

INSIGHTS

Ca²⁺tapulting HSCs into action

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In this issue of *JEM*, Umemoto et al. (<https://doi.org/10.1084/jem.20180421>) demonstrate that calcium influx stimulates mitochondrial metabolism and initiates proliferation in hematopoietic stem cells (HSCs). Extracellular adenosine, sourced from surrounding hematopoietic progenitors, inhibits this calcium influx, thereby suppressing mitochondrial metabolism and promoting HSC quiescence. This is the first demonstration that a calcium-mitochondria pathway regulates HSC division.

The ability to precisely control hematopoietic stem cell (HSC) quiescence, self-renewal, and differentiation is a prerequisite for the identification of effective means of expanding clinically relevant multipotent HSCs for widespread transplantation purposes. While a number of studies have revealed some of the key epigenetic and transcriptional regulators controlling HSC maintenance, the essential metabolic regulators of HSC fates and their utility in HSC expansion still remain poorly understood. Quiescent HSCs predominantly reside in perisinusoidal regions of the bone marrow (BM) where the oxygen tension is markedly low (Ding et al., 2012; Spencer et al., 2014). As a consequence, local hypoxia has been proposed to maintain HSC quiescence and a low metabolic state, at least in part through the HIF-1-PDK2/4 pathway (Simsek et al., 2010; Takubo et al., 2013). Mitochondria require oxygen to generate their membrane potential and produce ATP, but quiescent HSCs suppress mitochondrial metabolism and predominantly rely on glycolysis for energy production (Simsek et al., 2010). However, quiescent HSCs are also found in periarteriolar areas of the BM (Kunisaki et al., 2013), where the local oxygen tension is significantly higher compared with the perisinusoidal regions (Spencer et al., 2014). Furthermore, HSCs cultured under normoxic conditions also display low mitochondrial metabolic activity (de Almeida et al., 2017). Together, these observations indicate that hypoxia may not be the sole cause of low mitochondrial metabolism in quiescent HSCs.

In this issue, Umemoto et al. investigates the metabolic regulation of HSC fate deci-



Insight from Andrew J. Finch, Amelie V. Guitart, and Kamil R. Kranc.

sions between quiescence and proliferation. Gene expression analyses in quiescent HSCs from untreated mice and activated HSCs (sampled just before they enter cell division) from 5-fluorouracil (5-FU)-treated mice were performed to reveal molecular mechanisms underpinning the onset of HSC division. In addition to enrichment of cell cycle-related and pro-growth mTOR and glycolysis gene sets, notably, they found a strong enrichment of genes associated with oxidative phosphorylation. This important result focused the study on changes in mitochondrial metabolism that precede HSC transition from quiescence to cell cycle re-entry. The authors found that HSCs display significant calcium influx into their cytosol and mitochondria and a concomitant increase in mitochondrial potential as a prerequisite for cell division in vitro. In their in vivo experiments, they used the ability of 5-FU to trigger HSC cell cycle reentry following depletion of more mature cycling hematopoietic cells. Intriguingly, HSCs from 5-FU-treated mice had elevated levels of intracellular calcium and enhanced mitochondrial potential before their division. In accordance with this, HSCs stimulated with thrombopoietin (TPO) and stem cell factor

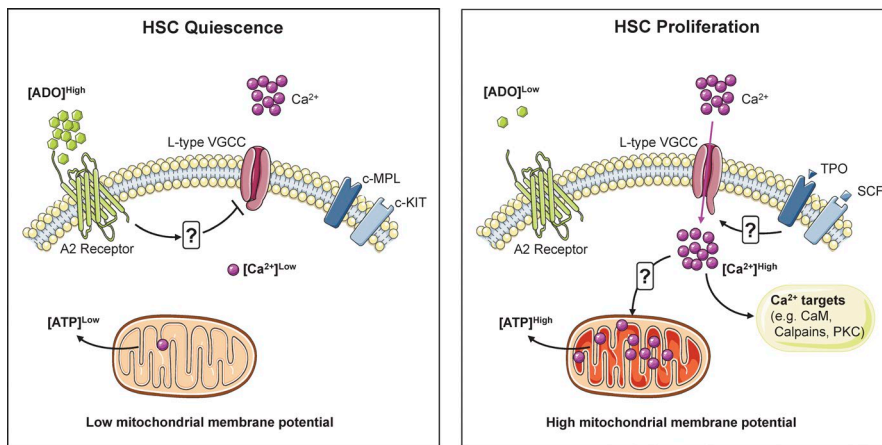
(SCF) in vitro also displayed the activation of this calcium-mitochondria pathway before undergoing cell division. The suppression of extracellular calcium influx into HSCs by Nifedipine or Isradipine (blockers of L-type voltage-gated Ca²⁺ channels) abolished the increase of intracellular Ca²⁺ and the resultant mitochondrial activation and prolonged the interval between the cell division through suppressing the expression of genes involved in late G1 phase of the cell cycle. While HSCs cultured in the absence of Ca²⁺ channel blockers lost their in vivo multilineage reconstitution potential following several divisions in vitro, those treated with Nifedipine maintained their undifferentiated immunophenotype and retained their stem cell activity upon transplantation. This suggests that calcium is a pivotal regulator of the balance between HSC quiescence and entry into the cell cycle.

