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604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

HDAC Inhibitor Synergizes with GL-V9, a Derivative of Wogonin, to Suppress Acute Myeloid Leukemia By Downregulating ESPL1 and PLK4

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Background
Developing more effective non-chemotherapy options is emerging needed for acute myeloid leukemia (AML). GL-V9, a newly synthetic flavonoid derivative, exhibits anticancer activities with low toxicity as its nature features, however, the anti-leukemia effect and molecular mechanism against AML remain elusive. HDACs inhibitors are promising effective therapies for cancers. Chidamide (CS055/HBI-8000) is a benzamide histone deacetylase (HDAC) inhibitor that induces growth inhibition, cell cycle arrest, and apoptosis in MDS and AML. Here, we sought to determine the potential synergistic anti-leukemia effect of Chidamide combined with GL-V9 in AML.

Methods
Cell proliferation assays were measured by Cell Counting Kit-8(CCK-8) in AML cells; CalcuSyn was used for synergy analysis. Cell cycle and apoptosis were measured by flow cytometry analysis. Transcriptome was analyzed by RNA-seq in U937 cells treated with 10μM Chidamide and in MV4-11 treated with 4μM GL-V9. qPCR was utilized for gene expression; R2 genomics analysis and visualization application were used for comparison of the expression difference and the Kaplan-Meier overall survival analysis was conducted by the Gene Expression Profiling Interactive Analysis (GEPIA) platform.

Results
GL-V9 had a dose-dependent effect on cell proliferation arrest in U937 and MV4-11 cells. Chidamide significantly sensitized this effect of GL-V9 versus single-drug controls, and CalcuSyn analysis showed the synergistic effect of the combination (Fig.1A). The combination also significantly increased the % of apoptotic cells versus single-drug controls ($p<0.0001$) (Fig1.B). Chidamide induced G0/G1-phase arrest and GL-V9 induced G2/M arrest, whereas the combination significantly decreased the % of S-phase cells (Fig.1C). These data indicate that Chidamide+GL-V9 combination results in the synergistic effect on cell growth inhibition, apoptosis and cell cycle arrest in AML. Next, the drug-induced change of the global transcriptome was analyzed. The significantly different expression genes (DEGs) were identified upon GL-V9 treatment and Chidamide treatment versus vehicle control. 1663 DEGs are identified upon GL-V9 treatment and 3756 DEGs upon Chidamide, in which 1405 were intersected regulated (1231 genes changed in the same direction but 174 changed in the opposite (Fig.2AB). The overlapped DEGs are mainly enriched in cell cycle regulation, DNA repair, chromosome segregation, spindle organization, DNA damage, etc. (Fig2.C). ESPL1 and PLK4 are at the top of the overlapped down-regulated DEGs; and qPCR results confirmed their mRNA levels are significantly downregulated by GL-V9+Chidamide, GL-V9, and Chidamide versus the vehicle control ($p<0.0001$) (Fig. 2D). ESPL1 encoded protein, Separase is an enzyme resolving sister chromatid cohesion, and plays a pivotal role in cell division and oncogenesis. PLK4 (polo-like kinase 4) is a key regulator of centrosome duplication and the substrate of histone acetyltransferases, and PLK4 overexpression leads to centrosome amplification in human cancer and contributes to multipolar spindle assembly. Our results not only indicated that the combination exerts its anti-tumor effect through downregulation of ESPL1 and PLK4 expression but also reveal their oncogenic roles in AML. Indeed, the mRNA levels of ESPL1 and PLK4 are significantly high expressed in AML patients versus normal BM controls, and ESPL1 high expression is correlated with poor prognosis (Log-rank $p=0.0039$) (Fig2.E). In addition, our data showed that the expression of the anti-apoptosis genes BCL2 and BCL2L1 are significantly decreased, and the early apoptosis indicators, Caspase-3 and Casepase-9 increased by

the combination versus the single-drug controls in U937 cells (Fig2.F). The proposed mechanism model is summarized in Fig. 2G.

Conclusion

Our data indicate that HDAC inhibitor Chidamide significantly synergizes GL-V9-induced cell growth inhibition, apoptosis, and cell cycle arrest in AML, and the underlying mechanism by suppressing the expression of ESPL1 and PLK4, the key regulators of cell division and proliferation. Our results also provide a potential chemotherapy-free option for AML patients.

