levels of impaired mitochondria while ensuring the rapid engagement of OXPHOS upon HSC stimulation.[1,69,70] However, it should be noted that quiescent HSC OXPHOS is not eliminated by these regulatory factors, as quiescent HSCs remain dependent on minimal mitochondrial activity for survival.[1,4,5,9,10]

Several studies suggest that once activated, HSCs may shift their metabolism to FAO and mitochondrial OXPHOS to support proliferation, differentiation, and the energetic and biosynthetic demands sustained by the increased concentration of mitochondria.[5,9,10,14] These demands include the expansion of mitochondrial mass, enhanced respiratory capacity, and increased ATP production in support of active cell cycle progression and lineage specification.[5,9,10,14] An increase in OXPHOS coincides with elevated ROS production, which represents a major step toward differentiation. ROS accumulation is known to drive HSCs out of quiescence by suppressing self-renewal through the activation of p38 downstream of the mitogen-activated protein kinase signaling pathway.[1,5,14] A study investigating the chemical uncoupling of mitochondrial OXPHOS demonstrated that *ex vivo* HSCs exhibited lower mitochondrial mass and reduced mitochondrial membrane potential while displaying increased self-renewal potential in cultures designed to induce differentiation.[72] This finding supports the role of limited mitochondrial activity as a key characteristic of HSC quiescence and heightened mitochondrial OXPHOS as a driver pushing HSCs out of quiescence.

2.2.1. Lipid metabolism

In addition to OXPHOS, mitochondria also play a major role in lipid metabolism, specifically FAO.[5,22] FAO is a metabolic process that breaks down fatty acids, supported by the presence of BM adipocytes, to generate the integral metabolite acetyl-coenzyme A (CoA), required for the tricarboxylic acid (TCA) cycle and downstream OXPHOS, as well as macromolecule biosynthesis.[22,73] In this process, fatty acids undergo a series of reactions that repeatedly shorten the fatty acid chain by two carbons while producing acetyl-CoA, nicotinamide adenine dinucleotide (NADH), and the reduced form of flavin adenine dinucleotide, which are needed for the TCA cycle and electron transport chain (ETC), respectively, in addition to generating precursors for macromolecule synthesis. In quiescent HSCs, FAO is the primary form of lipid metabolism. Here, FAO is sustained by the low levels of mitochondrial respiration and is regulated by the peroxisome proliferator-activated receptor-δ (PPARδ) transcription factor.[5,22] PPARδ promotes the expression of genes regulating fatty acid uptake, transportation, and oxidative catabolism to preserve HSC longevity and self-renewal capacity while in the hypoxic BM niche.[74] It then follows that loss of PPARδ results in a decline in HSCs with the ability to self-renew, thereby supporting the role of FAO in HSC dormancy.[74]

Upon activation, HSCs show a notable reconfiguration in their lipid metabolic profile. Specifically, activated HSCs are proposed to engage in a dynamic interplay between FAO and lipid macromolecule biosynthesis.[5,75,76] Sustained FAO assists in meeting the metabolic demands of activation[75] but also contributes to differentiation and fate determination through acetyl-CoA-dependent histone modifications.[76] Concurrently, lipid biosynthesis is upregulated in active HSCs to meet the increasing demand for building membranes required during rapid cell division.[77,78] Taken together, these findings further highlight the delicate balance of the metabolic profile required to either maintain HSC dormancy or promote differentiation.

2.3. Mitochondrial dynamics and ROS regulation

Mitochondrial dynamics enable mitochondria to adapt to changing metabolic demands and regulate ROS levels required to exit quiescence.[5,9,10] The term "mitochondrial dynamics" refers to the continuous process of balancing mitochondrial fusion, fission, mitophagy, and biogenesis to maintain mitochondrial function, shape, and distribution within healthy cells.[79] In HSCs, mitochondrial dynamics are critical for balancing fusion, fission, and mitophagy to preserve quiescence and self-renewal capacity, while also providing a structured means to enhance mitochondrial

function and biogenesis upon stimulation.[5,9,10] Disruption of this dynamic equilibrium can impair mitochondrial function and lead to stem cell exhaustion or aberrant differentiation.[10]

