News & Views

Stem cells "aclymatise" to regenerate the blood system

Livia E Lisi-Vega[1-3] and Simón Méndez-Ferrer[1-3,*]

[1] *Wellcome-MRC Cambridge Stem Cell Institute, Cambridge CB2 0AW, United Kingdom*

[2]*Department of Hematology, University of Cambridge, Cambridge CB2 0AW, United Kingdom*

[3]*National Health Service Blood and Transplant, Cambridge Biomedical Campus, Cambridge CB2 0AW, United Kingdom*

[*]Correspondence: < sm2116@cam.ac.uk >

Summary: How blood stem cells balance fate decisions between quiescence maintenance and differentiation during recovery from cancer treatment, remains poorly understood. A recent study by Umemoto et al (2022) uncovers an unexpected linkage between metabolic and epigenetic regulation of haematopoiesis, suggesting new targets in haematopoietic regeneration, with possible implications in leukaemogenesis and therapy resistance.

See also: **T Umemoto *et al* (2022)**

eToC: Recent work reports unexpected linkage between acetyl-CoA metabolism and epigenetic regulation of hematopoietic stem cells during recovery after myeloablation.

Main text:

At the apex of the haematopoietic system's hierarchy, haematopoietic stem cells (HSCs) are in charge of maintaining homeostasis by carefully regulating their capacity to self-renew and differentiate (Ito and Suda, 2014). After an insult, such as inflammation, or following myeloablation or irradiation for cancer treatment, HSCs need to rapidly replenish the haematopoietic system, while carefully maintaining the balance between self-renewal and differentiation. This "fine-tuning" is the result of a complex interplay between HSC-intrinsic mechanisms and signals from the specialised bone marrow microenvironment, which regulates HSC quiescence during haematopoietic regeneration (Fielding et al, 2022; Silverstein et al, 2016). In this issue, an elegant study by Umemoto *et al* (2022) investigates the contribution of HSC subsets to haematopoietic recovery and identifies unexpected links between metabolic and epigenetic HSC regulation dependent on acetyl-CoA and its biosynthetic enzyme, Acly.

Quiescent and proliferating HSCs have different metabolic requirements and are respectively required for long-term maintenance or short-term production of blood and immune cells. Quiescent HSCs preferentially rely on anaerobic glycolysis, while HSC proliferation and differentiation requires oxidative phosphorylation (OXPHOS), which is associated with increased mitochondrial potential (ΔΨm). Importantly, ΔΨm decreases in aged HSCs, critically influencing HSC transcription, engraftment and regenerative potential (Mansell et al, 2021). While the key roles of reactive oxygen species (ROS) or hypoxia in HSC fate have been studied in detail (Suda et al, 2011), the interplay between metabolism and epigenetic HSC regulation is comparatively less explored. A recent study has identified retinoic acid and its metabolite 4-oxo-retinoic acid as master regulators of metabolic hubs involved in epigenetic regulation of transcriptional networks controlling HSC fate (Schönberger et al, 2022). All in all, an important conclusion of these studies is that the balance between HSC self-renewal and differentiation cannot be thought of as a simple binary switch. Cellular metabolic status is reversible and so, by subjecting their fate decisions to metabolic regulation, HSCs achieve a continuum of cellular states. However, it is not yet clear how HSCs translate their metabolic status into cell fate decisions.

