Acute myeloid leukemia (AML) is a blood cancer derived from the hematopoietic stem/progenitor cells, whereby leukemia-initiating cells (LIC) produce large numbers of leukemic blasts to destroy normal bone marrow functions, leading to disease related mortality. Many patients with AML can enter into clinical complete remission after standard chemotherapy treatments, but no more than 30% of patients survive longer than 5 years (1). Even though significant therapeutic improvements have been achieved in younger patients with AML with specific molecular features using cytotoxic and targeted therapies, patients over 65 years still manifest poor survival rates and high incidence of relapse (1). AML is a genetically heterogenous disease with many oncogenic drivers. An individual patient may further harbor a mosaic of genetically distinct subclonal populations of leukemia cells, which creates a challenging scenario for “precision” therapy directed toward specific mutations. Among the complexities associated with AML, AML LICs uniquely exhibit metabolic dependency on mitochondrial oxidative phosphorylation and stringent control of reactive oxygen species (ROS) production while normal hematopoietic stem cells (HSC) rely on glycolysis for their self-renewal and quiescent states (ref. 2; Fig. 1A). This metabolic difference between LICs and HSCs inspired discovery or application of therapeutic agents directed toward mitochondrial processes, such as the BCL2 inhibitor venetoclax and the oxidative phosphorylation complex I inhibitor IACS-010759, for AML therapy (3). Mitochondrial oxidative phosphorylation is controlled by hundreds of enzymes and regulatory proteins, which may be functionally hijacked by AML cells as a form of non-oncogene addiction (2, 3). Hence, there are many opportunities to target such mechanisms to selectively disrupt AML dependencies on particular aspects of this metabolic process.

In this issue of Blood Cancer Discovery, Yan and colleagues discovered that mitochondrial deacylase, SIRT5, is required in AML cells to support mitochondrial oxidative phosphorylation, maintain redox homeostasis, and drive glutaminolysis. The new SIRT5 inhibitor, NRD167, can efficiently target SIRT5 in AMLs at micromolar range and may constitute a novel therapeutic approach to improve clinical outcomes of patients with AML.

See related article by Yan et al., p. 266 (4).
It is critical for AML LICs to prevent excessive production of ROS from the oxidative phosphorylation, which may otherwise impair self-renewal and trigger differentiation and cell death (2). SIRT5 was reported to protect cells from ROS by desuccinylating superoxide dismutase 1 (SOD1) and enzymes generating NADPH as reducing power. In this study, Yan and colleagues observed that loss of SIRT5 induced mitochondrial ROS and cell death in SIRT5-dependent AML cells. Addition of vitamin E and ectopic expression of SOD2, instead of SOD1, could partially rescue the SIRT5 knockdown effects. It is still a mystery how SIRT5 depletion induces ROS production because no direct connection was discovered between SIRT5 and SOD2. However, as it is known that SIRT3 can deacetylate SOD2 to increase its activity, it is possible that SIRT5 and SIRT3 may coordinate together to protect AML cells from ROS damage. This is consistent with recent findings showing that SIRT5 and SIRT3 can compensate for each other’s loss of function in specific biological conditions (6). SOD2 activity induced by SIRT3 deacetylation is required for aged HSCs to maintain their self-renewal ability. AMLs can thus hijack the activity of SIRT3 to support oxidative phosphorylation and SOD2 to facilitate chemoresistance (7). Interestingly, the expression of SIRT3 gradually decreases with age, but no such change is observed in SIRT5 expression, which may point to the importance of SIRT5 in scavenging ROS in aged AML cells (Fig. 1B).

On the other hand, the authors found that SIRT5-dependent AML cells more efficiently converted glutamine into glutamate as a source for anaplerotic metabolism. This enhanced glutaminolysis may be the result of SIRT5 desuccinylating its substrate glutaminase (GLS). Untargeted metabolomic profiling accordingly showed that SIRT5 loss of function resulted in reduced abundance of TCA cycle and amino acid metabolites. These metabolic changes may be explained by the diverse deacylase activities of SIRT5. Yan and colleagues observed increased expression of protein succinylation, malonylation, and even acetylation in SIRT5-depleted AML cells, which indicates that these posttranslational modifications may have an important functional impact on the respective metabolic enzymes. CMK cells (SIRT5-dependent) showed more profound increase of acetylation than KG1a cells (SIRT5-independent), so it might be possible to characterize SIRT5’s deacylated targets as a putative biomarker to determine the dependency of SIRT5 in AML cases. Such effects on metabolism-associated posttranslational modifications have also been documented as being regulated by SIRT3. Proteomics studies showed that lysine acetylation and succinylation are most frequently observed in mitochondrial proteins, and some proteins can be regulated by both
modifications (8, 9). It is unknown whether different acylation moieties (acetyl, malonyl, succinyl, and glutaryl) affect one another’s abundance in the mitochondria and hence induce different or nuanced impact on mitochondrial activity. Yan and colleagues observed that loss of SIRT5 in AML cells also affects mitochondrial protein acetylation, which may indicate further potential functional overlap with SIRT3 in maintaining AML mitochondrial functions. It would be of interest to further compare the role of both sirtuins to further understand their relative contributions to AML stem cell functions.

Several small-molecule inhibitors targeting sirtuin family proteins have been developed in recent years. However, it is relatively difficult to design tool compounds that achieve both high efficacy and sirtuin selectivity that have favorable pharmacologic properties. One route to achieve this involves the design of mechanism-based inhibitors using a thioacetyllysine peptide as substrate to form a stalled S-alkylamidate intermediate that blocks substrates from engaging the sirtuins catalytic pocket. This strategy was used to successfully generate inhibitors to selectively target SIRT2 and SIRT3 in vitro and in vivo (10). Similarly, Yan and colleagues started from a mechanism-based inhibitor/prodrug designed to target SIRT5, yielding a compound that could inhibit SIRT5 in vitro with an IC$_{50}$ of 110 nmol/L. After the authors modified the SIRT5 inhibitor to increase its cell permeability, the final compound, NRD167, could kill SIRT5-dependent AML cells at micromolar concentrations but exhibited less effect against SIRT3-independent AMLs or normal cord blood cells. Furthermore, NRD167 treatment phenocopied the inhibition of oxidative phosphorylation by SIRT5 shRNAs (Fig. 1C). NRD167 treatment also tends to prolong the survival in mice engrafted with primary human AML cells. Another SIRT5 inhibitor, DK1-04 (11), was reported to have an IC$_{50}$ in vitro of 340 nmol/L. NRD167 and DK1-04 shared a similar mechanism of action in forming stalled covalent intermediates with SIRT5. DK1-04e was also effective against breast cancer xenografts in vivo, so it would be interesting to compare the two SIRT5 inhibitors in future AML studies. SIRT5 requires mitochondrial NAD to exert its enzyme activity. A recent work from Jones and colleagues discovered that NAD metabolism is a crucial metabolic checkpoint helping refractory and relapsed AML cells escape from venetoclax treatment (3). It would be interesting to know whether SIRT5 may play a role in this NAD-dependent resistance to venetoclax. Along these lines, as SIRT5 and BCL2 may intersect in regulating leukemia stem cell mitochondrial metabolism functions, it would be interesting to test whether drugs such as NRD167 and venetoclax could be used in combination for greater eradication of AML stem cells.

**Authors’ Disclosures**

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Meng Li and Ari M. Melnick


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