Fusion allows mitochondria to merge and share mitochondrial contents, including DNA, proteins, and metabolites, thereby compensating for damaged or inefficient mitochondrial function and maximizing the ratio of metabolically healthy mitochondria.[9,10] In contrast, mitochondrial biogenesis provides mitochondria with the ability to produce more mitochondria, while fission enables division during cell proliferation or isolates damaged mitochondrial segments for removal through mitophagy.[9,10] A key consequence of improper regulation of mitochondrial dynamics is the accumulation of excess ROS beyond the levels needed for HSC stimulation. The physiology and metabolic state of mitochondria influence their morphology, dynamics, and turnover rate, which directly affect ROS production.[9,10] Although moderate ROS levels are required to drive HSCs out of quiescence, elevated ROS levels can induce DNA damage, impair self-renewal, promote senescence,[80] or induce oncogenic transformation.[80,81]

To mitigate excessive oxidative stress, cells deploy a selective form of autophagy termed "mitophagy." Mitophagy is a process by which the cell selectively degrades damaged or dysfunctional mitochondria.[10,82] It also prevents the buildup of ROS and eliminates dysfunctional mitochondria, thereby enabling HSCs to maintain homeostasis during quiescence and preserve a healthy mitochondrial population in anticipation of activation.[10,82] In addition, redox buffering systems, largely fueled by nicotinamide adenine dinucleotide phosphate (NADPH) reducing power, help to buffer accumulated ROS and prevent oxidative damage,[83] alongside other enzymatic (e.g., superoxide dismutase and catalase) or non-enzymatic (e.g., glutathione) antioxidant systems (e.g., superoxide dismutase 1/2). Taken together, mitochondrial dynamics and regulatory networks ensure mitochondrial function, health, and integrity, as well as the redox balance required to maintain functional, long-term HSCs.

3. Metabolic rewiring in LSCs

Compared to the activation of quiescent HSCs, the oncogenic transformation of LSCs entails significant metabolic reprogramming, allowing them to self-renew, survive in the BM niche, and resist therapeutic intervention (Table 1; Figure 2). However, unlike quiescent HSCs, which rely primarily on glycolysis for energy production, LSCs exhibit enhanced mitochondrial respiration associated with increased mitochondrial density. These changes are linked to elevations in FAO and OXPHOS.[84,85] However, the intrinsic organization of mitochondria in LSCs and their contribution to chemoresistance remain to be fully understood.[85,86]

3.1. Shared and divergent pathways between HSCs and LSCs

Quiescent HSCs and oncogenic LSCs both rely primarily on core metabolic pathways, including glycolysis and FAO, to maintain self-renewal and survival.[5,14] However, they differ in how these pathways are regulated. In HSCs, metabolism is tightly regulated by numerous extrinsic and intrinsic niche factors to preserve quiescence and genomic integrity, as discussed in previous sections.[5,14] Moreover, these quiescent stem cells are predominantly glycolytic, express high levels of glycolytic enzymes, and suppress mitochondrial membrane potential to minimize ROS production and prevent premature HSC activation.[5,14,89]

In contrast, LSCs rewire these metabolic programs to enhance mitochondrial efficiency, thereby sustaining elevated rates of oxidative metabolism, which may ultimately facilitate resistance to metabolic or therapeutic stress.[25] For example, LSC mitochondria depend primarily on components of the ETC to facilitate the generation of ATP and regulate redox balance.[14,25] In chronic myeloid leukemia (CML), LSCs exhibit elevated OXPHOS activity and increased catabolism of TCA cycle metabolites. An increase in mitochondrial respiratory flux to generate ATP sensitizes these cells to Complex I inhibition by phenformin.[90] Complex I, or NADH dehydrogenase, is a central regulatory step within the mitochondrial respiratory chain and a primary site of electron entry into the ETC through oxidation of NADH to NAD^+.[87] Complex I plays a key role in maintaining redox balance by transferring electrons to coenzyme Q (ubiquinone) while simultaneously pumping protons across the inner mitochondrial membrane.[87] The resulting proton gradient is utilized by ATP synthase to drive ATP production, as well as NADPH synthesis and metabolite transport. In LSCs, where glycolytic flexibility is limited, this dependency on intact Complex I function highlights a critical metabolic vulnerability. One study supports the notion that CML LSCs are highly dependent on mitochondrial oxidative metabolism for survival.[25] This finding confirms that mitochondrial activity and increased TCA cycle catabolism in CML LSCs are not merely passive characteristics but represent a critical energetic pathway that can be therapeutically targeted.

