# Mitochondria underlie different metabolism of hematopoietic stem and progenitor cells

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It is becoming increasingly clear that hematopoietic stem cells (HSC) are a heterogeneous set of cells with a diversity of functional states despite (so far seemingly) immunophenotypically identical cells. Long-term (LT-) HSC, those endowed with long-lasting self-renewal, have been shown to differ from more differentiated cells in energy metabolism. LT-HSC reside in a hypoxic microenvironment[1] in the bone marrow and preferentially use glycolysis to obtain energy.[2] Adjusting oxygen bioavailability and transitions between aerobic and anaerobic metabolism might be one of the ways by which HSC niche cells, such as sub-endothelial nestin$^+$ mesenchymal stem cells,[3] regulate HSC behavior. Intriguingly, nestin$^+$ mesenchymal stem cells are directly regulated by sympathetic nerve fibers, which control blood flow, oxygen availability and also circadian HSC traffic.[4] The role of the microenvironment in regulating HSC metabolism is, therefore, an exciting area of research.

During glycolysis, glucose is converted into pyruvate to generate ATP. Pyruvate can be transformed into acetyl-CoA - later utilized in the mitochondria - by pyruvate dehydrogenase, which is inhibited by pyruvate dehydrogenase kinases (Pdk). The characteristic hypoxia of LT-HSC stabilizes the hypoxia-inducible factor HIF-1α, promoting glycolysis and leading to Pdk activation, pyruvate dehydrogenase inhibition and a reduced supply of acetyl-CoA to mitochondria. These kinases have therefore emerged as critical metabolic regulators of HSC function.[5]

In the mouse, LT-HSC contain fewer mitochondria than do more differentiated hematopoietic progenitors.[2,6] In this issue of Haematologica, Romero-Moya *et al.* report a similar finding in human HSC-enriched cord blood CD34$^+$ cells.[7] In their study, human cord blood CD34$^+$ cells were sorted according to mitochondrial content. Concordantly with the results previously reported in the mouse,[2,6] CD34$^+$ cells with fewer mitochondria contained more immunophenotypically-defined (CD34$^+$ CD38$^-$) HSC.[7] Since the authors used immunomagnetically-enriched CD34$^+$ cells, it would be interesting to know whether more purified human HSC follow the same trend. Human HSC can be virtually isolated at single-cell resolution as Lin$^-$ CD34$^+$ CD38$^-$ CD45RA$^-$ Thy1$^+$ Rho$^{lo}$ CD49f$^+$ cells.[8] It would also be interesting to know whether those HSC are the ones containing fewer mitochondria and favoring glycolysis. In the study by Romero-Moya *et al.*, cord blood CD34$^+$ cells with fewer mitochondria generated slightly fewer colony-forming units in culture (a readout of hematopoietic progenitor activity), mostly of erythroid lineage, and reduced secondary colonies (reflecting more primitive hematopoietic progenitors). As expected, most of the differences between CD34$^+$ cells with more or fewer mitochondria were attenuated or disappeared when cells were cultured, presumably under 20% $O_2$.[7]

The gold standard assay to test HSC activity is long-term transplantation into mice. The kinetics of hematopoietic reconstitution in conditioned recipients is hierarchically organized in mice[9] and humans,[10] with more committed progenitors giving rise to blood cells soon after transplantation, while only LT-HSC can contribute to hematopoiesis in the long term. Romero-Moya *et al.* analyzed the bone marrow of immunodeficient mice 7 days after transplantation and surprisingly found a slight increase in human hematopoietic chimerism (mostly consisting of B cells) from CD34$^+$ cells with fewer mitochondria,[7] which would suggest that this population might not only contain HSC but also committed precursor cells. Further studies, including purification of HSC and long-term transplantations, could determine whether human LT-HSC and/or other hematopoietic progenitors have relatively fewer mitochondria and a distinct metabolic profile. Controlling HSC metabolism indirectly, e.g. through supporting mesenchymal stem cells, might facilitate expansion of human cord blood HSC for transplantation.[11]