In their study, Umemoto and colleagues (2022) analyse two different stages of haematopoietic recovery after 5-fluorouracil (5-FU), a drug commonly used to treat cancer, since it eliminates proliferative cells. In the haematopoietic system, 5-FU reduces proliferative HSCs expressing low levels of endothelial protein receptor ($EPCR^{low}$), while it spares quiescent $EPCR^{high}$ HSCs, which subsequently play a key role during the early phase of haematopoietic regeneration (3-7 days after 5-FU). It is only in a later phase of recovery (9-12 days) that $CD48^{+}$ progenitor cells markedly increase and become predominant. Comparison of the phenotypic

characteristics of HSCs in both phases revealed that early phase HSCs exhibit greater cell division capacity, mitochondrial metabolism and stem cell potential, compared with late phase HSCs. Correspondingly, upon division, late phase HSCs give rise to more $CD48^+$ progenitor cells and less phenotypic HSCs. Analysis of the chromatin accessibility patterns of early vs. late phase HSCs indicates an increased accessibility to progenitor-related genes (e.g. CD48) in late phase HSCs. However, these cells exhibit unaltered H3K27ac levels (a mark responsible for activating gene expression) within these same genetic regions, thereby allowing their stemness maintenance.

Interrogating potential mechanisms responsible for the dynamic changes observed in chromatin during haematopoietic regeneration, Umemoto *et al* (2022) focus on acetyl-CoA, which is a major regulator of histone acetylation, and its biosynthetic enzyme, Acly. They find that Acly mRNA, protein and phosphorylation (p-Ser455) levels in HSCs exhibit similar dynamics as H3K27ac – increasing during the early phase of haematopoietic recovery, to decrease later. Compared with early phase HSCs, late phase HSCs exhibit lower citrate and total acetylated lysine levels, which are consistent with altered Acly-dependent metabolism, and which correlate with suppressed ΔΨm. Supporting a causal relationship, inhibition of Acly, the mitochondrial citrate transporter, or histone acetyltransferases, converge into the same outcome: inhibition of early phase HSC expansion and $CD48^+$ progenitor generation. These findings identify Acly-mediated metabolism and its regulation of histone acetylation as important mediators in the dynamic HSC regulation during haematopoietic regeneration.

Overall, the observations put forward by Umemoto and colleagues (2022) illustrate the epigenetic "landscape" diversity during haematopoietic regeneration. Soon after myeloablation, early phase HSCs ($EPCR^{high}$) metabolically adapt to enable stem cell expansion by simultaneously enhancing ΔΨm, Acly-dependent metabolism and histone acetylation of stemness genes. Conversely, late phase HSCs repress Acly metabolism to favour priming or differentiation through increased chromatin accessibility of committed progenitor-associated genes, and a concomitant decrease in global H3K27ac levels within the same genetic regions. According to this model, it is the well-timed increase and decrease in H3K27ac marks within these regions mediated by Acly/acetyl-CoA metabolism what allows HSCs to either differentiate into $CD48^+$ progenitors or maintain their stem cell capacity during regenerative haematopoiesis.

The thorough study by Umemoto et al (2022) adds to cumulative evidence underscoring the interplay between mitochondrial activity and the epigenome in the regulation of HSC fate decisions. However, other metabolic regulators might be at play. For instance, primitive HSCs have been reported to rely on high levels of fatty acid oxidation (FAO) and low levels of fatty acid synthesis (FAS), with the FAO/FAS ratio acting as a "rheostat" that controls HSC proliferation (Ito et al, 2012). Acly is a lipogenic enzyme, and its activity very much influences cellular fatty acid metabolism. Interestingly, Umemoto et al detect increased H3K27ac marks in FAS genes of early phase HSCs, and treatment with a FAS inhibitor suppresses early phase HSC expansion. These results suggest a possible causal relationship between these two phenomena. Therefore, Acly might regulate HSC fate decisions through its role in lipid synthesis (either through FAO or epigenetic regulation of lipid synthesis genes), adding to its regulation of histone acetylation of stem/progenitor genes.

