



Investigation of the Antitumor Effect of Targeting PI3K-AKT-mTOR Pathway and Histone Deacetylase Enzymes on Acute Myeloid Leukemia Cells

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Abstract

Histone deacetylase inhibitors (HDACi) have promising anticancer activity for acute myeloid leukemia (AML). The combination of HDACi with small molecules targeting different signaling pathways is more effective in treating AML than single treatments. Here, we investigated the effect of combination of phosphatidylinositol-3-kinase (PI3K) inhibitor, LY294002, and different HDACis, SAHA , PCI-34051 and Tubastatin A, on two different AML cell lines, CMK and MOLM-13.

Background

AML is a complex disease and many chromosomal abnormalities and aberrant signaling pathways play a role in the pathogenesis of AML [1-2]. One of these pathways is PI3K which is one of the most important intracellular pathways that regulate cell growth, survival, and metabolism [3]. Although, AML has been considered a genetic disease, a number of recent studies have shown that epigenetic changes also play a role in the pathogenesis of AML [4-5]. In particular, HDACs are an important epigenetic regulators for chromatin remodeling and gene expression. Many studies have found abnormal expression of HDACs in AML and associated with disease progression and poor patient prognosis. Many studies in the literature support the anti-proliferative and anti-apoptotic effects of HDAC inhibitors on AML [6]. Especially the heterogeneous and complex pathogenesis of AML reveals that there is always a need for potential additional treatment methods. Here we examined the combination of two important driver of AML on two different AML subgroups.

