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604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Identification of Vulnerabilities for Pharmacological Inhibition of PIP4K2s in Acute Myeloid Leukemia

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Introduction and aims: Proteins of the PIP4K2s family (PIP4K2A, PIP4K2B and PIP4K2C) participate in the generation of PIP *4,5*P $_2$ (PIP $_2$), which acts as a secondary messenger in signal transduction, substrate for metabolic processes or has structural functions. In patients with AML, high expression of *PIP4K2A* and *PIP4K2C* is an independent marker of worse prognosis. Recently, our research group reported that THZ-P1-2 (pan-inhibitor of PIP4K2s) exhibits anti-leukemic activity by disrupting mitochondrial homeostasis and autophagy in AML models. In the present study, we describe the expression profile of PIP4K2s in normal and leukemic myeloid cells, as well as identify the association between PIP4K2s expression and the *ex vivo* response to several antineoplastic agents in AML.
Material and methods: *PIP4K2A*, *PIP4K2B* and *PIP4K2C* expression data in different hematopoietic cell populations were obtained from the study GSE24759. The expression of *PIP4K2s* in the different AML subgroups was obtained from the TCGA cohort and visualized by BloodSpot. Gene and protein expression of PIP4K2s was investigated in a panel containing twelve AML cell lines and a sample of normal CD34 $^+$ cells. Data from *ex vivo* treatments in patients with AML were obtained from BEAT AML. Kasumi-1, NB4 and U-937 cells were treated with vehicle or increasing concentrations of THZ-P1-2 and/or venetoclax for 48h. Cell viability was assessed by MTT, apoptosis by flow cytometry (annexin V/PI) and cell signaling by Western blot. Correlation analyzes and comparison between groups were performed by Spearman, Mann-Whitney or ANOVA tests, as appropriate. A p value <0.05 was considered significant.
Results: In myeloid cells, *PIP4K2A* expression was higher in erythroid and megakaryocytic cells ($p < 0.05$). *PIP4K2B* expression was higher in hematopoietic stem cells ($p < 0.05$). *PIP4K2C* expression was lower in common myeloid progenitors and erythroid cells ($p < 0.05$). In patients with AML, *PIP4K2A* and *PIP4K2C* expression was higher in complex karyotype groups, whereas *PIP4K2B* expression was higher in del(5q)/5q- and t(8;21)+others groups. In cell lines, PIP4K2A expression was higher in K-562, KU812, HEL and SET2 cells. The expression of PIP4K2B and PIP4K2C was similar between the evaluated cells. In ex vivo assays, the expression of PIP4K2s was related to sensitivity and resistance to several drugs, highlighting the association between high expression of *PIP4K2A* and resistance to venetoclax in AML ($p < 0.05$). The combination of THZ-P1-2 and venetoclax showed potentiating effects in reducing viability and inducing apoptosis in Kasumi-1, NB4 and U-937 cells ($p < 0.05$). In the molecular setting, the combination of THZ-P1-2 and venetoclax induced higher levels of cleaved PARP1 (marker of apoptosis) and γH2AX (marker of DNA damage) compared to monotherapies.
Discussion and conclusion: Our study characterized the expression of PIP4K2s in the myeloid compartment of hematopoietic cells, as well as in different molecular subsets of AML. The identification of the correlation between the expression of PIP4K2s and the response to antineoplastic agents in *ex vivo* assays in AML exposed vulnerabilities that can be exploited in combined therapies, which could result in better therapeutic responses. Support by FAPESP, CNPq, and CAPES.

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