Disclosures No relevant conflicts of interest to declare.

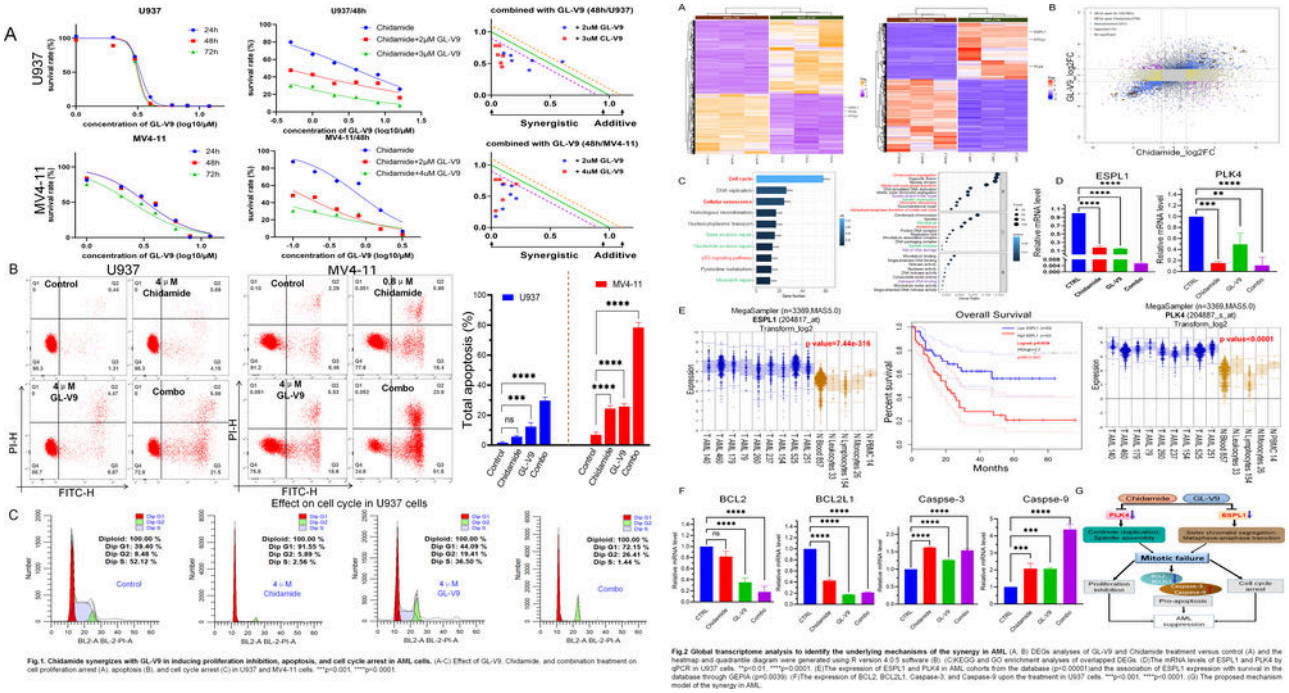

Fig.1. Chidamide synergizes with GL-V9 in inducing proliferation inhibition, apoptosis, and cell cycle arrest in AML cells. (A-C) Effect of GL-V9, Chidamide, and combination treatment on cell proliferation arrest (A), apoptosis (B), and cell cycle arrest (C) in U937 and MV4-11 cells. ***p<0.001, ****p<0.0001.

Fig.2 Global transcriptome analysis to identify the underlying mechanisms of the synergy in AML. (A, B) DEGs analyses of GL-V9 and Chidamide treatment versus control (A) and the heatmap and quadrantile diagram were generated using R version 4.0.5 software (B). (C)KEGG and GO enrichment analyses of overlapped DEGs. (D)The mRNA levels of ESPL1 and PLK4 by qPCR in U937 cells. **p<0.01, ***p<0.0001. (E)The expression of ESPL1 and PLK4 in AML cohorts from the database (p<0.00001)and the association of ESPL1 expression with survival in the database through GEPIA (p=0.0039). (F)The expression of BCL2, BCL2L1, Caspase-3, and Caspase-9 upon the treatment in U937 cells. **p<0.001, ****p<0.0001. (G) The proposed mechanism model of the synergy in AML.

Figure 1

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