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Poster Presentations: Single Cell Analysis of the TME

Abstract: P-004

Non-toxic concentrations of 3-bromopyruvate sensitize acute leukaemia cells to chemotherapy by inducing oxidative stress

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Introduction

Acute Myeloid Leukaemia (AML) displays an aggressive cancer phenotype with high relapse rates and no effective therapy. Metabolic rewiring is involved in cancer chemoresistance, but this issue has been poorly explored in AML. Our aim is to sensitize AML cells to daunorubicin (DNR) and cytarabine (Ara-C) using non-toxic concentrations of 3-bromopyruvate (3-BP), an anti-glycolytic agent.

Material and Methods

KG-1 and MOLM13 cells were exposed to different concentrations of 3-BP for 16h, and cell viability (Trypan Blue , MTS assay), cell apoptosis and necrosis (AnnexinV/PI) were assessed. The expression of apoptotic markers was evaluated by Western Blot and extracellular glucose and lactate levels were assessed using colorimetric kits. We also characterized the 3-BP effect on mitochondrial activity and formation of reactive oxygen species (ROS) by flow cytometry and glutathione (GSH) levels by colorimetric kit. AML cells were pre-treated with 3-BP (5μM) for 16h followed by different concentrations of DNR and Ara-C for 48h. Cell viability and IC_{50} values were determined by Trypan Blue.

Results and Discussions

3-BP (5μM) pre-treatment sensitized AML cells to the chemotherapeutic agents, decreasing their IC_{50} values. Aiming to unveil the sensitizing mechanism, we further characterized the effect of 3-BP low concentration in AML cells. In MOLM13 cells, the treatment increased cell viability by MTS, but not by the Trypan Blue assay, neither induced cell apoptosis/necrosis (AnnexinV/PI). For KG-1 cells, 3-BP did not affect cell viability, but these results still need to be confirmed by AnnexinV/PI. As 3-BP is an anti-glycolytic and alkylating agent, we assessed the 3-

BP effect in glycolytic profile, mitochondrial activity and GSH levels. 3-BP did not alter the glycolytic profile of KG-1 cells, but in MOLM13 cells the glucose consumption decreased. This suggests that 3-BP did not inhibit the fermentative pathway of these cells. Moreover, 3-BP decreased mitochondrial activity and increased ROS levels for KG-1 and MOLM13 cells, respectively. For GSH, an increase in the oxidized GSH was detected, meaning that 3-BP decreased the free-reduced GSH.

Conclusion

These data suggest that non-toxic concentrations of 3-BP reduce the antioxidant defences of AML cells, boosting the effect of the chemotherapeutic drugs.

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