The regulation of normal HSC proliferation and differentiation through this interplay of metabolism and epigenome has potential implications for haematological malignancies. In the leukaemic setting, being able to tip the balance in favour of differentiation becomes a powerful therapeutic strategy. Resembling their healthy more "primitive" counterparts, leukaemia stem cells rely on high fatty acid oxidation and low FAS activity (Ye et al, 2016). In line with this, ACC1, the enzyme that converts acetyl-CoA to manolyl-CoA, has been shown to suppress the self-renewal capacity of leukaemia-initiating cells by switching cellular energy flux towards high FAS, high NADPH consumption and ROS accumulation (Ito et al, 2021). Future studies will investigate the implications of Acly-mediated regulation of HSC fate in leukaemogenesis and therapy resistance.

Conflict of Interest

The author declares no conflict of interest.

Figure Legend

Figure 1. **Model of metabolic and epigenetic crosstalk during regenerative haematopoiesis[4].** Quiescent haematopoietic stem cells (HSCs) exhibit low levels of mitochondrial membrane potential ($\Delta\Psi_m$) and acetyl-CoA biosynthetic enzyme (Acly) under steady state. Acly-dependent chromatin accessibility favours differentiation of $EPCR^{low}$ HSCs and self-renewal of $EPCR^{High}$ HSCs. In the early phase after 5-fluorouracil (5-FU) administration,

EPCR^{High} HSCs undergo self-renewing divisions by enhancing histone acetylation under high mitochondria-Acly activity. In the late phase after 5-FU administration, HSCs acquire differentiation potential through increased accessibility of progenitor cell-related *cis*-regulatory regions, while histone acetylation suppression due to decreased mitochondria-Acly activity allows for HSC maintenance by repressing genes with increased accessibility. Figure based on Fig. EV5C of Umemoto *et al* (2022).

References

Fielding C, García-García A, Korn C, Gadomski S, Fang Z, Reguera JL, Pérez-Simón JA, Göttgens B, Méndez-Ferrer S (2022) Cholinergic signals preserve haematopoietic stem cell quiescence during regenerative haematopoiesis. *Nat Commun* 13: 543

Ito K, Suda T (2014) Metabolic requirements for the maintenance of self-renewing stem cells. *Nat Rev Mol Cell Biol* 15: 243–256

Ito K, Carracedo A, Weiss D, Arai F, Ala U, Avigan DE, Schafer ZT, Evans RM, Suda T, Lee CH et al (2012) A PML–PPAR-δ pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. *Nat Med* 18:1350-8.

Ito H, Nakamae I, Kato JY, Yoneda-Kato N (2021) Stabilization of fatty acid synthesis enzyme acetyl-CoA carboxylase 1 suppresses acute myeloid leukemia development. *J Clin Invest* 131: e141529