In search for mechanisms underlying the regulation of the calcium-mitochondria pathway in HSCs, Umemoto et al. (2018) discovered that myeloid progenitor cells cocultured with HSCs suppress the Ca²⁺ influx into HSCs. Intriguingly, this effect is mediated, at least in part, by adenosine produced in myeloid progenitors, which activates

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The role of the adenosine- $[Ca^{2+}]$ -mitochondria axis in the regulation of HSC division. Left: Adenosine (ADO) through adenosine A2 receptor prevents calcium entry to maintain low mitochondrial membrane potential and sustain HSC quiescence. Right: TPO and SCF induce calcium influx in HSCs through L-type voltage-gated Ca²⁺ channel (VGCC), which in turn activates mitochondria, leading to HSC division. Ca²⁺ influx may also regulate multiple other targets, including CaM, calpains, and the PKC family members, to exert its functions in HSCs. This figure was created using the Servier Medical Art database (licensed under a Creative Commons Attribution 3.0 Unported License).

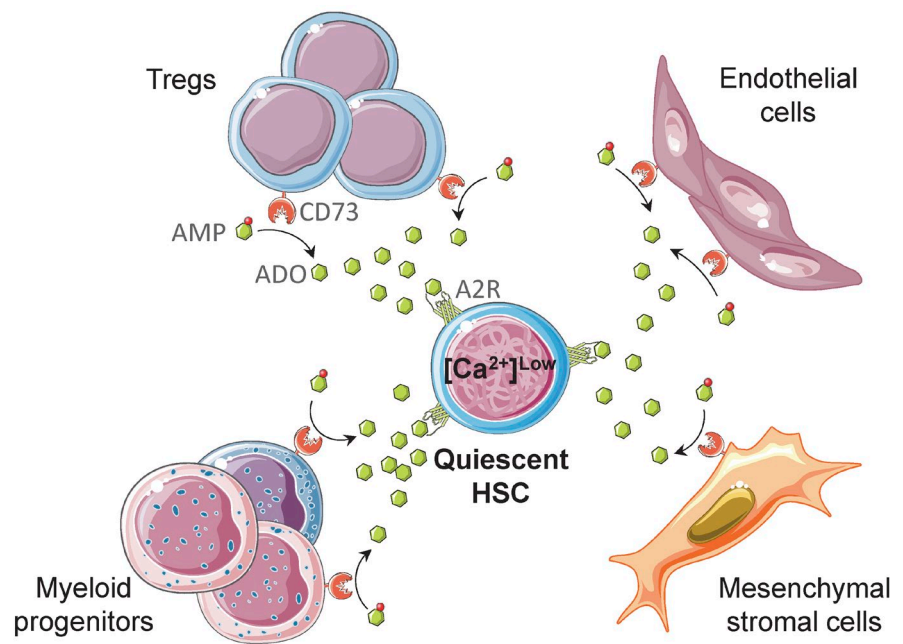
adenosine A2 receptors expressed by HSCs and thereby negatively regulates Ca²⁺ influx into HSCs and consequent mitochondrial activation. In their *in vivo* experiments, the authors demonstrated that following 5-FU-mediated myeloablation, the levels of adenosine drop, coinciding with an activation of the Ca²⁺-mitochondria pathway and HSC division. Notably, administration of an adenosine A2 receptor agonist following 5-FU treatment suppressed mitochondrial activity of HSCs and dampened their division. Thus, a decrease in extracellular adenosine (produced by myeloid progenitors that are depleted by 5-FU) within the BM microenvironment is at least one mechanism through which HSCs undergo cell division upon myeloablation.

