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605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Potency and Efficacy of Pharmacological PIP4K2 Inhibitors in Acute Lymphoblastic Leukemia

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Introduction and aims: Adult acute lymphoblastic leukemia (ALL) is a heterogeneous group of highly aggressive hematological malignancies with high recurrence rates and poor prognosis. Pharmacological inhibition of proteins of the phosphatidylinositol-5-phosphate 4-kinase type 2 family (PIP4K2s), through the compound THZ-P1-2, exhibits antileukemic activity by interrupting mitochondrial homeostasis and autophagy. Nevertheless, different pharmacological inhibitors with distinct affinities for PIP4K2s have been developed (PIP4K-IN-a131 and CC260), but their potential as antileukemic agents has been little explored. In the present study, we investigated the expression of PIP4K2s in ALL patients and cell models. Furthermore, the potency and efficacy of three PIP4K2s inhibitors were characterized in ALL cells.
Material and methods: The gene expression of *PIP4K2A, PIP4K2B* and *PIP4K2C* was evaluated in a cohort composed of 12 healthy donors and 101 patients with ALL by quantitative PCR. Gene and protein expression of PIP4K2s was investigated in a panel containing nine ALL cell lines. The potency and efficiency of three pharmacological inhibitors of PIP4K2s (THZ-P1-2, PIP4K-IN-a131, and CC260) were investigated in cell lines (Jurkat, MOLT4, NALM6, and Namalwa) and primary cells from patients with ALL. Cell viability was assessed by MTT assay, annexin V/PI labeling and mitochondrial membrane potential by flow cytometry, and proteins involved in cell signaling by Western blotting. Comparisons were performed by ANOVA and Bonferroni post-test and a p-value < 0.05 was considered significant.
Results: *PIP4K2A* mRNA levels were higher in ALL patients ($p < 0.05$), whereas *PIP4K2B* levels were lower ($p < 0.01$) compared to healthy controls. *PIP4K2C* expression did not differ between groups. Gene and protein expression of PIP4K2s showed a good correlation, and the expression of PIP4K2A and PIP4K2C showed the greatest variations between ALL cell lines. Pharmacological inhibitors of PIP4K2s showed reduced viability dependent on time and concentration, with THZ-P1-2 being the one with the highest pro-apoptotic activity (IC_{50} range: 1.4 - 8.1 μM), followed by PIP4K-IN-a131 (1.4 - >50 μM) and CC260 (13.3 - >50 μM). Similar results were observed in primary cells from patients with ALL. THZ-P1-2 also showed better efficacy in inducing apoptosis and mitochondrial damage compared to PIP4K-IN-a131 and CC260 ($p < 0.05$). From a molecular point of view, both THZ-P1-2 and PIP4K-IN-a131 similarly induced markers of apoptosis (PARP1 cleavage) and DNA damage (γH2AX). However, only THZ-P1-2 induced markers of blocking autophagic outflow (increased LC3BII and SQSTM1/p62) and reduced RPS6 phosphorylation (a target of the PI3K/AKT/mTOR pathway).
Discussion and Conclusion: A differential expression *PIP4K2A* and *PIP4K2C* gene expression profile was observed in a cohort of patients with ALL. THZ-P1-2 was the PIP4K2 inhibitor with the highest anti-leukemic activity and exerted its effects through a dose-dependent apoptosis induction and modulation of the autophagic process. An analysis of the structure-activity of the compounds may help to identify the most promising pharmacophores groups and promote advances in the optimization of this potential class of antileukemic agents. Support by FAPESP, CNPq and CAPES.

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