Mitochondria are the major source of reactive oxygen species (ROS), which are generated as a result of regular bioenergetic metabolism. ROS levels must be tightly regulated in the cells as they play a key role as second messengers, but are detrimental when their levels are too high. In fact, HSC are more sensitive to ROS exposure than are committed progenitors, and small increases in ROS can compromise HSC 'stemness' whereas high levels induce HSC apoptosis[12] (Figure 1). Thus, the comparatively lesser mitochondrial content in murine[2,6] and human[7] HSC might contribute to protect the cells from mitochondrial damage and subsequent apoptosis driven by ROS overproduction. Indeed, different studies in the mouse have shown that HSC have lower ROS levels than more differentiated progenitors.[13]

In addition, other protective mechanisms have been proposed to actively reduce ROS levels in HSC, mainly through the stimulation of antioxidant defenses. The serine/threonine protein kinase Ataxia telangiectasia mutated (ATM) is essential to maintain low ROS levels in HSC.[14] Following DNA damage, ATM is activated and phosphorylates several proteins responsible for DNA repair, cell cycle arrest or programmed cell death. HSC deficient in ATM exhibit increased ROS levels and reduced self-renewal.[14] In addition, the Foxhead O (FoxO) subfamily of transcription factors regulates ATM expression and ROS levels in HSC, since deletion of FoxO1,3 and 4 resulted in increased ROS production and decreased HSC self-renewal.[15]

Polycomb proteins, and more specifically Bmi-1, are master epigenetic regulators of HSC self-renewal and fate. This gave rise to the idea of a stem cell signature that defines the identity of the HSC. The ability of Bmi-1 to maintain the 'stemness' of HSC relies in part on the silencing of one of its major targets, the locus encoding the $p16^{INK4a}$ and $p19^{Arf}$ tumor suppressors, which promote cell senescence.[16] Notably, Bmi-1 also regulates mitochondrial ROS production independently of that pathway, further linking ROS and HSC fate.[17]

Another potential protective mechanism against oxidative

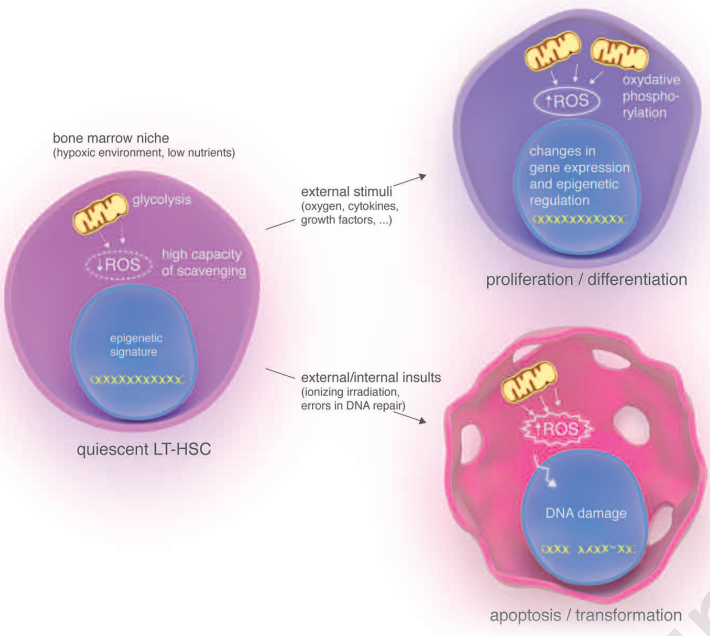


**Figure 1.** ROS produced during mitochondrial metabolism determine the fate of HSC. Quiescent LT-HSC possess protective mechanisms to prevent abnormally high ROS levels, including low mitochondrial content, preferential use of glycolysis and reinforced antioxidant defenses. Small changes in ROS content may affect HSC fate, stimulating these cells' proliferation and differentiation, and reducing their self-renewal capacity. High levels of ROS may lead to either HSC apoptosis or transformation.

stress that has been less explored is represented by mitochondrial DNA (mtDNA) variants. In mice, frequent and non-pathological mtDNA variants determine differences in the performance of mitochondrial oxidative phosphorylation and ROS production.[18] Some inherited mtDNA variants have been related to healthy aging in human centenarians,[19] whereas a certain somatic mtDNA mutation accumulation in leukocytes was suggested to enhance immunity and promote longevity.[20] Recently, CD34$^+$ cells from members of the same family were shown to share several unique mtDNA variants, but the overall age-related accumulation of mtDNA mutations in CD34$^+$ cells varied in different families. Future study of mtDNA variants could pave the way to discerning susceptibility to age-related HSC failure/dysfunction mediated by ROS.[21]