Recent investigations revealed that although quiescent HSCs have reduced mitochondrial respiration compared with progenitor cells, their mitochondrial content is unexpectedly high and mitochondrial turnover is low (de Almeida et al., 2017). However, why quiescent HSCs would require high mitochondrial mass and how the mitochondrial activity is suppressed (even under normoxic conditions) in HSCs remains puzzling. The work by Umemoto et al. (2018) suggests that quiescent HSCs sense extracellular adenosine from the BM microenvironment to suppress Ca²⁺ influx and inhibit mitochondrial activation. However, high mitochondrial content in quiescent HSCs may poise them for rapid induction of

the oxidative phosphorylation necessary for cell cycle reentry. The activities of three key energetic mitochondrial dehydrogenases (pyruvate dehydrogenase, NAD-isocitrate dehydrogenase, and oxoglutarate dehydrogenase) are stimulated by Ca²⁺ ions (Denton, 2009), and this offers a mechanism whereby influx of Ca²⁺ would stimulate mitochondrial ATP generation. Thus, in the scenario

portrayed by the authors, division-inducing stimuli such as myeloablation, bleeding, cytokines, or bacterial pathogens induce Ca²⁺ influx and activate ATP production necessary to undergo HSC division. There are, however, many outstanding questions that merit further investigations. How are Ca²⁺ levels regulated during different phases of the cell cycle and between sequential cell divisions in HSCs? What are the events downstream of the Ca²⁺-mitochondria pathway that determine HSC fate decisions between self-renewal and differentiation? Do HSCs pump out Ca²⁺ to sustain quiescence? Finally, considering the role of the Ca²⁺-mitochondria pathway in HSC division, how does Ca²⁺ influx impact on leukemic transformation and leukemic stem cell functions?

The paper by Umemoto et al. (2018) reinforces an emerging role of adenosine as a key extracellular regulator of adult HSC quiescence. The authors demonstrate that myeloid progenitor cells maintain HSC quiescence by producing adenosine. The ectonucleotidase CD73, which converts AMP into adenosine, is expressed in several other cell types surrounding HSCs. The CD150^{High} regulatory T cell (T reg) population, which is frequently adjacent to HSCs within their niches, promotes HSC quiescence and enhances allo-HSC engraftment upon trans-



Adenosine maintains HSC quiescence *in vivo*. Under physiological condition, T reg cells, myeloid progenitors, endothelial cells, and mesenchymal stromal cells express ecto-5'-nucleotidase (CD73), which converts AMP to adenosine. Adenosine presence in the BM microenvironment is sensed by adenosine A2 receptor (A2R), thereby inhibiting influx of extracellular Ca²⁺ and promoting HSC quiescence. This figure was created using the Servier Medical Art database (licensed under a Creative Commons Attribution 3.0 Unported License).

plantation (Hirata et al., 2018). These T reg cells produce adenosine, which in turn activates adenosine A2 receptors (highly expressed in HSCs) to promote HSC quiescence. CD73 is also highly expressed in mesenchymal stromal cells and sinusoidal endothelial cells, which are in close contact with quiescent HSCs and are essential for HSC maintenance (Breitbach et al., 2018). Thus, adenosine production may be a more general feature of hematopoietic cells and the BM microenvironment supporting HSC quiescence. Finally, Umemoto et al. (2018) indicate that one mechanism of adenosine's action in HSCs is to suppress the Ca²⁺-mitochondria pathway. However, how adenosine A2 receptor inhibits Ca²⁺ influx into HSCs to regulate HSC fates remains an open question for future investigations.

