Effect of Adjuvant Epigenetic Therapy on KG-1 Acute Myeloid Leukemia Cells *in Vitro*

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Introduction

Dysregulated epigenetic mechanisms are central in the pathogenesis of acute myeloid leukemia (AML). They impair the differentiation of myeloid progenitors and promote their uncontrolled clonal proliferation leading to bone marrow failure. Histone deacetylation, as ruled by histone deacetylases (HDACs), is accompanied with a non-permissive condensed chromatin configuration and subsequent transcriptional gene silencing. Promoter CpG island hypermethylation of tumor suppressor genes, as mediated by DNA methyltransferase enzymes (DNMTs), is also well-reported to associate with a heterochromatin architecture and potent transcriptional silencing. The reversible nature of such epigenetic alterations highlight epigenetic modulators as promising attractive avenues for targeted therapy. Decitabine (DAC) is an azanucleoside with demethylating activity that depleted DNMT1, induced DNA damage and triggered apoptosis in AML cell lines. Suberoylanilide hydroxamic acid (SAHA), a pan HDAC inhibitor, reduced cell viability, induced apoptosis in AML cells and enhanced the expression of numerous genes. Augmentation of anti-tumorigenic effect with low drug dosages may be achieved through the synergistic use of HDAC and DNMT inhibitors. Combined DAC-SAHA treatment synergistically decreased cell proliferation, induced apoptosis, enhanced acetylation of histones and decreased DNMT1 protein in AML cells, however, the underlying mechanism(s) for these anti-tumor effects awaits further investigation. Unfolding the molecular mechanism(s) upholding their synergistic anti-tumor effect is the major scope of the current work.

Objectives

In the present study, we aimed to examine the effects of SAHA and DAC when applied separately and when combined on:

- Cellular proliferation
- Cell cycle
- Cell death
- Expression of apoptosis-related proteins

SAHA & DAC Reduce the Proliferation of KG-1 Cell Line

Fig. 1. KG-1 cells were grown in 96-well plates and treated with SAHA (A) (1, 2, 4 or 6 μM) or DAC (B) (0.1, 0.5, 1, 2 or 5 μM) for different time points. Cell proliferation was measured by WST-1 assay. Data were mean ± SD of five independent experiments.

SAHA & DAC Induce Cell Cycle Arrest in KG-1 Cell Line

Fig. 2. KG-1 cells were treated with SAHA (A) or DAC (B) at the indicated concentrations. The distribution of cell cycle following SAHA (C) or DAC (D) treatment was detected by flow cytometry following PI staining. The percentages of sub-G1, G0/G1, S and G2/M phases were calculated using C Flow software relative to controls. Two separate experiments were performed for each treatment condition, reported as the mean ± SD. *, **, *** indicate $p < 0.05$, $p < 0.001$, $p < 0.0001$; respectively.

SAHA & DAC Induce Cell Cycle Arrest of KG-1 Cell Line

Fig. 3. KG-1 cells were treated with SAHA (A) or DAC (B). Apoptosis was detected by flow cytometry following Annexin V/PI double staining. Total apoptosis following SAHA (C) or DAC (D) treatment was indicated by the sum of proportions of Annexin V+/PI- and Annexin V+/PI+ cells. Two separate experiments were performed for each treatment condition, reported as the mean ± SD. *, **, *** indicate $p < 0.05$, $p < 0.001$, $p < 0.0001$; respectively.

SAHA & DAC Alter the Expression of Apoptosis-Related Protein in KG-1 Cell Line

Fig. 4. Western blot for procaspase 3, caspase 3, p53, Bcl-2, cleaved PARP and β-actin from SAHA or DAC treated KG-1 cells

SAHA and DAC Synergize to Inhibit Proliferation of KG-1 Cell Line

Fig. 5. KG-1 cells were treated with different concentrations of DAC for 48h. 1μM SAHA was added for an additional 24h. Cell viability was measured using WST-1 assay.

SAHA and DAC Synergize to Induce Apoptosis of KG-1 Cell Line

Fig. 5. KG-1 cells were treated with 1 μM DAC for 72h or 1 μM SAHA 24h or with their sequential combination. Apoptosis was measured using Annexin-V/PI double staining flow cytometry.

Conclusion and Future Work

- Each of DAC and SAHA caused a dose and a time dependent decrease in the proliferation of KG-1 cells by inducing cell cycle arrest and apoptotic cell death.
- SAHA induced a increase in the cleavage of procaspase 3, increased the expression of p53 and cleaved PARP and decreased the expression of anti-apoptotic Bcl-2.
- DAC induced a increase in the cleavage of procaspase 3 and decreased the expression of anti-apoptotic Bcl-2.
- The sequential combination of DAC and SAHA resulted in synergistic growth inhibition and apoptosis of KG-1 cells.
- Future work will mechanistically examine the synergistic anti-neoplastic effect of SAHA and DAC on KG-1 cells.