In addition to enhanced oxidative metabolism and TCA cycle activity observed in CML, LSCs in acute myeloid leukemia (AML) also rewire upstream metabolic inputs to

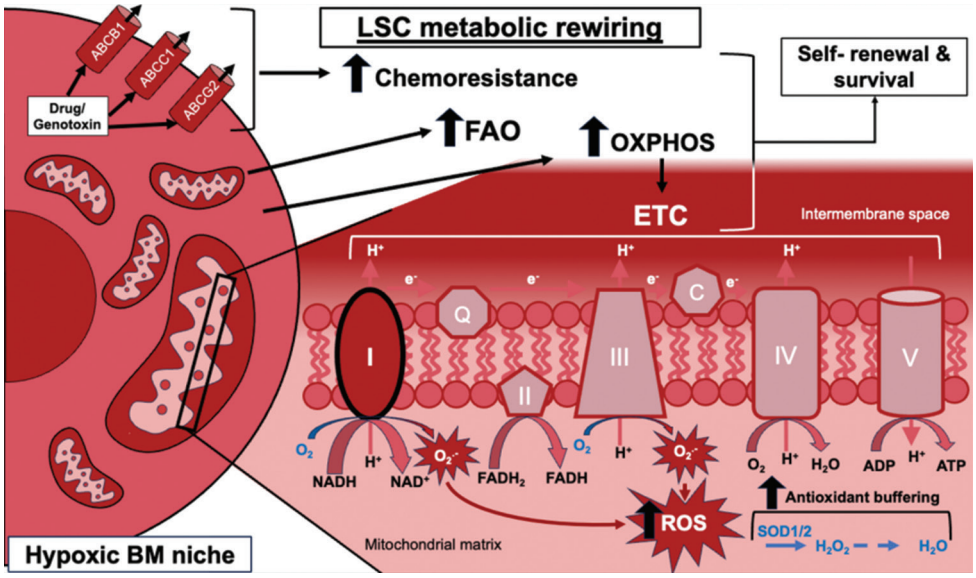

Figure 2. LSC metabolic rewiring. A graphical depiction of an LSC located within the hypoxic BM niche.[13,14] Here, LSCs rely heavily on upregulated FAO, OXPHOS, and ATP production for self-renewal and survival.[84,85] Complex I of the ETC plays an essential role in driving ATP production by acting as the primary site for NADH oxidation and electron (e^-) entry into the ETC.[87] From here, electrons are transferred to coenzyme ubiquinone (Q), while protons (H^+) are simultaneously pumped across the inner mitochondrial membrane, generating the proton gradient necessary for ATP synthesis.[87] During this process, LSCs also exhibit elevated OXPHOS activity and increased levels of ROS.[25] To enhance mitochondrial efficiency and sustain these elevated rates of OXPHOS, LSCs rewire conventional metabolic programs and mitigate elevated ROS levels through increased antioxidant buffering, which enables them to tolerate ROS at levels conductive to self-renewal while exhibiting oxidative resistance.[14,75,83,88] LSCs also remodel their microenvironment, leading to the increased expression of ABC transporters (e.g., ABCB1, ABCC1, and ABCG2), resulting in chemoresistance.[85,86]
Abbreviations: BM: Bone marrow; ETC: Electron transport chain; FADH: Flavin adenine dinucleotide; $FADH_2$: Reduced form of flavin adenine dinucleotide; FAO: Fatty acid oxidation; H_2O: Water; H_2O_2: Hydrogen peroxide; LSC: Leukemia stem cell; NADH: Nicotinamide adenine dinucleotide; OXPHOS: Oxidative phosphorylation; ROS: Reactive oxygen species; SOD1/2: Superoxide dismutase ½.

fuel mitochondrial respiration.[14,25] One such pathway that further differentiates HSCs from LSCs is FAO. In LSCs, FAO is upregulated to support mitochondrial respiration and promote chemoresistance.[14,26] To sustain this elevated FAO activity, LSCs highly express Cluster of Differentiation (CD)-36 for increased fatty acid transport and lipid scavenging and rely on carnitine palmitoyltransferase 1A-driven lipid oxidation.[85,86] AML LSCs exhibit increased carnitine palmitoyltransferase 1A expression to facilitate long-chain fatty acid breakdown and express CD36 when localized to adipocyte-rich niches, enabling the scavenging of extracellular lipids.[85,86] Notably, LSCs tolerate moderate levels of intracellular ROS by exhibiting enhanced oxidative resistance, which fuels proliferation without triggering oxidative damage, in contrast to the ROS sensitivity of normal quiescent HSCs.[5,14,88]