Objective

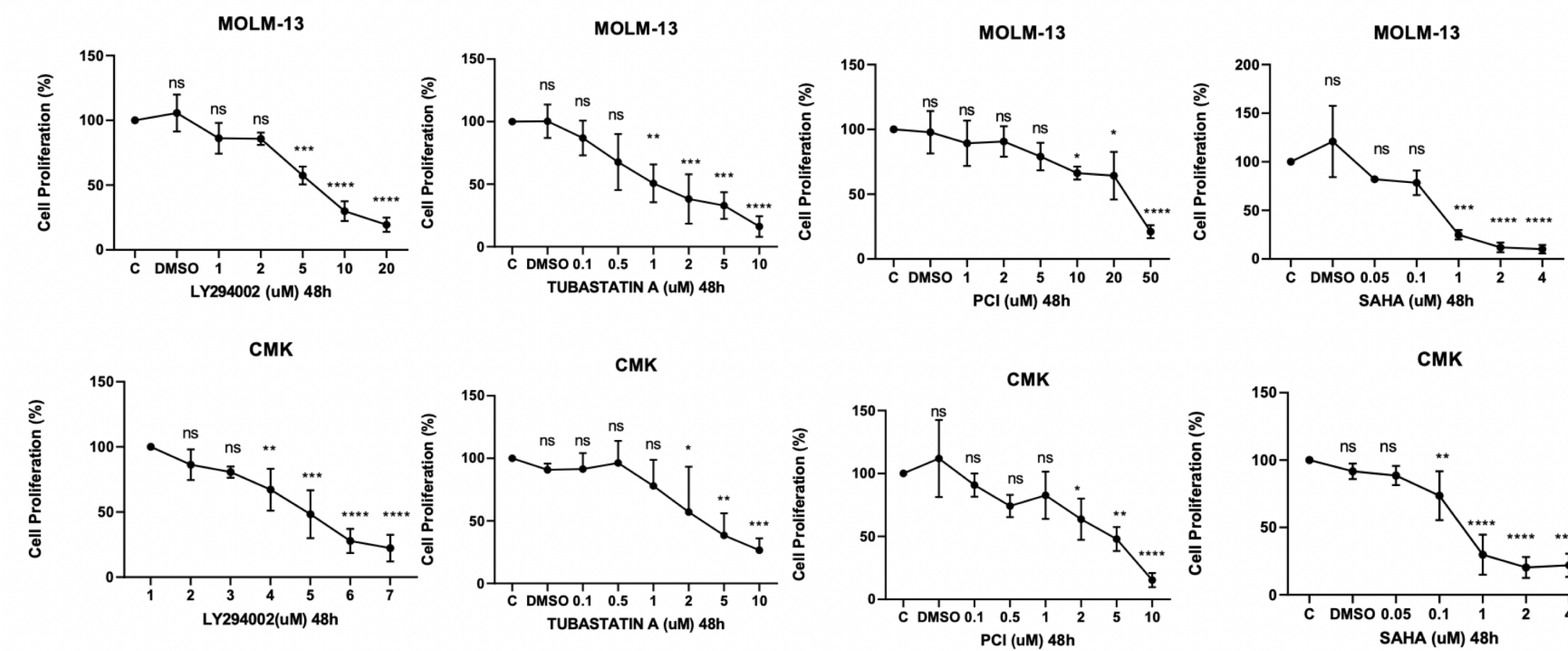


Figure 1: The effect of LY294002, Tubastatin A, SAHA and PCI-34051 treatment on the proliferation of MOLM-13 and CMK cells after 48h. Normalization was done per control and shown as percentage of proliferation cells. 3 independent experiments were performed and each experiment consisted of 3 replicate. The significance of the test was evaluated as $p < 0.05$ by using GraphPad Prism 8 ($n=3$). (ns= $P > 0.05$, *= $P \leq 0.05$, **= $P \leq 0.01$, *= $P \leq 0.001$, ****= $P \leq 0.0001$)**

The results show that, LY294002 led to a decrease in both AML cells. Subsequently, we investigated the effect of HDAC inhibition on CMK and MOLM-13 cell lines, and it was demonstrated that the HDACis SAHA, Tubastatin A, and PCI-34051 cause a decrease in the proliferation of CMK and MOLM-13 cell lines.

