Metformin-induced ferroptosis is a therapeutic vulnerability in AML linked to metabolic rewiring towards fatty acid oxidation

Introduction: Metabolic rewiring is a hallmark of cancer, essential for sustaining leukemogenesis. In acute myeloid leukemia (AML), high dependency on oxidative phosphorylation (OXPHOS) is often linked to poor outcomes and inhibiting mitochondrial respiration has shown significant efficacy in AML therapy. This study aimed to assess the efficacy of metformin, an FDA-approved drug known for inhibiting OXPHOS, in a genetically diverse panel of AML patients. The goal was to identify metabolic profiles predicting susceptibility to metformin and elucidate its mechanism of action in AML.
Methods: Using label-free quantitative proteome analysis from 26 patients to generate single-sample Gene Set Enrichment Analysis focused on metabolic terms, we correlated enrichment scores with metformin sensitivity data. Functional studies were performed in cell lines harboring *IDH2* or *FLT3*-ITD mutations and a genetically diverse set of AML patient samples.
Results: Increased response to metformin was associated with disrupted lipid metabolism and ferroptosis, with *FLT3*-ITD and *IDH1/2* mutant AMLs showing the highest enrichment scores for these signatures. Extracellular flux analysis showed that metformin treatment completely abolished mitochondrial metabolism and promoted glycolysis in wild-type samples, whereas *FLT3* and *IDH1/2* mutants appeared to be less efficient in rewiring their metabolism upon OXPHOS inhibition. To better understand the association between metformin and ferroptosis, we focused on lipid peroxidation, reactive oxygen species (ROS) formation, iron metabolism and lipid composition. Metformin treatment increased both lipid peroxidation and ROS levels, especially in *FLT3* and *IDH1/2* mutant samples. As iron is an essential co-factor in ferroptosis, combining metformin with an iron quelator led to a strong antagonistic effect. Metabolome analysis revealed an imbalance in the lipid pool, with higher poly-unsaturated fatty acids in this subset of mutant patients, increasing their susceptibility to lipid peroxidation at baseline. Since these samples also showed increased expression of *CD36*, an important fatty acid transporter, we disrupted lipid dynamics to evaluate the consequences for metformin treatment. Co-treatment with palmitate, a saturated fatty acid, increased metformin sensitivity, and *CD36* knockdown rendered *IDH2*^{R140Q} cells more resistant. Finally, combination with a *DGAT1* inhibitor, an enzyme involved in lipid droplet formation, showed additive-to-synergistic effects in cell lines and primary samples, emphasizing the importance of lipid homeostasis for metformin sensitivity.
Conclusions: OXPHOS inhibition and ferroptosis induction by metformin is effective across genetically diverse AMLs, particularly those with disturbed lipid metabolism. Defective metabolic rewiring towards glycolysis, along with increased lipid peroxidation, ROS production, and lipid droplet formation, are mechanisms associated with enhanced metformin sensitivity in AML. These findings highlight the potential of targeting metabolic vulnerabilities in AML for more effective and personalized therapies.