One mechanism that enables LSC tolerance to ROS is the integrated stress response (ISR).[91-93] The ISR is a conserved cellular pathway that balances innate biological processes, such as protein synthesis and gene expression, in response to stressors such as ROS-induced oxidative damage, nutrient deprivation, and mitochondrial dysfunction.[91,92] In LSCs, the ISR sustains cell survival under metabolic and therapeutic pressure. Phosphorylation and activation of components such as eukaryotic translation initiation factor 2α and the stress-adaptive activating transcription factor 4 reduce ROS-induced apoptosis, maintain redox balance, and support mitochondrial metabolism.[92-94] This process also enhances resistance to therapeutic intervention and promotes the long-term persistence of LSCs, serving as a critical mediator of survival.[92,93]

These fundamental differences in metabolic regulation demonstrate how LSCs are uniquely equipped for survival. Despite this, the notion that FAO is critical to LSC survival remains under scrutiny. AML LSCs exhibit a distinct metabolic phenotype compared with both normal HSCs and bulk leukemia blasts, characterized by a dependence on mitochondrial respiration rather than glycolysis.[95] Previous studies have demonstrated that AML LSCs maintain low ROS levels while displaying elevated oxidative metabolism and adenosine monophosphate-activated protein kinase (AMPK) activation.[95] These features are consistent with a mitochondria-centric bioenergetic program. Similarly, another study showed that the activation of the signal transducer and activator of transcription 3 (STAT3)–MYC axis enhances *SLC1A5*-mediated glutamine import, thereby reinforcing TCA cycle flux and mitochondrial metabolism.[96] While LSCs retain functional glycolysis

through GLUT1, its contribution to ATP production remains undefined. Moreover, other studies reported that dual inhibition of GLUT1 and OXPHOS is required to fully eliminate LSCs *in vivo*.[97] Although FAO remains a viable metabolic target, its precise contribution relative to other substrates, such as glutamine, remains an open question. Further investigation is needed to explore the specific fuel preferences of LSCs and to identify context-specific therapeutic vulnerabilities across leukemia subtypes.

3.2. Role of nutrient-sensing pathways in HSCs and LSCs

In addition to metabolic pathways, evidence suggests that nutrient-sensing pathways also play a role in regulating stem cell metabolism.[5,98,99] These include the AMPK and mechanistic target of rapamycin complex 1 (mTORC1) signaling pathways. In quiescent HSCs, suppression of mTORC1 activity is essential for maintaining dormancy and preventing premature differentiation by reducing protein synthesis and inhibiting cell growth.[5,100] Conversely, AMPK activation plays a pivotal role in preserving energy homeostasis during metabolic stress by promoting catabolic processes such as FAO and glycolysis to generate ATP, while inhibiting anabolic processes to preserve energy.[101] This balance supports both quiescence and the long-term self-renewal capacity of HSCs under physiological conditions.

In LSCs, however, this balance between nutrient-signaling pathways—namely, mTORC1 and AMPK—is reprogrammed. The mTORC1 pathway acts as a central regulator of anabolic metabolism, enhancing amino acid uptake, protein synthesis,[102] and RNA and DNA biosynthesis.[103] In contrast, AMPK inhibits fat and protein biosynthesis and promotes FAO and glycolysis to maintain energy balance.[104] Previous research has shown that chemical activation of AMPK in $CD34^+$ AML cells inhibits mTORC1 signaling and enhances sensitivity to cytarabine and idarubicin,[105] suggesting that therapeutic manipulation of these pathways can impair LSC maintenance and viability. Similar observations have been reported in solid tumor models, where AMPK activation reduces cancer stem cell survival.[106] Collectively, these findings underscore the conserved yet opposing roles of AMPK and mTOR1 signaling in regulating stemness. Moreover, as nutrient availability and metabolic signaling pathways are tightly linked to stemness and therapeutic resistance, targeting these pathways offers a promising strategy for selectively eliminating LSCs without compromising normal hematopoiesis.

3.3. Mechanisms of adaptation to hypoxia and chemoresistance

Previous reviews have highlighted that the hypoxic BM niche plays a significant role in maintaining HSC quiescence and regulating LSC localization.[2,27] As discussed in Section 2.2, quiescent HSCs downregulate mitochondrial function to minimize the negative effects of ROS accumulation. In contrast, LSCs maintain robust mitochondrial metabolism even under hypoxic conditions in the BM niche. In xenograft studies, AML LSCs that survive cytarabine treatment retain high mitochondrial activity and strong BM adhesion capacity, underscoring their ability to resist therapy through persistent mitochondrial function.[107] Unlike quiescent HSCs, LSCs maintain ROS at levels conducive to self-renewal and mitigate ROS-induced damage by upregulating antioxidant defenses, including non-enzymatic antioxidant systems similar to those of active HSCs.[14,75,83,88]

