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POSTER ABSTRACTS

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

Metformin-Induced Ferroptosis Is a Therapeutic Vulnerability in *IDH2*-Mutant AML Linked to Metabolic Rewiring Towards Fatty Acid Oxidation

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Metabolic rewiring is an essential feature of leukemic cells to sustain tumorigenesis and is largely influenced by mutational status. We previously demonstrated this phenomenon in *FLT3*-ITD acute myeloid leukemia (AML) upon combined inhibition of complex II activity and lactate import (Erdem et al., 2022). Mutations in isocitrate dehydrogenase (*IDH1/2* ^{mut}) occur in about 20% of AML cases, and despite the emergence of targeted therapies, patients often relapse. The molecular and epigenetic outcomes of 2-R-hydroxyglutarate accumulation and competitive inhibition of histone demethylases have been extensively studied in *IDH1/2* ^{mut} AMLs, but the metabolic consequences are still largely unexplored. Here, we applied multi-omics studies on cell line models and primary AML samples combined with functional studies to unravel the metabolic rewiring of *IDH1/2* ^{mut} AMLs.

Transcriptional characterization of isogenic TF1 ^{wt} and *IDH2* ^{R140Q} cell lines (10978 genes) revealed increased expression of CD36, a major fatty acid (FA) transporter, and gene set enrichment analysis (GSEA) associated *IDH2* ^{R140Q} cells with terms related to FA processes and activity of mitochondrial complex I. Quantitative metabolome analysis of TF1 ^{wt} and *IDH2* ^{R140Q} (180 metabolites) revealed lower levels of acylcarnitines of variable carbon lengths, suggesting increased FA oxidation, and upregulation of glycerophospholipid and glycerolipid metabolism in *IDH2* ^{R140Q} cells. Next, we validated our findings by performing a metabolomic profiling (172 metabolites) on primary AML samples (n=26, including 4 *IDH1* ^{mut} and 2 *IDH2* ^{mut}), for which label-free quantitative proteome data (11272 proteins) was also generated. Proteome analysis confirmed increased CD36 expression and enrichment for FA and mitochondrial processes in *IDH1/2* ^{mut} patients. In line, metabolome data also revealed increased oxidation of branched-chain FAs in this sample group. Altogether, these findings suggested a profound disturbance in lipid metabolism and supported the notion that *IDH1/2* mutant cells rely on mitochondrial respiration, whereby FAs seem to be the preferred carbon source.

Next, we performed an *in vitro* drug screen targeting the main metabolic pathways, which revealed increased sensitivity of *IDH2* ^{R140Q} cells to the FDA-approved complex I inhibitor metformin. Extracellular flux analysis indicated no significant difference in the oxygen consumption rate (OCR) between TF1 ^{wt} and *IDH2* ^{R140Q} upon metformin treatment, while the viability of mutant cells was significantly diminished. Contrarily, the basal extracellular acidification rate (ECAR) was increased in TF1 ^{wt} cells upon complex I inhibition, suggesting that these cells rewire their metabolism towards glycolysis more efficiently than mutant cells. Furthermore, we knocked down CD36 expression to investigate whether disrupting lipid metabolism in *IDH2* ^{R140Q} cells would constitute a metabolic vulnerability. Notably, CD36 knockdown resulted in increased resistance to metformin in *IDH2* ^{R140Q} cells. These data suggested that enhanced lipid uptake mediated by CD36 in *IDH2* ^{R140Q} cells not only boosts OCR but may also disrupt lipid homeostasis, causing cells to become more susceptible to lipid peroxidation. Since the role of

metformin-induced ferroptosis in AML is still unclear, we performed an RNA-seq analysis on TF1 ^{wt} and *IDH2* ^{R140Q} cells treated with metformin (5 mM) for 72 hours (10326 genes). Single-sample GSEA associated the transcriptome of metformin-treated cells with ferroptosis signatures in both cell lines but to a significantly higher extent in *IDH2* ^{R140Q}. We functionally validated this finding by using the BODIPY C11 probe, a lipid peroxidation sensor. After 24h of metformin treatment, both TF1 ^{wt} and *IDH2* ^{R140Q} displayed increased lipid ROS. Lipid peroxidation levels were further enhanced by combining metformin with palmitate, a saturated FA, supporting the notion that elevated lipid availability and uptake may increase cell death via ferroptosis.
Altogether, we identified a new metabolic vulnerability in *IDH1/2* ^{mut} AMLs associated with a profound disturbance in lipid metabolism. Moreover, we show that treatment with metformin not only inhibits complex I but also induces cell death via ferroptosis in *IDH2* ^{mut} cells providing an alternative treatment option in combination with available *IDH2* ^{mut} targeted therapies for this subgroup of patients.

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