The authors propose direct links between elevation of cytosolic Ca²⁺, elevation of mitochondrial Ca²⁺, and mitochondrial ATP production, but important questions remain unanswered. First, a recent paper by Luchsinger et al. (2016) indicated that Mitofusin 2 regulates HSC maintenance by tethering of mitochondria to the endoplasmic reticulum and thereby promoting buffering of intracellular Ca²⁺. The interplay between the calcium-mitochondria pathway described by Umemoto et al. (2018) and the intracellular Ca²⁺ buffering described by Luchsinger et al. (2016) and how it impacts on HSC division merit further investigations. The second question is whether the transfer of Ca²⁺ is indeed direct and, if so,

through what channels. Mitochondrial Ca²⁺ has been reported to respond optimally to pulsatile, rather than sustained, cytosolic Ca²⁺ (Hajnoczky et al., 1995), and this suggests that the sustained influx of extracellular Ca²⁺ described by Umemoto et al. (2018) would be unlikely to result in transfer of the Ca²⁺ signal to the mitochondrion. Another outstanding question is what role other Ca²⁺-activated pathways play in the promotion of proliferation in HSCs. Ca²⁺ has long been known to drive proliferation in many cell types through direct activation of calmodulin (CaM), which promotes both G1/S and G2/M transition through several mechanisms, including Ca²⁺-dependent activation of the cyclin/cyclin-dependent kinase complexes. Calpains and members of the protein kinase C (PKC) family are also regulated by Ca²⁺, and thus the possibility for multiple Ca²⁺-activated signaling pathways to impact upon HSC proliferation exists.

The key future application of the study by Umemoto et al. (2018) is the expansion of HSCs for transplantation purposes. HSC transplantation (HSCT) often remains the only curative therapy in blood malignancies or severe immunodeficiencies. However, the shortage of histocompatible donors and limited numbers of HSCs that can be obtained from their sources (e.g., umbilical cord blood) often limit the widespread applications of HSCT. Therefore, the ex vivo expansion of clinically relevant HSCs is an unmet clinical need. The current study indicates that in vitro HSC culture in the pres-

ence of Ca²⁺ channel blocker, adenosine, or adenosine A2 receptor agonist allows for HSC divisions while maintaining their undifferentiated state. This discovery offers an exciting possibility that these agents may synergize with currently applicable HSC expansion approaches (e.g., those using an aryl hydrocarbon receptor antagonist StemRegenin-1 [Wagner et al., 2016] or the pyrimidoindole derivative UMI71 [Fares et al., 2014]) to provide unprecedented numbers of clinically relevant multipotent HSCs. Equally exciting is the prospect of modulating cellular Ca²⁺ levels to either protect HSCs from myeloablative chemotherapies or expand them in vivo following allogeneic HSCT.

- Breitbach, M., et al. 2018. *Cell Stem Cell*. 22:262–276. <https://doi.org/10.1016/j.stem.2018.01.008>
- de Almeida, M.J., et al. 2017. *Cell Stem Cell*. 21:725–729. <https://doi.org/10.1016/j.stem.2017.11.002>
- Denton, R.M. 2009. *Biochim. Biophys. Acta*. 1787:1309–1316. <https://doi.org/10.1016/j.bbabi.2009.01.005>
- Ding, L., et al. 2012. *Nature*. 481:457–462. <https://doi.org/10.1038/nature10783>
- Fares, I., et al. 2014. *Science*. 345:1509–1512. <https://doi.org/10.1126/science.1256337>
- Hajnoczky, G., et al. 1995. *Cell*. 82:415–424. [https://doi.org/10.1016/0092-8674\(95\)90430-1](https://doi.org/10.1016/0092-8674(95)90430-1)
- Hirata, Y., K. Furuhashi, H. Ishii, H.W. Li, S. Pinho, et al. 2018. *Cell Stem Cell*. 22:445–453.
- Kunisaki, Y., et al. 2013. *Nature*. 502:637–643. <https://doi.org/10.1038/nature12612>
- Luchsinger, L.L., et al. 2016. *Nature*. 529:528–531. <https://doi.org/10.1038/nature16500>
- Simsek, T., et al. 2010. *Cell Stem Cell*. 7:380–390. <https://doi.org/10.1016/j.stem.2010.07.011>
- Spencer, J.A., et al. 2014. *Nature*. 508:269–273. <https://doi.org/10.1038/nature13034>
- Takubo, K., et al. 2013. *Cell Stem Cell*. 12:49–61. <https://doi.org/10.1016/j.stem.2012.10.011>
- Umemoto, T., et al. 2018. *J. Exp. Med.* <https://doi.org/10.1084/jem.20180421>
- Wagner, J.E. Jr., et al. 2016. *Cell Stem Cell*. 18:144–155. <https://doi.org/10.1016/j.stem.2015.10.004>