In addition to intrinsic metabolic adaptations, LSCs that exhibit resistance to chemotherapy exploit extrinsic cues from the microenvironment.[28] Adipocyte-rich BM provides LSCs with an exogenous source of fatty acids to support FAO and sustain mitochondrial respiration.[85] Furthermore, LSCs actively remodel their microenvironment by engaging in stromal interactions and altering chemokine gradients, thereby promoting niche retention and therapeutic evasion.[28] Through the increased expression of ABC transporters (e.g., ABCB1, ABCC1, and ABCG2), LSCs efflux chemotherapy drugs, reducing treatment efficacy and contributing to drug resistance across multiple cancers.[1,108] In LSCs, oncogenic drivers such as c-MYC enhance the activity of these transporters, enabling more efficient drug efflux and thereby increasing chemoresistance and relapse potential.[109] These strategies highlight the diverse resistance mechanisms by which LSCs resist hypoxia and chemotherapy, suggesting that targeting both intrinsic metabolic programs and extrinsic niche interactions may be necessary to overcome LSC-mediated relapse.

3.4. Genomic alterations in LSCs

Metabolic changes in LSCs do not occur passively but are instead typically driven by genetic mutations or modifications that actively influence cellular energy processing. In chronic lymphocytic leukemia, although genetic mutations do not directly alter the expression of genes involved in metabolic pathways, STAT3 becomes constitutively activated, with reduced levels of microRNA-125 playing a major role. This ultimately promotes the transition of LSCs toward more efficient fatty acid utilization for energy production.[110]

The HIF pathway helps healthy blood stem cells remain in a glycolysis-dependent resting state; however, its role in LSCs is more complex.[111,112] Although LSCs reside in hypoxic niches, elimination of HIF-1α accelerates disease

progression after chemotherapy-based treatment in mouse models of AML.[112,113] Thus, it remains unclear if HIF-1 is a feasible therapeutic target.

Other genomic alterations contribute to chemoresistance through well-established mechanisms. Activating mutations in metabolic enzymes such as isocitrate dehydrogenase (IDH) 1 and IDH2 lead to the accumulation of the oncometabolite 2-hydroxyglutarate (2-HG). The presence of 2-HG induces hypermethylation of specific genes, preventing cellular differentiation and triggering widespread epigenetic changes.[112] In addition, 2-HG upregulates B-cell lymphoma 2 (BCL-2), conferring resistance to apoptosis and increasing sensitivity to BCL-2 inhibitors.[112] Overall, elevated BCL-2 expression is crucial for LSC survival, supporting a resting, low-ROS state that depends on OXPHOS for energy production.[112]

4. Metabolomic approaches

Recent advances in metabolomic technologies have enabled more precise and comprehensive studies of metabolism in HSCs and LSCs. Metabolomics allows for highly precise measurements of small molecules and metabolites that reflect the metabolic state at the cellular or tissue level.[15,16,114] These measurements provide critical insights into processes that regulate energy production, biosynthesis, redox balance, and metabolism, thereby enabling thorough characterization of the metabolic states of quiescent and active HSCs as well as LSCs.[17] This is particularly important because both HSCs and LSCs rely on distinct metabolic patterns and pathways[14] (Table 1).

In addition, metabolic profiling can be used to identify diagnostic biomarkers, reveal alterations in metabolic pathways associated with treatment, monitor therapeutic responses, and uncover vulnerabilities that may be exploited for the development of novel therapies[17] (Figure 3). To better understand the metabolic profile of HSCs and LSCs, researchers employ technologies such as mass spectrometry (MS), single-cell and multi-omics approaches, and live-cell metabolic assays.

4.1. Targeted versus untargeted metabolomics

One widely used technology to study metabolism is MS, an analytical technique that ionizes chemical compounds and measures their mass-to-charge (m/z) ratios with high sensitivity and throughput.[115] Metabolomic analysis using MS typically employs two main approaches: targeted and untargeted metabolomics. Targeted metabolomics focuses on quantifying a specific set of metabolites using known standards for each compound under investigation, thereby providing a quantitative snapshot of the metabolome.[121,122] This approach enables profiling of metabolite concentrations, including glycolytic, FAO, or TCA cycle intermediates, as well as amino acids, nucleotides, lipids, and small molecules or drugs, at specific time points.

