Pharmacological PIP4K2 inhibition and venetoclax synergistically induces apoptosis in leukemia cells

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Background

Venetoclax, a selective inhibitor of BCL2, has been introduced in clinical practice for the treatment of patients with acute myeloid leukemia (AML). PIP4K2s comprise a family of lipid kinases responsible to produce phosphoinositides (PtdIns4,5P2) with structural and signal transduction functions. The combined expression of PIP4K2A and PIP4K2C has been identified as an independent prognostic factor in AML. Recently, a selective pharmacological inhibitor of PIP4K2s was developed (THZ-P1-2).

Methodology

OCI-AML3 leukemia cell line that is resistant to venetoclax was used. Cell viability was evaluated by MTT assay and apoptosis by annexin V/PI and flow cytometry. OCI-AML3 were treated with graded doses of THZ-P1-2 (Ø, 1.6, 3.2, 6.4, 12.5, and 25 μM) and/or venetoclax (Ø, 3.2, 6.4, 12.5, 25, and 50 μM) for 48 hours. The data obtained from at least three independent experiments were analyzed by linear regression for determination of IC50, and statistical analysis was performed by ANOVA and Bonferroni post-test using GraphPad Prism software. A p-value < 0.05 was considered significant.

Objective

To characterize the antineoplastic potential of THZ-P1-2 in combination with venetoclax in a cellular model of AML.

Results

Pharmacological PIP4K2s inhibition reduces cell viability

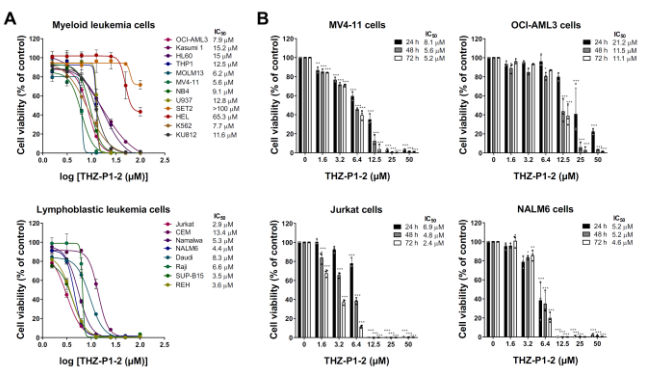

Figure 1. THZ-P1-2 reduces cell viability. (A) Dose-response cytotoxicity analyzed using a methylthiazoletetrazolium (MTT) assay in a panel of myeloid and lymphoblastic leukemia cell lines treated with vehicle or increasing concentrations of THZ-P1-2 (1.6, 3.2, 6.4, 12.5, 25, 50, and 100 μM) for 72 h. Values are expressed as the percentage of viable cells for each condition relative to vehicle-treated cells. The IC50 values and leukemia cell lines used are described. **(B)** Dose- and time-response cytotoxicity was evaluated by methylthiazoletetrazolium (MTT) in MV4-11, OCI-AML3, Jurkat, and NALM6 cells treated with vehicle (Ø) or with increasing concentrations of THZ-P1-2 (1.6, 3.2, 6.4, 12.5, 25, and 50 μM) for 24, 48, and 72 h. Bar graphs represent values expressed as a percentage for each condition relative to vehicle-treated controls. Results are presented as the mean ± SD of at least three independent experiments. The p values and cell lines are indicated in the graphs; * p < 0.05; ** p < 0.01; *** p < 0.001, ANOVA and Bonferroni post-test.

THZ-P1-2 triggers apoptosis and disrupts mitochondrial homeostasis and autophagic flux

