Old drug, new parasite: targeting leukemia with antifungals

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Main text

In this issue of *Blood*, Himonas et al identify antifungal agents as potent inhibitors of mitochondrial metabolism in acute myeloid leukemia (AML) cells, offering new therapeutic options through drug repurposing.[1]

Leukemic transformation of hematopoietic cells is accompanied by extensive metabolic reprogramming, which commonly features changes in mitochondrial oxidative phosphorylation (OXPHOS) activity.[2] OXPHOS encompasses two closely-coupled biochemical processes: the electron transport chain, where electrons from NADH and $FADH_2$ are transferred to oxygen through a series of four multi-subunit protein complexes (I-IV) generating a proton gradient across the inner mitochondrial membrane, and chemiosmosis, in which ATP synthase (also known as complex V) uses the energy of the proton gradient to synthesize ATP from ADP and phosphate. In general, AML cells show higher OXPHOS activity compared to their non-malignant counterparts, and this is even more pronounced in leukemic stem cells and in residual AML cells persisting after therapy, creating a particular metabolic vulnerability.[3] Interestingly, complex V in AML mitochondria hydrolyzes ATP rather than generating it.[4] This indicates that maintaining a high mitochondrial membrane potential may be the primary function of OXPHOS in these cells, likely to suppress pro-apoptotic signaling.

The high dependency on OXPHOS in AML cells has inspired many therapeutic attempts to exploit this vulnerability. Unfortunately, despite promising preclinical efficacy, recent clinical

trials using electron transport chain complex I inhibitors, including BAY87-2243, ASP4132 and IACS-010759, as well as other approaches targeting mitochondrial metabolism (e.g. DHODH inhibition), have failed to yield therapeutic benefit for solid tumor and hematological cancer patients, largely due to dose-limiting toxicities.[5, 6] Identifying safe and effective drugs to target mitochondrial metabolism in AML therefore remains a critical challenge.

In the Himonas et al study, an elegant screening method was designed to find known drugs that could be repurposed to target mitochondrial metabolism in AML cells. AML cell lines with different genetic backgrounds were cultured in regular medium or in medium containing galactose instead of glucose, thus forcing the cells to a more oxidative phenotype. Cells were then exposed to a library of 1,274 FDA-approved compounds, evaluating differential effects on cell viability. As such, the authors identified several azole antifungals as potent OXPHOS inhibitors in AML, able to significantly induce apoptosis when used alone or in combination with standard chemotherapeutic agents such as cytarabine. This effect was confirmed in primary AML patient samples and proved independent of p53 mutational status. Subsequent mechanistic studies revealed that itraconazole and ketoconazole antifungals target OXPHOS in multiple ways. Firstly, the drugs were found to directly inhibit complex I, leading to a lower NAD^+/NADH ratio, decreased mitochondrial membrane potential and reduced respiration. However, restoring electron transport chain activity by overexpressing the yeast mitochondrial NADH dehydrogenase-1 was not sufficient to rescue OXPHOS or AML cell viability, although it did restore the NAD^+/NADH ratio. Secondly, itraconazole and other azoles were found to inhibit CYP51A1, a known target of these drugs, in AML cells. CYP51A1, also known as lanosterol 14α-demethylase, is part of the cytochrome P450 family and is involved in cholesterol biosynthesis. Interestingly, while Himonas and colleagues discovered that loss of CYP51A1 strongly reduced OXPHOS activity in AML cells, most likely through a yet unknown role in supporting complex I activity, the effects of the antifungals were found to be independent of cholesterol levels and were maintained in the absence of CYP51A1. Thus, while itraconazole and related azoles inhibit complex I activity in AML cells though at least two

independent mechanisms, this is still not sufficient to explain the full capacity of these drugs to block OXPHOS, suggesting additional mitochondrial targets. One possibility is a direct inhibition of Krebs cycle activity, which generates the NADH that fuels OXPHOS, as suggested by the stable isotope tracing experiments included in the study, although this aspect will require further in-depth exploration. Using xenograft mouse models, the authors finally show that itraconazole also reduces human AML growth *in vivo*. The effects were particularly evident in the leukemic stem cell population, in line with the higher OXPHOS dependency of these cells.

Taken together, Himonas et al have identified itraconazole and related azole antifungals as potent OXPHOS inhibitors with strong anti-leukemic activity. These drugs join the list of therapeutic options to reduce mitochondrial activity of AML cells, which recently has seen several other interesting additions including drugs that disrupt mitochondrial structure[7, 8] or heme biosynthesis[9]. One aspect that particularly favors azole drugs for further clinical exploration is the observation that itraconazole did not significantly increase the extracellular levels of lactate, suggesting that it may not exert drug toxicities related to lactic acidosis, a common limitation observed with OXPHOS inhibitors in the clinic[5]. Azole antifungal compounds also have excellent safety profiles and are routinely used in fungal prophylaxis in AML patients, which will likely facilitate further clinical testing. Yet this exciting study also raises several new questions. What are the additional mitochondrial targets that make itraconazole and related compounds such powerful OXPHOS inhibitors? Do these drugs indeed specifically target leukemic stem cells and, if so, what is the best treatment regimen to exploit this effect? Do certain mutations confer a stronger vulnerability to, or protection from, antifungals? And why does itraconazole not affect extracellular lactate levels? The future will hopefully provide an answer to these questions and will show whether azole antifungals can achieve what so many other OXPHOS inhibitors have failed to do: bring new hope to AML patients.

References

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