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Can we selectively target AML stem cells?

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ABSTRACT

De novo acute myeloid leukemia (AML) leukemia stem cell (LSC) populations are uniquely dependent on amino acid metabolism to drive the TCA cycle and oxidative phosphorylation. Oxidative phosphorylation can be selectively downregulated in de novo AML LSC populations by perturbing amino acid metabolism via BCL2 inhibition with venetoclax. While venetoclax-based therapies have shown high response rates, not all patients achieve remission. It may be possible to prospectively identify the patients who will most likely respond to venetoclax-based treatment by analyzing the metabolic properties of individual patients. Specifically, it appears that patients who are likely to be resistant to venetoclax-based therapy are able to employ alternate metabolic pathways to drive oxidative phosphorylation.

Introduction

Twenty years ago, the task of selectively targeting leukemic stem cells (LSC) was incredibly daunting, but in the subsequent 20 years, there have been numerous reports in the preclinical literature that suggest there are biological properties of LSC that can be identified and targeted. These approaches to targeting LSCs have included immunophenotypes and immune therapy; targeting specific mutations; epigenetic targets; targeting the microenvironment; and proteasome inhibition. However, the translational steps into the clinical setting have been fairly limited. This may be largely due to the intrinsic heterogeneity of this population and various other biological features. The current functional definition of an LSC could be a cell that has tumor-initiating potential in the context of some kind of animal model. These cells should be able to recapitulate disease after transplantation in an immune-deficient mouse.

Energy metabolism

Energy metabolism is a fundamental biological process with LSC-specific characteristics. This fundamental process can be regulated in specific cell types. In LSC, energy metabolism is regulated very differently than in a normal stem cell, creating therapeutic opportunities. Energy metabolism is driven through the tricarboxylic acid (TCA) cycle, and electron transport is the main oxidative form of energy metabolism. Leukemic stem cell populations depend on oxidative phosphorylation and are sensitive to inhibition of this process [1–4]. There are three basic ways to fuel oxidative phosphorylation: by virtue of burning or metabolizing amino acids, carbohydrates typically in the form of glucose, or lipids.

To determine which fuel drives oxidative phosphorylation in LSCs, de novo primary human AML specimens were sorted into LSCs and mature AML blasts by flow cytometry (Fig. 1). LSCs have low levels of reactive oxygen species (ROS), while mature AML blasts that do not have functionally defined stem cells have high levels of ROS. These two cell populations were then put through the mass spectroscopy for global metabolomic analysis. The metabolites that were uniquely prevalent in the ROS-low or LSC compartment are amino acids [5]. This invites the hypothesis that LSCs are preferentially dependent on amino acids. To test this, the ROS-low and ROS-

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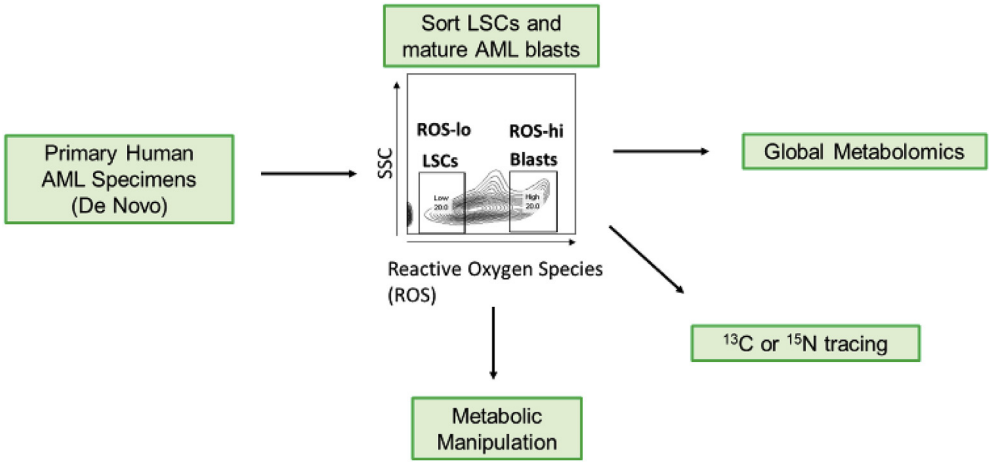

Fig. 1. Experimental design to determine metabolic drivers of oxidative phosphorylation in AML cells.

high cell populations were independently cultured in the absence of amino acids. While all cells need amino acids, the ROS-high population had near 100% survival for the first 24–48 h, while the ROS-low population was much more sensitive to the amino acid deprivation, with only a quarter of LSCs remaining viable at 24 h.