Abnormally high ROS levels can decrease HSC self-renewal through activation of the p38 mitogen-activated protein kinase (MAPK) pathway.[22] In fact, treatment with a p38 inhibitor has been shown to restore normal function in HSC with a high ROS content.[13,22] High ROS levels have also been associated with increased expression of the mammalian target of rapamycin (mTOR).[13] In hypoxic HSC, mTOR is inhibited by its major negative regulator Tuberous sclerosis complex (TSC1). TSC1 mutations have been shown to increase mitochondrial biogenesis and ROS accumulation, while reducing HSC self-renewal.[23] In addition, mTOR is negatively regulated by the AMP-activated protein kinase (AMPK). When nutrients are low, AMPK is activated by the tumour suppressor Lkb1, leading to mTOR repression and reduced cell proliferation. Therefore, multiple pathways regulating ROS production and/or directly affected by ROS can control HSC energy metabolism and function.

Recent studies have shown that Lkb1 regulates quiescence and energy metabolism in HSC but, unexpectedly, not through the AMPK-mTOR pathway but, instead, by largely unknown mechanisms.[24-26] Deletion of Lkb1[24-26] or AMPK[26] decreases mitochondrial biogenesis and energy production in HSC. This seems to be mediated, at least partially, through the master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma (PPARγ) coactivator protein 1α (PGC-1α).[25] During stress hematopoiesis, PGC-1α is required to induce mitochondrial biogenesis and stimulate glucose uptake and rapid proliferation of HSC and hematopoietic progenitors, despite the relatively low oxygen availability.[27] Altogether, these studies have coupled ROS levels and master regulators of energy metabolism to HSC function. Controlling HSC metabolism has, therefore, emerged as a promising avenue to stimulate engraftment[28] and also to sensitize malignant cells.[29] Nevertheless, important metabolic differences have been noted between normal and malignant HSC. Unlike normal HSC, leukemic stem cells and their derivatives have been proposed to contain a greater mitochondrial mass, have a higher $O_2$ consumption and be particularly sensitive to inhibition of mitochondrial translation.[30] Future studies will determine whether these promising avenues for obtaining metabolic control of normal and malignant HSC are therapeutically valuable.