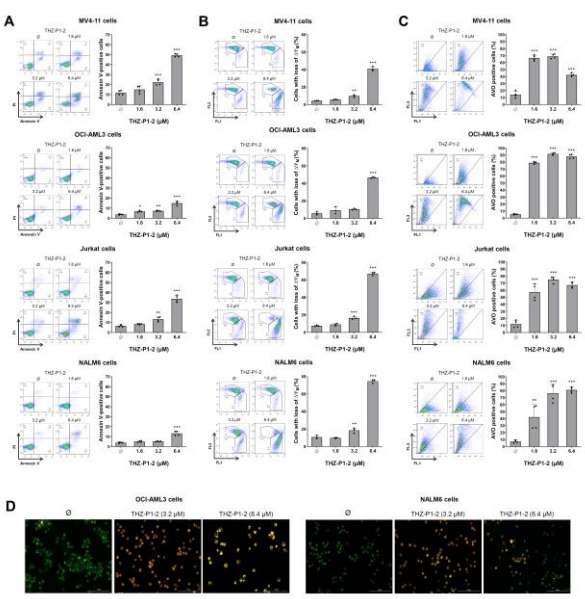

Figure 2. THZ-P1-2 induces apoptosis and dysfunction on mitochondria and autophagic flux. (A) Apoptosis detected by flow cytometry in MV4-11, OCI-AML3, Jurkat, and NALM6 cells treated with vehicle (Ø) or with increasing concentrations of THZ-P1-2 (1.6, 3.2, and 6.4 μM) for 24 h using an annexin V/PI staining method. Representative dot plots are shown for each condition. The upper and lower right quadrants (Q2 plus Q3) cumulatively contain the apoptotic population (annexin V+ cells). Bar graphs represent the mean ± SD of at least three independent experiments. The p values and cell lines are indicated in the graphs; * p < 0.05, ** p < 0.01, *** p < 0.0001; ANOVA and Bonferroni post-test. **(B)** Mitochondrial membrane potential (ΔΨM) analysis was evaluated using the JC-1 staining method and flow cytometry. Leukemia cells were treated with vehicle or THZ-P1-2 (1.6, 3.2, and 6.4 μM) for 24h. Representative dot plots are shown for each condition; the gate FL-2 contains cells with intact mitochondria and the gate FL-2/FL-1 contains cells with damaged mitochondria. Bar graphs represent the mean ± SD of at least three independent experiments and the p values are indicated; ** p < 0.01, *** p < 0.0001; ANOVA and Bonferroni post-test. **(C)** The evaluation of acidic vesicular organelles (AVOs) was investigated through acridine orange labeling and flow cytometry in AML and and ALL cell lines treated with vehicle (Ø) or THZ-P1-2 (1.6, 3.2, and 6.4 μM) for 24 h. Bar graphs represent the mean ± SD of at least three independent experiments and the p values are indicated; * p < 0.05, ** p < 0.01, *** p < 0.001; ANOVA and Bonferroni Post-test. **(D)** Alternatively, the presence of AVOs was confirmed by immunofluorescence on OCI-AML3 and NALM6 cell lines treated with vehicle (Ø) or THZ-P1-2 (3.2 and 6.4 μM) for 72 h on a Lionheart FX automated microscope at magnification, ×400. The overlapping RFP and GFP channels are shown.

THZ-P1-2 increases markers of apoptosis, DNA damage and blockage of autophagic flow