Techniques such as liquid chromatography–MS (LC-MS) and gas chromatography–MS (GC-MS) are commonly used for metabolite separation and detection[115,116] (Figure 3). LC-MS separates metabolites using a liquid mobile phase and a stationary phase based on properties such as charge, polarity, or hydrophilicity,[116] whereas GC-MS uses a gas mobile phase to separate compounds according to volatility and boiling point.[115] These targeted MS-based methods are especially valuable for examining the metabolic profiles of quiescent and active HSCs or LSCs, where subtle changes in glycolysis, OXPHOS, FAO, or amino acid metabolism can influence stemness, activation, proliferation, or therapeutic resistance.

However, a major limitation of these approaches is the low abundance of HSC and LSC populations within the BM.[123,124] This rarity makes it difficult to obtain sufficient cellular material for MS, often necessitating strategies such as pooling samples from multiple donors to achieve the required input for metabolomic analyses[125] or extensive cell sorting for metabolic flux measurements.[124] Consequently, the feasibility of single-sample untargeted workflows in these rare cell populations remains a significant challenge.

Untargeted metabolomics is a bottom-up approach that focuses on identifying and quantifying potentially 100 or 1000 metabolites along with novel metabolic features.[126] This technique allows researchers to identify and analyze shifts in the metabolic profile and metabolic pathways, as well as the effects of different treatments on the entire system.[15] This technique enabled the identification of chemotherapy-induced metabolic shifts in osteosarcoma stem cells using untargeted metabolomics by LC-MS that would not have been detected by targeted metabolomics.[127] A prominent example is the identification of 2-HG, which accumulates in IDH1/2-mutant AML and serves as both a key biomarker and a direct therapeutic target.[88] Similarly, analyses of CML stem cells have identified distinct metabolic enzyme signatures compared to normal HSCs, highlighting novel vulnerabilities for therapeutic exploitation.[112] It should be noted that choosing between targeted and untargeted approaches ultimately relies on the biological question, as both are suitable for testing hypotheses.[15] In the context of exploring the metabolic profile of HSCs and LSCs, either method can be used to map metabolic changes associated with HSC activation or oncogenic transformation. Furthermore, these technologies provide foundational data for enhancing our understanding of HSC and LSC metabolism.

