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ORAL ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Metabolism-Related Features Identify the Combination Metformin Plus NAMPT Inhibitors As a Selective Treatment Strategy in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a heterogeneous disease, which remains difficult to treat due to its heterogeneous nature. Recently, metabolic adaptation has been recognized as a trademark of leukemogenesis. Notably, altered function of mitochondria (mt) in leukemic stem cells (LSCs) has been linked to chemoresistance in some patients, causing relapse of disease. Thus, targeting the rewired energy metabolism of leukemic cells has gained a lot of attention in recent years.

Here, using a large cohort of adult de novo AML samples (n=502 patients, all intensively treated with 3+7 scheme), we identified a subset of patients with increased mitochondrial DNA content (mtDNAcn, n=163) compared to age- and sex-matched healthy controls (n=308). mtDNAcn was significantly higher in patients harboring FLT3-ITD (31%) and IDH1 mutations (50%). Patients with high mtDNAcn were associated with worse clinical outcomes regardless of European Leukemia Net (ELN) risk stratification, sex, and age. In contrast, high mtDNAcn was not a predictive survival marker in a cohort of adult AML patients treated with venetoclax (VEN) + hypomethylating agents (HMA), indicating that these metabolic features are specifically linked to the resistance to intensive chemotherapy. Using label-free quantitative proteome analysis (11272 proteins) combined with metabolomic screening (173 metabolites) on sorted CD34 $^{+}$/CD117 $^{+}$ AML blasts (n=26), we found that AMLs with high mtDNAcn were enriched for signatures like "GMP-like" and "mt complex I activity", being metabolically enriched for long-chain fatty acids, suggesting that these cells rely more on oxidative phosphorylation (OXPHOS) metabolism. Functionally, AML blasts with high mtDNAcn displayed increased levels of BCL2, mt mass, and mt membrane potential, which was associated with increased oxygen consumption rate (OCR) measured by extracellular flux analysis.

Considering the impact of mtDNAcn on AML survival in patients treated with intensive chemotherapy, which was not observed in the context of VEN+HMA, and considering the increased reliance of LSC on mtOXPHOS, we studied the impact of mtDNAcn on chemotherapy response. Ex vivo analysis of primary AML samples with high mtDNAcn indicated increased

resistance to AraC-induced apoptosis, which was reverted upon knock-down of the DNA polymerase gamma gene (POLG) encoding the catalytic subunit of the mtDNA polymerase. Due to the link between mtDNAcn and mt complex I activity, we investigated if the treatment with metformin, known to inhibit complex I, would sensitize AML patients with high mtDNAcn to the standard of care AML drugs, such as Cytarabine (AraC), FLT3-inhibitors and VEN. Treatment with low dose of metformin (1 mM) effectively reduced the mtDNAcn in AML cells (n=40), resulting in decreased OCR, while glycolysis was increased, suggesting metabolic rewiring. Furthermore, the combination of metformin with AraC, VEN and FLT3-inhibitors synergistically increased drug-induced apoptosis in AML cells with high mtDNAcn and helped to overcome VEN resistance in AML cells. In vivo, combination of metformin with VEN prolonged the survival of mice transplanted with K562 cells (VEN resistant), resulting in decreased leukocyte counts and blood chimerism.

Since treatment with metformin resulted in metabolic rewiring, with increased glycolysis and NAD^+ consumption, we wondered if the combination of metformin with glycolytic (2-DG/DAP) and NAMPT (KPT-9274) inhibitors would impede the metabolic rewiring, enhancing cytotoxicity. Among the different treatment combinations, we find that the combination of metformin and KPT-9274 gave the best therapeutic window by significantly increasing drug-induced apoptosis, with more pronounced effects in patients with high mtDNAcn, while sparing healthy $CD34^+$ cells. Concomitantly, OXPHOS and glycolysis were diminished upon KPT-9274+metformin, suggesting that the combination was able to bypass the metabolic rewiring of the AML cells.

In conclusion, we uncover a subgroup of patients with high mtDNAcn linked to increased mtOXPHOS that is resistant to chemotherapy-induced apoptosis and is characterized by poor clinical outcomes, which can be overcome by the inhibition of the mt complex I. Moreover, the quantification of mtDNAcn can be easily incorporated into clinical practice as a simple and cost-effective metabolic readout of AML patients to identify metabolic vulnerabilities.

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