(PS1586) SYNERGISTIC EFFICACY OF HDACI CHIDAMIDE WITH GL-V9 BY SUPPRESSING MYBL2 EXPRESSION IN ACUTE MYELOID LEUKEMIA

Topic: 03. Acute myeloid leukemia - Biology & translational research

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Background

Traditional high-dose chemotherapy is limited to elderly and unfit patients with acute myeloid leukemia (AML). Therefore, it has significant clinical relevance to have a new targeted therapy and combined therapy for AML. Chidamide is a benzamide histone deacetylase (HDAC) inhibitor that induces growth inhibition, cell cycle arrest, and apoptosis in AML. GL-V9 is a synthesized flavonoid derived from wogonin, which is one of the main active components of Scutellarin baicalensis and exhibits anticancer activities with low toxicity as its natural features.

Aims

This study sought to explore the synergistic efficacy and underlying mechanisms of Chidamide with GL-V9 in AML.

Methods

Cell Counting Kit-8 was used for cell proliferation assay; CalcuSyn was applied for the synergy analysis of the two drugs. Cell cycle and apoptosis were measured by flow cytometry analysis. Cellular senescence was assessed by senescence-associated β-galactosidase (SA-β-gal) staining. The human AML cell U937-xenograft (CDX) mouse model was developed to examine the therapeutic efficacy of chidamide with GL-V9 in vivo. Transcriptome was analyzed by RNA-seq analysis in MV4-11 cells treated with chidamide or GL-V9, respectively. WB and qPCR were utilized for gene expression. Co-immunoprecipitation (Co-IP) assays were performed to verify the interaction between the two proteins in AML.

Results

GL-V9 had a dose-dependent effect on cell proliferation arrest in U937, Molm13, and MV411 cells. GL-V9 significantly sensitized this effect of chidamide vs single-drug controls, and CalcuSyn analysis showed the synergistic effect of the combination (Fig. 1A). The combination also significantly increased the % apoptotic cells vs single-drug controls (Fig. 1B). Chidamide induced G0/G1-phase arrest and GL-V9 induced G2/M arrest, whereas the combination significantly decreased the % S-phase cells (Fig. 1C). Also, combined treatment with drug chidamide and GL-V9 increased the number of SA-β-gal–positive cells (Fig. 1D). Moreover, the combination of chidamide with GL-V9 vs either single drug controls significantly extended the survival (Fig. 1F), reduced the spleen sizes and weight (Fig. 1E), % $hCD45^{+}hCD33^{+}$ leukemia cells of the spleen and bone marrow (Fig. 1G) in the U937-xengraft mouse models. The combination vs single drug controls also significantly inhibited % cell viability of the primary cells from AML patients (Fig. 1H), and CalcuSyn analysis showed the combination had a synergistic effect.

To better understand the underlying mechanism of the synergy, the transcriptome changes upon the drug treatment were analyzed, and the significantly different expression genes (DEGs) were identified upon either GL-V9 or Chidamide treatment vs vehicle control. The overlapped DEGs are mainly enriched in cell cycle and cell senescence (Fig. 1I), and MYBL2, a member of the MYB family of transcription factors, is one of the down-regulated top DEGs. MYBL2 is a central regulator of cell cycle progression, cell senescence and cell survival, dysregulated in a wide range of cancers. Combining chidamide with GL-V9 significantly down-regulated MYBL2 mRNA and protein levels compared with single drug and vehicle control (Fig. 1K). Co-IP analysis confirmed the interaction between HDAC1 and MYBL2(Fig. 1L).

Summary/Conclusion

Combining HDAC inhibitor chidamide with GL-V9 demonstrates synergistic efficacy by suppressing MYBL2 expression in AML. Our results reveal a new potential combined therapeutic option for AML.