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## References


1. Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc Natl Acad Sci USA. 2007;104(13):5431-6.
2. Simsek T, Kocabas F, Zheng J, Deberardinis RJ, Mahmoud AI, Olson EN, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell. 2010;7(3):380-90.
3. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010;466(7308):829-34.
4. Mendez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. Nature. 2008;452(7186):442-7.
5. Takubo K, Nagamatsu G, Kobayashi CI, Nakamura-Ishizu A, Kobayashi H, Ikeda E, et al. Regulation of glycolysis by Pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. Cell Stem Cell. 2013;12(1):49-61.
6. Norddahl GL, Pronk CJ, Wahlestedt M, Sten G, Nygren JM, Ugale A, et al. Accumulating mitochondrial DNA mutations drive premature hematopoietic aging phenotypes distinct from physiological stem cell aging. Cell Stem Cell. 2011;8(5):499-510.
7. Romero-Moya D, Bueno C, Montes R, Navarro-Montero O, Iborra FJ, Lopez LC, et al. Cord blood-derived CD34+ hematopoietic cells with low mitochondrial mass are enriched in hematopoietic repopulating stem cell function. Haematologica. 2013;98(7):1022-9.
8. Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science. 2011;333(6039):218-21.
9. Nibley WE, Spangrude GJ. Primitive stem cells alone mediate rapid marrow recovery and multilineage engraftment after transplantation. Bone Marrow Transplantation. 1998;21(4):345-54.
10. Martinez C, Urbano-Ispizua A, Rozman C, Marin P, Rovira M, Sierra J, et al. Immune reconstitution following allogeneic peripheral blood progenitor cell transplantation: comparison of recipients of positive CD34+ selected grafts with recipients of unmanipulated grafts. Exp Hematol. 1999;27(3):561-8.
11. Isern J, Martín-Antonio B, Ghazanfari R, Martín AM, López JA, del Toro R, et al. Self-renewing human bone marrow mesenspheres promote hematopoietic stem cell expansion. Cell Rep. 2013. pii: S2211-1247(13)00165-4.
12. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell. 2011;9(4):298-310.
13. Jang YY, Sharkis SJ. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood. 2007;110(8):3056-63.
14. Ito K, Hirao A, Arai F, Matsuoka S, Takubo K, Hamaguchi I, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004;431(7011):997-1002.
15. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell. 2007;128(2):325-39.
16. Oguro H, Iwama A, Morita Y, Kamijo T, van Lohuizen M, Nakauchi H. Differential impact of Ink4a and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. J Exp Med. 2006;203(10):2247-53.
17. Liu J, Cao L, Chen J, Song S, Lee IH, Quijano C, et al. Bmi1 regulates mitochondrial function and the DNA damage response pathway. Nature. 2009;459(7245):387-92.
18. Moreno-Loshuertos R, Acin-Perez R, Fernandez-Silva P, Movilla N, Perez-Martos A, Rodriguez de Cordoba S, et al. Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants. Nat Genet. 2006;38 (11):1261-8.
19. De Benedictis G, Rose G, Carrieri G, De Luca M, Falcone E, Passarino G, et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. Faseb J. 1999;13(12):1532-6.
20. Zhang J, Asin-Cayuela J, Fish J, Michikawa Y, Bonafe M, Olivieri F, et al. Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. Proc Natl Acad Sci USA. 2003;100(3):1116-21.
21. Yao YG, Kajigaya S, Feng X, Samsel L, McCoy JP, Jr., Torelli G, et al. Accumulation of mtDNA variations in human single CD34(+) cells from maternally related individuals: Effects of aging and family genetic background. Stem Cell Res. 2013;10(3):361-70.
22. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med. 2006;12(4):446-51.
23. Chen C, Liu Y, Liu R, Ikenoue T, Guan KL, Liu Y, et al. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med. 2008;205(10):2397-408.
24. Gurumurthy S, Xie SZ, Alagesan B, Kim J, Yusuf RZ, Saez B, et al. The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. Nature. 2010;468(7324):659-63.
25. Gan B, Hu J, Jiang S, Liu Y, Sahin E, Zhuang L, et al. Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. Nature. 2010;468(7324):701-4.
26. Nakada D, Saunders TL, Morrison SJ. Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. Nature. 2010;468(7324):653-8.
27. Basu S, Broxmeyer HE, Hangoc G. Peroxisome proliferator-activated-gamma coactivator-1alpha-mediated mitochondrial biogenesis is important for hematopoietic recovery in response to stress. Stem Cells Dev. 2013;22(11):1678-92.
28. Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP. Pharmacologic stabilization of HIF-1alpha increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation. Blood. 2013;121(5):759-69.
29. Frolova O, Samudio I, Benito JM, Jacamo R, Kornblau SM, Markovic A, et al. Regulation of HIF-1alpha signaling and chemoresistance in acute lymphocytic leukemia under hypoxic conditions of the bone marrow microenvironment. Cancer Biol Ther. 2012;13(10):858-70.
30. Skrtic M, Sriskanthadevan S, Jhas B, Gebbia M, Wang X, Wang Z, et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell. 2011;20(5):674-88.


# Circulating microparticles in children with sickle cell anemia: a heterogeneous procoagulant storm directed by hemolysis and fetal hemoglobin


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Chronic hemolytic anemias are made up of sickle cell anemia (SCA), beta (β)-thalassemia, paroxysmal nocturnal hemoglobinuria, autoimmune hemolytic anemia, and unstable hemoglobinopathies. They are associated with a high thrombotic risk. In SCA patients, a high rate of both venous and arterial thrombosis (deep vein thrombosis, pulmonary embolism, stroke, pregnancy-related venous thromboembolism) has been reported.[1] Interestingly, these subjects commonly present with laboratory features of a subclinical hypercoagulable state,[2] charac-