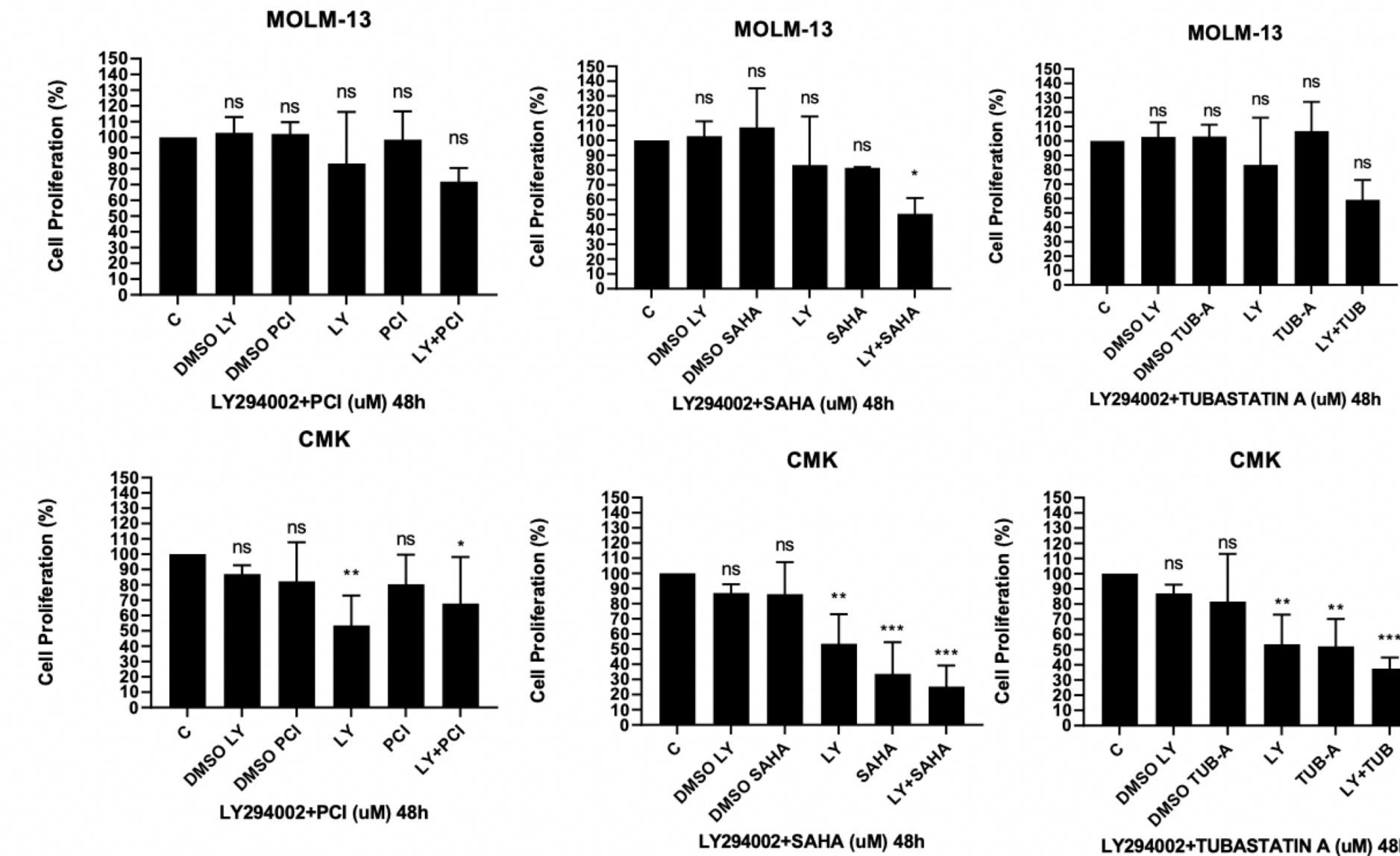


Figure 2: The effect of PI3K inhibitor and HDACi treatment on the proliferation of MOLM-13 and CMK cells after 48h. Normalization was done per control and shown as percentage of proliferation cells. 3 independent experiments were performed and each experiment consisted of 3 replicate. The significance of the test was evaluated as $p < 0.05$ by using GraphPad Prism 8 ($n=3$). (ns= $P > 0.05$, *= $P \leq 0.05$, **= $P \leq 0.01$, *= $P \leq 0.001$, ****= $P \leq 0.0001$)**

While the results demonstrated that combination treatment with LY294002+SAHA decreases cell proliferation by 50% in MOLM-13 cells, CMK cells showed a 25% decrease when compared to the control. LY294002+tubastatin A treatment showed 65% and 40% decreases in the cell proliferation of MOLM-13 and CMK cells, respectively. The proliferation of both cell lines decreased by approximately 70% upon treatment with LY294002+PCI-34051 for 48 h.

Materials & Methods

Cell Maintenance

The experiments were performed on AML cell lines originating from different AML subgroups;

- MOLM-13 AML M5 cell line with an FLT3-ITD mutation.
- CMK are AML M7 cell line from down-syndrome patients (DS- AMKL)

MTT Cell Viability Assay

In order to check the effect of combination of PI3K inhibitor which is LY294002 and different HDACi, SAHA , PCI-34051 and Tubastatin A on AML cells, we used to MTT cell viability assay. The cells were seeded in triplicates in 96-well plates at a plating density of 10^4 cells per each well for 48h. MTT solution was added to each well and the 96-well plates were incubated for 2h at 37°C . The absorbance was measured with a Varioskan™ LUX multimode microplate reader (Thermo Scientific™) at 570 nm.

Statistical analysis

The statistical analyses were done by unpaired GraphPad Prism 8. Comparisons of more than two groups were performed by 2-way ANOVA with Dunnett's multiple comparisons test. The level of significance was set at $P < 0.05$. All data were presented as mean $\pm$ s.d.

Summary

As, a summary, the result demonstrated that treatment with LY204002 combined with PCI-34051, SAHA, and Tubastatin A significantly affected the proliferation of both MOLM-13 and CMK cells.

Discussion

In conclusion, it is expected that this combination will lead to a more specific targeted combination therapy that results in the abolition of AML. Although there is a need for follow-up studies and further investigation, our preliminary results suggest that targeting HDAC enzymes and PI3K pathway could be potential therapeutic target for treating AML.

References

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