This was further demonstrated when LSCs were subject to amino acid depletion for 24 h and were then transplanted to immune-deficient mice; when compared with LSCs that were not subject to amino acid depletion, grafting potential was significantly impaired [5]. The same did not hold true for normal hematopoietic stem cells, suggesting a therapeutic index with respect to utilization of amino acids.

When the same experiment was repeated with carbohydrate depletion and lipid depletion instead of amino acid depletion, there was no effect on oxygen consumption, a marker for oxidative phosphorylation, in the glucose- and lipid-deprived ROS-high and ROS-low specimens (Fig. 2). This suggests that the LSC population relies on amino acids to drive oxidative phosphorylation, and that amino acids are the only fuel source that has this particular effect, making amino acid metabolism an attractive target in AML patients.

Targeting amino acid metabolism in AML patients

Amino acids are essential for oxidative phosphorylation in LSC, and BCL2 inhibition decreases oxidative phosphorylation in LSCs [1]. BLC2 is inhibited by venetoclax, but it was unclear whether venetoclax decreased oxidative phosphorylation by virtue of interfering with amino acid metabolism in AML patients. To determine whether the mechanism of venetoclax is to interfere with amino acid metabolism, samples were taken from elderly untreated AML patients who had enrolled in a dose escalation and expansion phase 1b study of venetoclax and the hypomethylating agent azacitidine [6]. The clearance of blasts is remarkably fast for patients on this regimen, so patients who had a large amount of peripheral disease prior to treatment were sampled at baseline and just 24 h after the initiation of therapy [5,7]. ROS-low cells were isolated both at baseline and 24-h after initiation of therapy, and oxidative

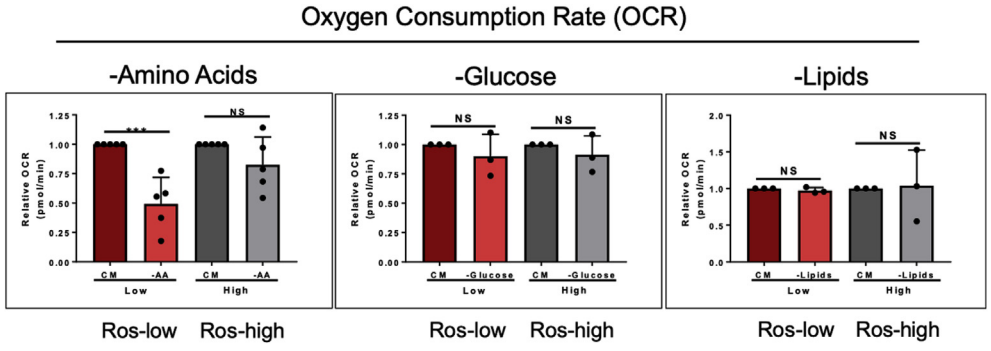

Fig. 2. ROS-low populations display unique dependence on amino acids for energy metabolism.
ROS-low and ROS-high cells from primary AML specimens were isolated and cultured for 24 h in media deprived of the indicated metabolites (AA, amino acids; glucose; lipids). Oxygen consumption rate (OCR) was then determined by respirometry. Data reproduced from Jones et al., 2018, Cancer Cell 34, 724–740 [5].
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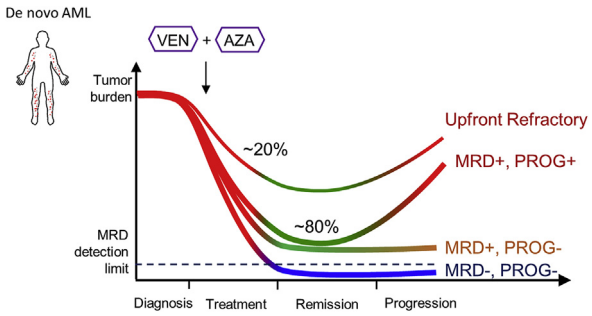

Fig. 3. Schematic representation of venetoclax/azacitidine response in de novo AML patients.
MRD: minimal residual disease; PROG: disease progression.

phosphorylation was profoundly reduced after treatment with venetoclax and azacitidine. Furthermore, in specimens from 3 patients, the combination of venetoclax and azacitidine significantly reduced amino acids in LSCs. As a control, these results were compared to patients who received conventional 7 + 3 therapy. There was no reduction in amino acids 24 h post-treatment, and there was actually an increase in amino acids in one patient.