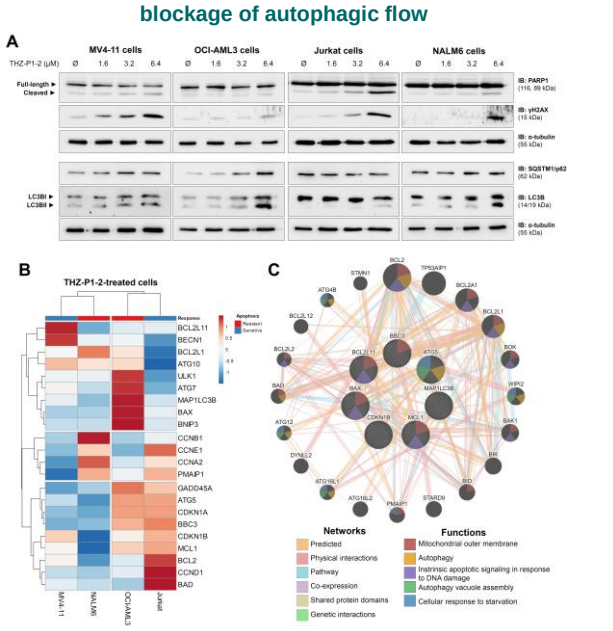

Figure 3. THZ-P1-2 induces markers of apoptosis, DNA damage and blockage of autophagic flow. (A) Western blot analysis for PARP1, γH2AX, p62/SQSTM1 and LC3B in total extracts from MV4-11, OCI-AML3, Jurkat, and NALM6 cells treated with vehicle (Ø) or with increasing doses of THZ -P1-2 (1.6, 3.2, and 6.4) for 24 h. Membranes were re-incubated with α-tubulin antibody and developed with the SuperSignal™ West Dura Extended Duration Substrate system and GBox. **(B)** Heatmap for gene expression in leukemia cell lines treated with vehicle (Ø) or THZ-P1-2 (6.4 μM) for 24 h. Blue color in the heat map indicates repressed mRNA levels while red indicates induced mRNA levels, which were normalized by vehicle-treated cells (n = 4). **(C)** Network for THZ-P1-2 modulated genes constructed using the GeneMANIA database. A total of seven genes were significantly modulated in all cell lines tested and are illustrated as crosshatched circles; the interacting genes included by modeling the software are indicated by circles without crosshatched. The main biological interactions and associated functions are described..

THZ-P1-2 potentates venetoclax-induced apoptosis in leukemia cells

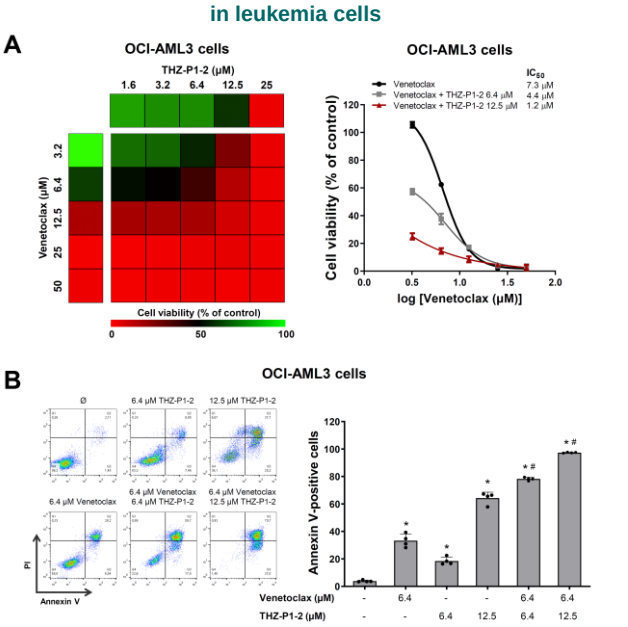

Figure 4. THZ-P1-2 potentates venetoclax-induced apoptosis in OCI-AML3 cells. (A) Dose-response cytotoxicity for combined treatment were analyzed by methylthiazoletetrazolium (MTT) assay for OCI-AML3 cells treated with graded concentrations of venetoclax and THZ-P1-2 alone or in combination with each other for 48 h, as indicated. Values are expressed as the percentage of viable cells for each condition relative to vehicle-treated cells. Results are shown as the mean of at least three independent experiments. Note that the inhibitory concentration of 50% (IC50) for venetoclax was reduced in combination with THZ-P1-2 in OCI-AML3 cells. **(B)** Apoptosis was detected by flow cytometry in OCI-AML3 cells treated with venetoclax and/or THZ-P1-2 for 48 hours using an annexin V/PI staining method. Representative dot plots are shown for each condition; the upper and lower right quadrants (Q2 plus Q3) cumulatively contain the apoptotic population (annexin V+ cells). Bar graphs represent the mean±SD of at least three independent experiments. The p values and cell lines are indicated in the graphs; *p < 0.05 for venetoclax- and/or THZ-P1-2-treated cells vs. vehicle-treated cells, #p < 0.05 for venetoclax- or THZ-P1-2-treated cells versus combination treatment at the corresponding doses; ANOVA and Bonferroni post-test.

Conclusion

The pharmacological inhibition of PIP4K2 potentiates venetoclax-induced apoptosis in acute myeloid leukemia cells. These findings represent new opportunities for overcoming venetoclax resistance and improving therapeutic interventions in AML.

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