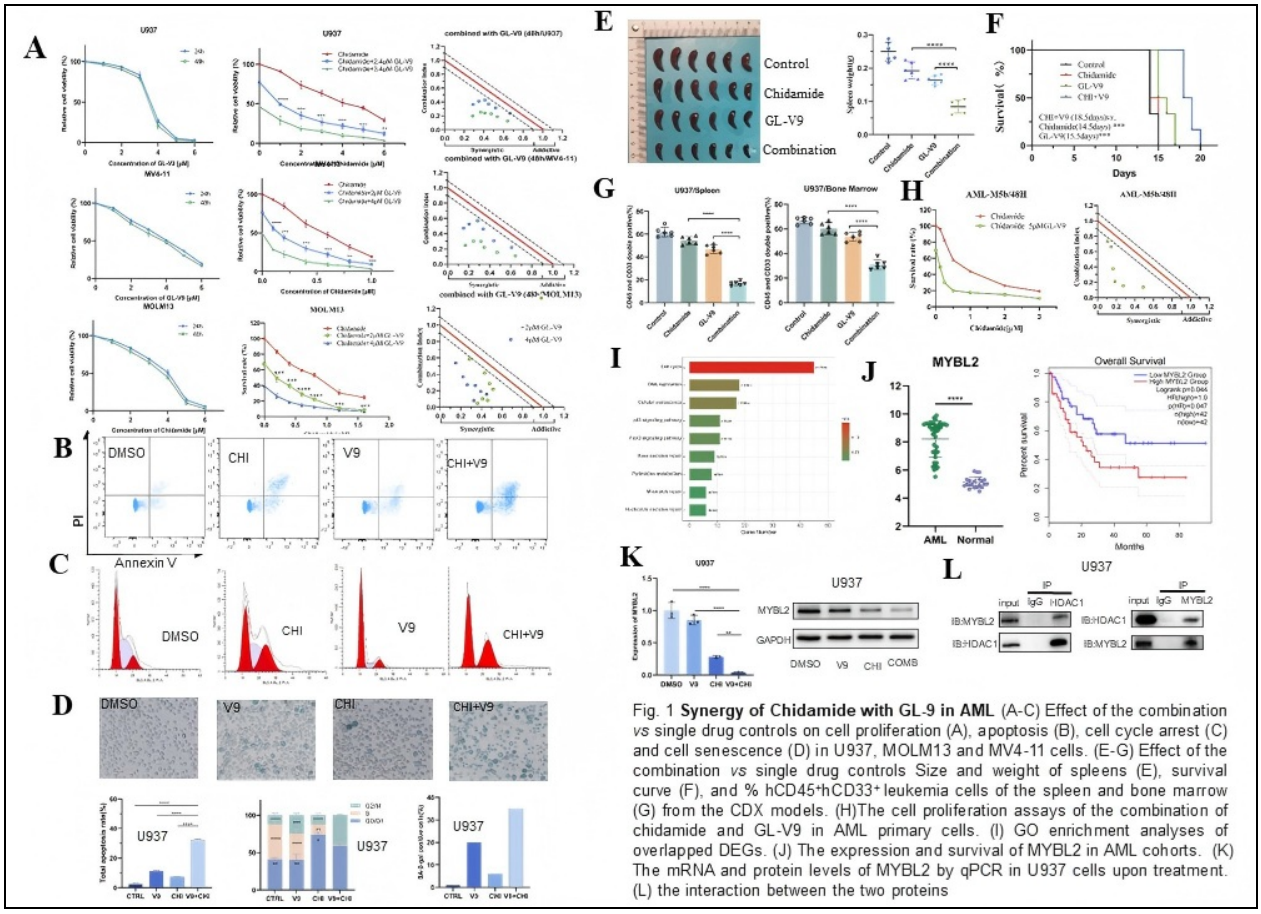

Fig. 1 **Synergy of Chidamide with GL-9 in AML** (A-C) Effect of the combination *vs* single drug controls on cell proliferation (A), apoptosis (B), cell cycle arrest (C) and cell senescence (D) in U937, MOLM13 and MV4-11 cells. (E-G) Effect of the combination *vs* single drug controls Size and weight of spleens (E), survival curve (F), and % hCD45^{+}hCD33^{+} leukemia cells of the spleen and bone marrow (G) from the CDX models. (H)The cell proliferation assays of the combination of chidamide and GL-V9 in AML primary cells. (I) GO enrichment analyses of overlapped DEGs. (J) The expression and survival of MYBL2 in AML cohorts. (K) The mRNA and protein levels of MYBL2 by qPCR in U937 cells upon treatment. (L) the interaction between the two proteins