To further understand the effect of venetoclax, primary AML cells were preincubated for 2 h with a 10-fold excess of amino acids to artificially raise the intracellular pools of amino acids [5]. When cells were pretreated with amino acids, there was no reduction in viability or oxidative phosphorylation after challenge with venetoclax and azacitidine. This implies by virtue of manipulating just amino acids, one can rescue the cytotoxic effects and can also rescue the oxidative phosphorylation inhibition phenotype. The functional consequence of this is a clear reduction in cells phenotypically defined as LSC after 24 h of treatment with venetoclax and azacitidine [7]. In xenograft studies, venetoclax and azacitidine obliterate the functional ability to graft LSCs in immune-deficient mice. Most importantly, LSC-targeting therapy with venetoclax and azacitidine correlates with better survival for AML patients, though it is impossible to determine whether these outcomes are a direct result of targeting the LSC compartment.

Predicting response

Venetoclax is most successful in the de novo AML population, but even in this upfront setting, it does not work for every patient. Approximately 20% of de novo AML patients are refractory to venetoclax upfront, while the remaining 80% will attain complete remission or at least a significant response to treatment (Fig. 3). Those patients can be further fractionated into the patients who have a good response but remain minimal residual disease (MRD)-positive using highly sensitive patient-specific mutation analysis by polymerase chain reaction (PCR). Some of those patients will progress, while some will not. There will also be a small number of patients who will attain MRD-negative status and will have a very stable remission. Therefore, there may be biological properties that can be used to restratify the patient population to best determine who might benefit from treatment with venetoclax.

To analyze this, a number of de novo AML specimens have been challenged with venetoclax/azacitidine regimens and bifurcate between sensitive and resistant. The ROS-low cell compartment was isolated from both resistant and sensitive samples and both RNA-sequence and metabolic analyses were performed. Notably, specimens that respond to venetoclax/azacitidine treatment appear to be solely reliant on amino acid metabolism to drive oxidative phosphorylation. In contrast, resistant specimens are able to also employ fatty acid oxidation as a means to fuel energy metabolism. Consequently, we tested dual inhibition of both amino acid metabolism

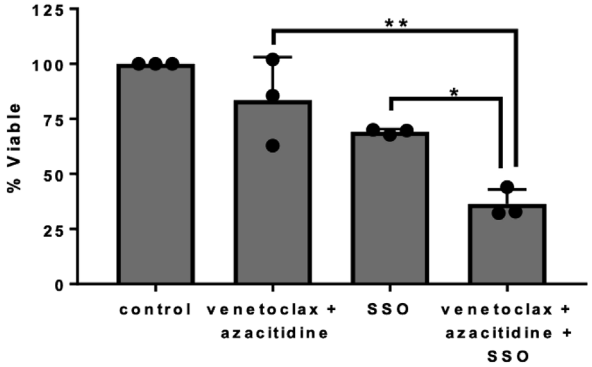

Fig. 4. Metabolic properties of venetoclax/azacitidine resistant primary AML cells.
ROS-low cells from primary ven/aza resistant AML specimens were culture for 24 h in venetoclax + aza, in CD36 inhibitor (SSO), or both conditions, and viability was determined. Data reproduced from Jones et al., 2018, Cancer Cell 34, 724–740 [5].
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and fatty acid metabolism using venetoclax/azacitidine with the fatty acid transport inhibitor sulfo-N-succinimidyl oleate (SSO). Addition of SSO acts to resensitize ROS-low AML cells to treatment with venetoclax/azacitidine (Fig. 4).

Conclusion

De novo AML LSC populations are uniquely dependent on amino acid metabolism to drive oxidative phosphorylation. A critical activity of venetoclax is to inhibit that amino acid metabolism, thereby downregulating oxidative phosphorylation and leading to selective targeting of LSCs. The AML patients who will likely be most responsive to venetoclax-based regimens can be prospectively identified by metabolic profiling. In some cases, it appears that both amino acid and fatty acid metabolism can be employed by AML LSC to drive energy metabolism. In those instances, therapies that inhibit both pathways may be required in order to effectively eradicate LSC populations.

Declaration of competing interest

Dr. Jordan has no relevant financial relationships with any commercial interest.

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