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

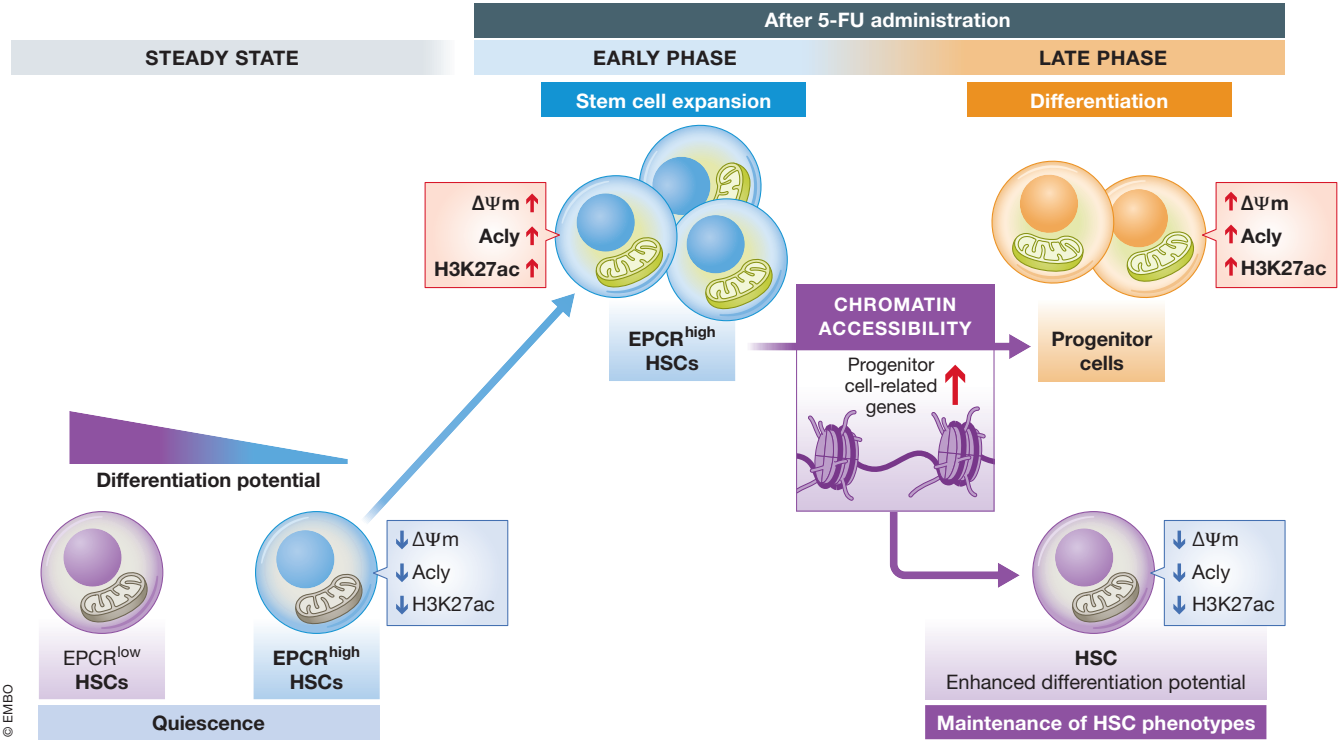
Schönberger K, Obier N, Romero-Mulero MC, Cauchy P, Mess J, Pavlovich PV, Zhang YW, Mitterer M, Rettkowski J, Lalioti ME, et al (2022) Multilayer omics analysis reveals a non-classical retinoic acid signaling axis that regulates hematopoietic stem cell identity. *Cell Stem Cell* 29:131-148

Silberstein L, Goncalves KA, Kharchenko PV, Turcotte R, Kfoury Y, Mercier F, Baryawno N, Severe N, Bachand J, Spencer JA, et al (2016) Proximity-Based Differential Single-Cell Analysis of the Niche to Identify Stem/Progenitor Cell Regulators. *Cell Stem Cell* 19:530-543

Suda T, Takubo K, Semenza GL (2011) Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell*. 9: 298-310

Umemoto T, Johansson A, Adil Ahmad S, Hashimoto M, Kubota S, Kikuchi K, Odaka H, Era T, Kurotaki D, Sashida G *et al* (2022) ATP citrate lyase controls hematopoietic stem cell fate and supports bone marrow regeneration. *EMBO J* doi: xxx

Ye H, Adane B, Khan N, Sullivan T, Minhajuddin M, Gasparetto M, Stevens B, Pei S, Balys M, Ashton JM, et al (2016) Leukemic Stem Cells Evade Chemotherapy by Metabolic Adaptation to an Adipose Tissue Niche. *Cell Stem Cell* 19: 23-37


After 5-FU administration
STEADY STATE
EARLY PHASE
LATE PHASE
Stem cell expansion
Differentiation
ΔΨm ↑
Acly ↑
H3K27ac ↑
EPCR^high HSCs
↑ΔΨm
↑Acly
↑H3K27ac
Progenitor cells
CHROMATIN ACCESSIBILITY
Progenitor cell-related genes
Differentiation potential
EPCR^low HSCs
EPCR^high HSCs
↓ΔΨm
↓Acly
↓H3K27ac
HSC
Enhanced differentiation potential
Quiescence
Maintenance of HSC phenotypes