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Blood 142 (2023) 2739–2740

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Chronic Mitochondrial Dysfunction Renders Acute Myeloid Leukemia Resistant to Multipronged Inhibition of Mitochondrial Metabolism

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Acute Myeloid Leukemia (AML) is a genetically diverse and aggressive myeloid neoplasm that awaits novel therapeutics to improve patient survival. Targeting AML metabolic aberrations as a genotype-agnostic strategy has shown promise, evidenced by the clinical success of venetoclax and the rapid pre-clinical development of other mitochondrial (mt) metabolism targeted therapies. However, the mechanism of how AML develops resistance to drugs targeting key mt metabolic pathways is not fully understood. We previously identified sirtuin 5 (SIRT5), a deacylase that regulates several pathways of mt metabolism in AML including oxidative phosphorylation and glutamine metabolism. In AML, it functions as a master metabolic regulator that is necessary for leukemic stem cell survival. Genetic or pharmacological inhibition of SIRT5 leads to rapid AML cell apoptosis in vitro and disease regression in multiple AML mouse models. However, a subset of AML cell lines and primary samples can survive SIRT5 inhibition, indicating an unusual intrinsic resistance to mt dysfunction.

To provide a mechanistic explanation, we first compared the molecular features between SIRT5-dependent and independent cases. Among the 25 primary AML samples we tested, NPM1c mutation, but not FLT3-ITD, TP53, WT1, NRAS, or IDH1 mutations, is significantly enriched in SIRT5-independent samples. Accordingly, among the panel of 22 AML cell lines we tested, the only NPM1 mutant cell line (OCI-AML3) sits at the most SIRT5-independent extreme of the spectrum. NPM1 mutant AML cells have increased cytoplasmic mitochondrial DNA (mtDNA) and fragmented mt morphology, indicating chronic mt damage (Wu H et al. Cancer Discovery. 2021). Because SIRT5 exerts its metabolic maintenance functions within the mt, we hypothesize that AML cells that can adapt to chronic, sublethal mt dysfunction will be capable of proliferation without SIRT5. To test this, we took three orthogonal approaches: (1) introducing NPM1c mutation, (2) using low concentrations of ethidium bromide (EtBr) to selectively block mtDNA replication, or (3) genetically inducing chronic mt dysfunction by silencing the essential mt transcription factor TFAM in 3 AML cell lines that are heavily dependent on SIRT5 - OCI-AML2, CMK, and SKM-1.

SIRT5-dependent cells expressing ectopic NPM1c exhibit mt dysfunction as evidenced by decreased oxygen consumption rate (OCR) measured using the Seahorse metabolic flux assay. Also, the lethal effect of SIRT5 depletion is rescued in these cells. Pharmacologically blocking NPM1c nuclear export with selinexor in the typically SIRT5-independent OCI-AML3 cells sensitizes them to the lethal effect of SIRT5 depletion. After culturing in 100 ng/mL EtBr for 3, 5, or 11 days, cells had significantly decreased mt abundance as measured by mtDNA content relative to nuclear DNA and had significantly decreased OCR and extracellular acidification rate (ECAR). SIRT5-dependent cells cultured in EtBr survived shRNA-induced SIRT5 depletion, in contrast to the rapid lethality of cells not cultured in EtBr. Additionally, cells initially cultured in EtBr and allowed to recover in the absence of EtBr assume their original phenotype of SIRT5 dependence. However, inhibiting specific subunits of the mt using potent complex I and III inhibitors (rotenone and antimycin A) shows no effect on SIRT5 dependence. Together these data suggest that mt damage, rather than loss of function in one pathway, is needed to convert SIRT5-dependent cells to SIRT5 independence. TFAM, a nuclearly coded transcription factor for mtDNA, is necessary for the replication and transcription of mt genes. We used cas13d-mediated knockdown of TFAM to deplete mtDNA and induce mt dysfunction. Experiments that investigate how this method of inducing mt dysfunction affects OCR, ECAR, and SIRT5-dependent cell line proliferation are currently underway and will be presented at the ASH conference.

In summary, we present a mechanism of how NPM1 mutation-associated or experimentally induced chronic sublethal mt dysfunction renders AML resistant to therapies targeting vital mt metabolic pathways, such as SIRT5 inhibitors, demonstrating AML's metabolic flexibility. Experiments are scheduled to validate these findings in primary samples and additional metabolomic and transcriptional studies will be done to deduce the mechanism for SIRT5 dependence in AML.

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https://doi.org/10.1182/blood-2023-179883

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