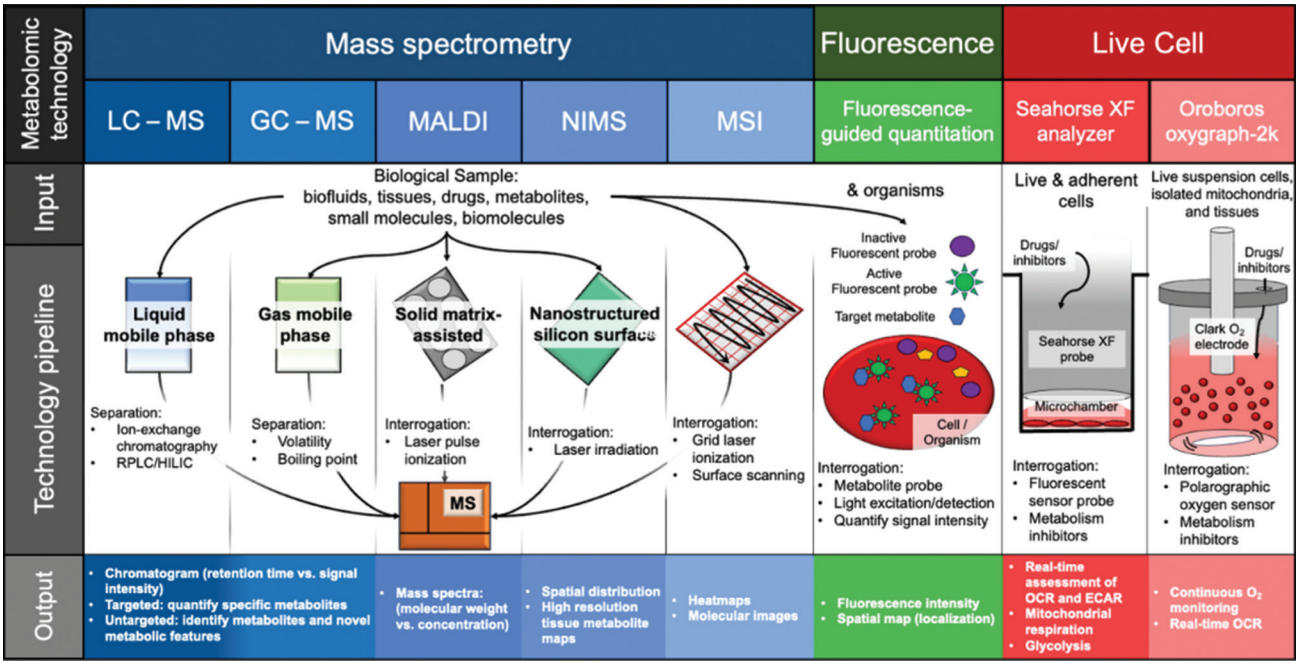

Figure 3. Overview of metabolomic technologies used to characterize metabolic features in HSCs and LSCs. This schematic overview shows the metabolomic platforms used to resolve the metabolic states of HSCs and LSCs, organized by detection modality: MS, fluorescence-guided quantitation, and live-cell metabolic assays. Under MS, LC–MS and GC–MS utilize liquid or gas mobile phases, respectively, for targeted or untargeted metabolite separation based on polarity, charge, or volatility.[115,116] Matrix-assisted technologies, such as MALDI and NIMS, enable surface-based ionization with spatial resolution of metabolite distributions.[117,118] MSI combines ionization with spatial scanning to generate molecular images.[117,118] Fluorescence-based techniques leverage metabolite-specific dyes and optical detection to produce intensity maps, while the Seahorse XF Analyzer and Oroboros Oxygraph-2k platforms provide real-time, high-resolution assessments of mitochondrial function, OCR, and ECAR in live cells or tissues.[119,120] Inputs and outputs for each platform are shown to further illustrate how these technologies enable comprehensive profiling of metabolism.
Abbreviations: ECAR: Extracellular acidification rate; GC: Gas chromatography; HSC: Hematopoietic stem cell; LC: Liquid chromatography; LSC: Leukemia stem cell; MALDI: Matrix-assisted laser desorption/ionization; MS: Mass spectrometry; MSI: Mass spectrometry imaging; NIMS: Nanostructure-initiator mass spectrometry; OCR: Oxygen consumption rate.

4.2. Single-cell and multi-omics platforms

Other useful techniques to explore metabolism include single-cell profiling and multi-omics integration. Single-cell metabolomic profiling allows for analysis at the single-cell level to better define the metabolic state, function, and interactions within the microenvironment.[128] This technique is a useful addition to targeted and untargeted metabolomics that helps resolve the metabolic heterogeneity within cells of a microenvironment down to a single-cell type, providing higher resolution and specificity.[129,130] Alternative techniques, such as matrix-assisted laser desorption/ionization, MS imaging (MSI), and nanostructure-initiator MS, enable the direct probing of low-abundance metabolites at subcellular resolution[117,118] (Figure 3). The feasibility of spatially mapping key metabolites within the hypoxic BM niche is supported by complementary approaches. For instance, high-resolution imaging has been used to correlate the localization of HSCs with specific hypoxic zones,[131] while MSI can be adapted to visualize how oncometabolites are distributed within BM tissue, allowing researchers to observe how LSCs metabolically reprogram their microenvironment.[132] These technologies also enable the identification of activation markers and metabolic rewiring associated with oncogenic transformation.[133,134] Furthermore, fluorescence-guided quantitation is an additional single-cell metabolomic technique designed to enhance specific metabolite detection through the use of spatial biology and fluorescence labeling strategies[119] (Figure 3). This technique enables researchers to improve the measurement accuracy of metabolite quantification in complex tissues.

Although metabolomics alone can provide highly sensitive and quantitative information on the metabolic state, it is important to consider the interplay between metabolomics and other omics processes to further decode specific metabolic phenotypes. Multi-omics platforms include the integration of transcriptomics, proteomics, and epigenomics, allowing for unprecedented resolution of the entire -omics profile within a given cell or tissue.[135] While each multi-omics platform provides unique data on its respective targets, integrating them with machine learning algorithms and computational workflows

can provide a comprehensive metabolic map to better understand the heterogeneity within HSCs and LSCs in the BM niche microenvironment, discern key metabolites and metabolic pathways, and develop novel therapeutic interventions.[136-138]

4.3. Technologies for live cells in mitochondria and metabolism

Live-cell technologies provide real-time functional insights into cellular processes and mitochondrial activity, making them powerful tools for studying metabolically dynamic populations like LSCs.[120] Instruments such as the Oroboros Oxygraph-2k (O2k) and the Seahorse XF Analyzer are widely used to assess bioenergetic function in intact or permeabilized cells, tissues, and isolated mitochondria under physiologically relevant conditions[120] (Figure 3). These platforms allow for the determination of oxygen consumption rate, enabling detailed profiling of ATP-linked respiration, proton leak, and spare respiratory capacity. The Seahorse technology is also capable of measuring the extracellular acidification rate to determine glycolytic activity.

