P75 3-Bromopyruvate pre-treatment sensitizes MOLM13 and KG-1 cell lines to chemotherapy

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Introduction: Acute Myeloid Leukemia(AML) is the most common adult acute leukemia and it is characterized by clonal expansion of immature myeloblasts1. The most common treatment is the combination of daunorubicin(DNR) with cytarabine(AraC). Unfortunately, the relapse rate is high and the outcome is poor2. Most cancer cells present altered energetic metabolism relying on aerobic glycolysis, even under aerobic conditions ("Warburg effect"). 3-Bromopyruvate(3BP) is an alkylating agent that targets cancer cell metabolism, and has been demonstrated to be a powerful antitumor agent either in in vitro and in vivo models, but little is known about its effect in leukemia models3. Our aim was to evaluate a new therapeutic approach, in which cells are pre-treated with a 3BP non-toxic concentration followed by DNR or AraC treatment and understand the mechanism of action of 3BP pre-treatment.

Experimental: MOLM13 and KG-1 cell lines were treated with 5μM 3BP for 16h or 0.5mM 2-deoxyglucose(2DG) for 24h. After that, 10^{5}cells were cultured in 24-well plates and treated with a range of DNR or AraC concentrations for 48h. Cell viability and IC50 values were determined by the Trypan Blue assay. For metabolic characterization, we measure extracellular glucose and lactate concentrations (commercial kits, according to the manufacturer's instructions), reactive oxygen species(ROS) and mitochondrial activity by flow cytometry. The molecular probes Dihydroethidium(DHE) and red/green Mitotracker used were for ROS level determination and mitochondrial activity, respectively.

Results: 3BP pre-treatment enhanced the effect of chemotherapy drugs, decreasing cell viability and IC50 values for KG-1 and MOLM13 cell lines. After incubation with 3BP, only MOLM13 cells presented a decrease in glucose consumption, which was not reflected in the decrease of lactate levels. However, 5μM of 3BP disrupted mitochondrial activity and increased ROS levels.

Conclusions: Pre-treatment with non-toxic concentrations of 3BP sensitize AML cells to chemotherapeutic agents likely not by alteration in the glycolytic profile but by mitochondrial activity disruption.

References

1. Arber DA,Orazi A,Hasserjian R,et al.(2016).The 2016revision to the World Health Organization classification of myeloid neoplasms and acute leukemia.Blood.127, 2391-2405.
2. Lichtman MA.(2013).A historical perspective on the development of the cytarabine(7 days) and daunorubicin(3 days) treatment regimen for acute myelogenous leukemia: 2013 the 40th anniversary of 7+3.BloodCellsMolDis.50,119–130.
3. Fan T,Sun G,Sun X,et al.(2019).Tumor Energy Metabolism and Potential of 3-Bromopyruvate as an Inhibitor of Aerobic Glycolysis:Implications in Tumor Treatment.Cancer.11,1-24.

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