Norchelerythrine from *Corydalis incisa* (Thunb.) Pers. promotes differentiation and apoptosis by activating DNA damage response in acute myeloid leukemia

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Abstract. Acute myeloid leukemia (AML) is the most prevalent form of leukemia in adults. The cornerstone of first-line chemotherapy for AML has poor survival rates, underscoring the urgent need for development of novel therapeutic agents. Differentiation therapy targets the blockade of differentiation in myeloid progenitor cells. The present study screened 100 plant extracts native to South Korea to search for those with differentiation-inducing activity in AML. Differentiation-inducing activity was assessed by measuring CD11b expression using fluorescence activated cell sorting. Of these, *Corydalis incisa* (Thunb.) Pers. (CIP) exhibited the highest efficacy. CIP induced myeloid differentiation, decreased viability and increased cell apoptosis and cell cycle arrest in HL-60, U937 and THP-1 cells. Furthermore, ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry identified norchelerythrine as the primary anti-leukemic compound in CIP. Norchelerythrine induced differentiation and promoted cell cycle arrest and apoptosis, mirroring the tumor-suppressive effects of CIP, and notably decreased cell viability in patients with various genetic abnormalities. The present mechanistic study showed that norchelerythrine stimulated reactive oxygen species generation, leading to activation of DNA damage signaling and upregulation of $p21^{cip1}$, a cyclin-dependent kinase inhibitor. Overall, norchelerythrine isolated from CIP may be a novel therapeutic option in AML.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of the hematopoietic system that is characterized by arrest of differentiation in myeloid progenitors (blasts) in bone marrow and peripheral blood (1,2). Such cells infiltrate the bone marrow, blood or other tissues, ultimately leading to hematopoietic failure (2-4). The incidence of AML increases with age. In 2022, an estimated 20,050 new cases of AML and 11,540 deaths were reported worldwide. The 5-year survival rate for AML is 30.5%, the lowest 5-year relative survival rate among all leukemias. Currently, ~40% of patients with AML under the age of 60 and 15% of aged >60 years are cured (4-6). Patients with AML are treated primarily with chemotherapy, called the 7 + 3 regimen (7 days of cytarabine and 3 days of anthracycline) (7-9). Nevertheless, these chemotherapeutic agents have limitations of poor efficacy and high toxicity (10).

Recently, more specific targeted therapies such as fms-like tyrosine kinase 3 (FLT3) and isocitrate dehydrogenase (IDH) inhibitor have been developed based on better understanding of the molecular pathogenesis of AML (11). Mutations in FLT3 genes occur in one-third of patients with AML. These mutations cause constitutive activation of signaling, which promotes cell proliferation and survival and inhibits differentiation. FLT3 mutation in AML is associated with increased risk of relapse and adverse prognosis (12,13). FLT3 inhibitors (sorafenib, midostaurin and quizartinib) have improved overall survival as single agents and in combination with the 7 + 3 regimen in patients with FLT3-mutated AML (13). Another mutation frequently occurring in AML is isocitrate

dehydrogenase (IDH), which has been identified in 15-20% of patients. Mutations in this gene cause abnormal enzyme activity, producing a metabolite 2-hydroxyglutarate (2-HG). 2-HG blocks the differentiation of myeloid precursors and causes uncontrolled proliferation. IDH1 inhibitor (ivosidenib) and IDH2 inhibitor (enasidenib) have been used in patients with relapsed or refractory AML with the IDH mutation and improved median overall survival (3.3-9.0 and 3.3-9.3 months, respectively) (13,14). CD33 is a cell surface marker highly expressed on leukemic blasts in almost all patients with AML (15). Gemtuzumab ozogamicin, a CD33 antibody conjugated to the cytotoxic drug calicheamicin, was developed based on molecular studies of AML (16-20), but withdrawn due to lack of improvement in survival rates and concerns about liver toxicity. These targeted therapies also have limited therapeutic efficacy because of the genomic complexity and clonal architecture of AML (15,21).

Differentiation therapy is a therapeutic method that stimulates differentiation in undifferentiated cancer cells to eliminate tumor phenotypes (22,23). One of the hallmarks of AML is blockade of differentiation, suggesting that induction of differentiation may be a feasible therapeutic option for AML subtypes. Differentiation therapy notable improves survival of patients with acute promyelocytic leukemia (APL) (23), a subtype of AML characterized by t (15;17) chromosomal translocation, resulting in fusion of the promyelocytic leukemia (PML) gene with a retinoic acid receptor (RARα) to form the PML-RARα fusion protein. All trans-retinoid acid (ATRA) and arsenic trioxide reverse the differentiation blockade induced by the fusion protein, leading to APL differentiation and clearance (24,25). However, ATRA and arsenic trioxide have limited effects in other subtypes of AML (25), necessitating novel agents with low toxicity and high efficacy for differentiation therapy.