The Agilent Seahorse XFe96 Analyzer was recently employed in CML LSCs, unveiling a deeply quiescent subset leukemia initiators (LI) characterized by suppressed Complex I activity yet enhanced FAO dependency.[139] Single-cell metabolomic profiling in parallel confirmed that this LI subset maintained low ROS levels despite high FAO flux, suggesting that mitochondrial complex I suppression is a protective adaptation within a functionally discrete LSC subpopulation.[139] Previous research has shown that cytarabine-resistant AML cells maintain a high oxygen consumption rate, indicating that persistent mitochondrial respiration is a hallmark of chemoresistant LSCs.[107]

Alternative to the Seahorse, the Oroboros O2k high-resolution respirometry provides a further depth of understanding by directly measuring Complex I–IV activity and coupling efficiency in primary AML samples.[140] In contrast, the Seahorse provides high throughput, dynamic stress testing. The Oroboros O2k offers extensive control over substrate and inhibitor addition, enabling precise dissection of respiratory chain function.[120,141] This makes Oroboros particularly valuable for evaluating subtle mitochondrial defects or drug-induced changes in electron transport that may underlie LSC persistence.[142,143] In the context of HSCs and LSCs, Oroboros O2k live-cell approaches not only reveal vulnerabilities in oxidative metabolism but also allow investigators to assess dynamic responses to metabolic inhibitors in real time. Today, the use of live-cell functional assays offers a critical readout for evaluating therapeutic efficacy and identifying bioenergetic escape mechanisms. Incorporating these technologies into HSC and LSC research helps bridge mechanistic insight with significant translational potential.

4.4. Problems and opportunities in therapeutic translation

Despite the advancements in metabolomics toward defining the metabolic profiles of HSCs or identifying LSC-specific vulnerabilities, translating these findings into effective therapies remains a challenge. The wide range and complexity of the metabolome can hide low-abundance but functionally critical metabolites. Furthermore, flux-based metabolic insights usually depend on stable isotope tracing, which is technically difficult to perform on limited clinical patient samples. Reproducibility also depends heavily on the standardization of sample preparation, instrumentation, and data analysis across platforms.

Nevertheless, recent technological advances are driving basic research toward clinical translation. An example of this innovation is the use of *in vivo* ^{13}C tracing to reveal metabolic circuits that are essential to LSC survival and to directly demonstrate the value of metabolomics in identifying targetable metabolic flux *in vivo.*[144] Additional research demonstrates the efficacy of co-targeting mitochondrial sirtuin 3 and cholesterol homeostasis to selectively disrupt mitochondrial function in AML LSCs,[145] while other studies demonstrate the efficacy of metformin for activating the AMPK pathway in AML to enhance chemosensitivity.[105] Together, these studies show a growing capacity to move from metabolomic discovery toward mechanistically driven interventions. As metabolomic technologies become more sensitive, especially in single-cell and live-cell contexts, they hold potential to define new biomarkers and therapies tailored specifically to LSCs, thereby advancing translational leukemia research.

5. Conclusion

The study of stem cell metabolism offers critical insight into the networks that govern HSCs and the precise regulation of quiescence and activation. Disruption of these tightly regulated metabolic programs leads HSCs to acquire abnormal metabolic profiles that contribute to oncogenic transformation. While LSCs maintain features of normal stemness, such as quiescence and drug resistance, they exhibit distinct metabolic characteristics that enable them to survive and resist therapeutic intervention. Recent advances in metabolic profiling, including single-cell analytics and live-cell functional assays, have enabled high-resolution assessment of these divergent metabolic states. Novel and emerging technologies supporting metabolic profiling not only reveal alterations in the biochemical pathways that contribute to HSC quiescence

and LSC survival but also highlight specific bioenergetic vulnerabilities within LSCs that may serve as therapeutic targets. By gaining a deeper understanding of the metabolic characteristics that distinguish healthy HSC activity from dysregulated LSC activity, researchers can better understand the mechanisms that sustain healthy function and identify those that lead to oncogenic transformation and other hematological disorders.

Collectively, these findings suggest that while LSCs are metabolically altered for survival, these unique adaptations may serve as targets for therapeutic intervention. Strategies that impair mitochondrial metabolism disrupt FAO or target nutrient-sensing pathways may offer promising approaches for selectively eliminating LSCs without compromising healthy hematopoiesis. As our understanding of stem cell metabolism deepens, these insights will guide the development of next-generation therapies to improve treatment durability and prevent relapse in hematologic malignancies such as AML and CML.

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Conflict of interest

Kathleen M. Sakamoto is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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