Understanding How Cellular Metabolism Affects Clofarabine Treatment in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) has a poor 5-year survival rate of only 40%. The poor survival rate is in part due to leukemic stem cells (LSCs) surviving traditional chemotherapeutic treatment; a high proportion of patients experience relapse because LSCs appear refractory to therapy. Previous research has indicated heterogeneous, but distinct metabolic profiles in hematopoietic stem cells (HSCs), LSCs, and AML blasts. The impact of these different metabolic pathways on therapeutic response to agents used to treat AML is not well understood. We hypothesized that the AML metabolic state could impact response to chemotherapy. We have shown that various AML cell lines of the same French American British (FAB) subtype have different metabolic states by using the Seahorse bioanalyzer. The basal metabolism of these cells can be shifted to reliance on oxidative phosphorylation (OXPHOS) by culturing cells in medium with galactose rather than glucose; cells can also be shifted to rely on glycolysis by treating cells with the complex I inhibitor, IACS-010759. Utilizing these approaches to shift metabolic state, the chemotherapeutic response based on cellular metabolism has been assessed. The current standard-of-care in AML includes a “7+3” regimen treatment with cytarabine (AraC) and an anthracycline as the backbone agents. We screened 4 M5 FAB subtype AML cell lines with various chemotherapeutic agents under “normal” glucose media culture conditions, or galactose media culture conditions. As previously reported, resistance to AraC was increased under OXPHOS conditions.1 In contrast, the anthracycline Idarubicin showed no difference in response under conditions favoring neither glycolysis nor OXPHOS. In 2004, the purine nucleoside analog antimetabolite—clofarabine—was approved for use in leukemia patients with relapsed or refractory disease. While this drug is not currently the standard of care for AML treatment, clofarabine is regularly used in AML treatment regimens. Clofarabine was tested and response was different under either glycolytic or OXPHOS conditions. In the more glycolytic U937 AML cell line, we have shown that shifting the cells to rely on OXPHOS leads to increased clofarabine resistance. However, the key determinants of this change in clofarabine sensitivity (e.g., dCK, RRM1) were unchanged by the altered metabolic state of the cells. This indicates a previously unidentified mechanism of clofarabine toxicity is operative under different metabolic conditions. An unbiased approach to determine which metabolic pathways affect drug sensitivity can be conducted by a metabolic-focused CRISPR screen. The metabolic CRISPR screen includes a library containing 29,790 gRNAs which target 2,981 metabolic genes. By conducting a “drop-out” CRISPR screen, genes important to clofarabine response can be identified. Based on the results from the CRISPR screen our studies should reveal previously unidentified clofarabine mechanism of action that can be exploited to improve AML treatment with clofarabine.

References

1 Farge T, et al. Chemotherapy-Resistant Human Acute Myeloid Leukemia Cells Are Not Enriched for Leukemic Stem Cells but Require Oxidative Metabolism. Cancer Discov. 2017

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