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Beyond adenosine triphosphate: unveiling the pleiotropic effects of pyruvate kinase activation in sickle cell anemia

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In this issue, D’Alessandro et al. describe detailed multi-omics data on 15 individuals with sickle cell anemia (SCA), treated with mitapivat, a novel oral activator of pyruvate kinase (PK).

SCA, being the most prevalent genetic hematological disease worldwide, inflicts a devastating toll on global health, particularly affecting childhood survival rates in low-income countries. The unmet need for improved care is evident. Despite recent advancements, current treatment remains mostly limited to infectious prophylaxis, hydroxyurea, and transfusion therapy. The main global challenge lies in ensuring early diagnosis and widespread access to these critical treatments. Access to curative treatments, like hematopoietic stem cell transplantation and gene therapy, is for the foreseeable future confined to a small fraction of young patients in high-income countries. Meanwhile, there is a critical need to develop accessible and scalable pharmacological treatment options.

PK is the rate-limiting step of glycolysis and inhibition of PK has been extensively studied as an anti-neoplastic strategy through countering the Warburg effect. Contrarily, mitapivat activates all isotypes of PK. This is particularly relevant for mature red blood cells (RBCs), which rely solely on glycolysis for ATP generation. Patients with PK deficiency lack the RBC specific isoform of PK (PKR) and are unable to produce sufficient ATP in RBCs resulting in lifelong hemolytic anemia. Here, the RBC metabolome shows a buildup of glycolytic intermediates upstream of PK, most notably increasing 2,3-diphosphoglycerate (2,3-DPG) levels, which effectively decrease hemoglobin oxygen affinity and thereby ameliorate anemia symptoms. Unsurprisingly, mitapivat can effectively treat patients with PK deficiency, although the response is much dependent on PKLR genotype and residual PK protein.

Benefits of PK activation in anemias beyond PK deficiency may seem less evident. However, numerous studies have found insufficient glycolytic capacity and ATP generation in a range of hereditary anemias. In SCA, activating PK exerts multifaceted effects (Figure 1). It not only enhances ATP production in RBCs but also reduces 2,3-DPG levels, which in turn increases the oxygen affinity of hemoglobin. As deoxygenation is a key trigger of sickling, this could also be a contributing mechanism for mitapivat. Additionally, PK activation has been suggested to improve the glutathione pool and thereby have an antioxidant effect, though this until now has only been shown in a mouse model of β-thalassemia. Overall, PK activators are a promising drug class for treating SCA. Currently, three PK activators – mitapivat, etovapivat, and AG-946 – are undergoing clinical trials for SCA.

The study by D’Alessandro et al. examines mitapivat’s extensive molecular effects on SCA patients over up to two years, utilizing metabolomics, lipidomics, and proteomics within the framework of a long-term extension phase 1 study (NCT04610866).

Unsurprisingly, a decrease in 2,3-DPG combined with increased ATP levels were confirmed along with improved hematologic and sickling parameters. Notably, a rise in reduced glutathione and activation of Lands cycle points to improvement in oxidative stress, which provides some evidence to a central proposed
benefit of PK activators. Less intuitive is the reported decrease in mitochondrial proteins detected as RBCs are – at least in healthy individuals – mostly known for their absence of mitochondria. However, a range of recent studies have highlighted the frequent occurrence and potential negative clinical impact of mitochondrial retention in SCA\(^8\). In PK deficiency, patients lack the PKR isoform, but this is during early-stage erythropoiesis likely compensated by expression of the PKM2 isoform. Nonetheless, shortages in pyruvate or ATP in late-stage erythropoiesis might impair reticulocyte maturation and mitophagy\(^9\) and could underly the extreme reticulocytosis measured in patients with PK deficiency post-splenectomy\(^7\). If so, replenishing pyruvate and ATP with PK activators should promote mitophagy. This seemed to be the case in a preclinical study of Townes mice – a well-known model of SCA – where mitapivat ameliorated both mitochondrial retention and oxidative stress\(^10\). As the authors speculate, mitochondrial proteins in RBCs might play a role in SCA by promoting inflammation, adding another potential benefit of the PK activation in SCA to be further studied.

One might argue that the expanded lifespan of RBCs in this study might lead to a decrease in mitochondrial proteins merely by reducing the fraction of reticulocytes. The authors claim that the leucocyte depletion used minimized this problem by removing most reticulocytes. This was, however, not formally demonstrated in the study.

Interestingly, mivapivat levels measured in RBCs correlated with a range of measures of positive outcomes including glycolytic activation (including higher ATP and lower 2,3-DPG), and acyl-carnitines. The depleted carnitine pools found could be interpreted as a rationale for testing supplementation on top of mitapivat, but until then it remains speculative.

A significant and noted study limitation was lack of control for RBC age, which complicates the precise evaluation of mitapivat’s effects, one example being the PK enzyme itself, which decrease during RBC lifespan. Ongoing and future studies will explore the potential of PK activation across various hemolytic anemias, hopefully adding more details to the multi-omics effects associated with PK activation while adjusting for RBC lifespan variations during treatment.

Collectively, the insights provided by D’Alessandro et al. are a significantly step forward, enhancing our understanding of the complex remodeling provided by in vivo PK activation.
References:


Figure 1: Pyruvate kinase activation in sickle cell anemia. Pyruvate kinase catalyzes the glycolytic pathway conversion of phosphoenolpyruvate to pyruvate. This facilitates three key outcomes: (1) Increased ATP availability for red blood cells (RBCs), improving energy supply and cellular functions. (2) Decreased levels of 2,3-diphosphoglycerate (2,3-DPG), leading to increased oxygen affinity of hemoglobin, an well-known anti-sickling mechanism. (3) Augmented antioxidant capacity and reduced reactive oxygen species (ROS), contributing to a decrease in oxidative stress within the RBCs.
Pyruvate kinase activators
- Mitapivat
- Etopivat
- AG-946

Phosphoenolpyruvate → Pyruvate kinase → Pyruvate

1. More ATP for RBCs
2. Less 2,3-DPG which increases hemoglobin $O_2$-affinity
3. Antioxidant less ROS