Plants provide a source of drug research and development. They contain natural bioactive compounds, such as vitamins, carotenoids, terpenoids, flavonoids, alkaloids, tannins and minerals, which are associated with anti-oxidant, anti-microbial, anti-inflammatory and anti-tumor activity (26-28). Plants belonging to the *Corydalis* genus exert tumor-suppressive effects. Tetrahydrocoptisine from *C. impatiens* has shown anti-inflammatory activity by inhibiting the NF-κB and MAPK signaling pathways (29). In addition, corynoline isolated from *C. bungeana* Turcz. decreases pro-inflammatory mediators by regulating the Nrf2/MAPK pathway (30). Furthermore, *C. edulis* Maxim exerts an anti-diabetic effect by increasing insulin secretion via protein kinase C activation (31). *C. yanhusco* extract inhibits cell proliferation in breast cancer and exhibits anti-proliferative and anti-tumor effects in liver carcinoma cells (32,33).

Alkaloids are bioactive compounds found frequently in natural herbs that elicit anti-tumor effects in various types of cancers by inducing DNA damage, cell cycle arrest and apoptosis. Taxol, camptothecin and vinblastine are among the most widely used alkaloids to treat cancer (34,35). Alkaloids also exert anti-leukemic effects in AML (36-40). Securinine is a major alkaloid isolated from the roots of *Securinega suffruticosa*. This compound induces differentiation by activating the DNA damage response (39). Tetrandrine, an alkaloid isolated from the roots of *Stephaniae tetrandrae*, promotes reactive oxygen species (ROS) accumulation and inhibits c-myc expression, inducing autophagy and differentiation (40).

The present study investigated the effect of *Corydalis incisa* (Thunb.) Pers. (CIP) and norchelerythrine, an alkaloid isolated from CIP, on differentiation and apoptosis in AML cells.

Materials and methods

Preparation of plant extracts. Plant extracts (Table SI) were obtained from the Korea Plant Extract Bank at the Korea Research Institute of Bioscience and Biotechnology. Each extract was dissolved in DMSO.

Cell culture and chemicals. Human AML cell lines HL-60, U937 and THP-1 were purchased from Korean Cell Line Bank (Seoul, Korea) and cultured in RPMI-1640 medium (Cytiva, cat. no. SH30027.01) supplemented with 10% FBS (HyClone, Cytiva; cat. no. SH30919.03), 1% HEPES (cat. no. 15630-080), 1% penicillin/streptomycin (cat. no. 15140-122) and 1% L-glutamine (all Gibco, cat. no. 25030-81; Thermo Fisher Scientific, Inc.) at 37˚C in a 5% CO_2 incubator. U937 cell line was authenticated using STR profiling by Cosmogenetech Co., Ltd. Normal bone marrow cells from wild-type C57BL/6 mice were cultured in RPMI-1640 medium with 10% FBS as aforementioned. Norchelerythrine and peltatoside were purchased from ChemFaces (cat. no. CFN92737 and CFN70318). Phorbol-12 myristate-13 acetate (PMA) (cat. no. P8139) and ROS scavenger N-acetylcysteine (NAC) was purchased from Sigma-Aldrich (cat. no. A9165; Merck KGaA).

Flow cytometry assay. The induction of AML cell differentiation was determined by assessing CD11b and CD14 expression on the cell surface. Briefly, cells were treated with DMSO, CIP (20 μg/ml for 96 h), norchelerythirne (2, 5 or 10 μM for 96 h) or *Corydalis speciosa* Maxim. (20 μg/ml for 96 h) at 37˚C, harvested and washed with PBS, followed by staining with FITC-conjugated CD11b (cat. no. 101206) or PE-cy7 conjugated CD11b (both Biolegend, Inc.; cat. no. 101216) or PercP-Cy5.5-conjugated CD14 (BD Biosciences, cat. no. 550787) for 1 h at 4˚C in the dark. To assess the effect of norchelerythrine on normal hematopoiesis in mice, bone marrow cells were treated with 5 μM norchelerythrine at 37˚C for 96 h. Cells were harvested and washed with PBS, then stained with PE-conjugated CD45 (eBioscience, cat. no. 12-0451-82; Thermo Fisher Scientific, Inc.), PE-Cy7-conjugated CD11b (BioLegend, Inc.; cat. no. 101216), APC-conjugated Ly-6G (BD Biosciences, cat. no. 560599) and FITC-conjugated CD14 (BioLegend, Inc.; cat. no. 123307) for 1 h at 4˚C in the dark. The samples were analyzed using a FACSDiva Fusion Flow Cytometer (BD Biosciences). At least 10,000 cells were analyzed for each data point. Data analysis was carried out using FlowJo software version 10.8.1 (Treestar Inc.).

Reverse transcription-quantitative (RT-q) PCR. Total RNA was isolated from U937 cells using Tri-RNA reagent (Favorgen, cat. no. FATRR001) and cDNA was synthesized using the PrimeScript RT reagent kit (Takara Bio, Inc., cat. no. RR047A) according to the manufacturer's protocol. Equal amounts of cDNA were used for transcript PCR amplification, which

Table I. Primers used in reverse transcription-quantitative PCR.

Gene	Forward, 5'→3'	Reverse, 5'→3'
CSF1R	GTGGCTGTGAAGATGCTGAA	CCTTCCTTCGCAGAAAGTTG
MAFB	GCCTGCGCTAATTGTAGGAG	CGCACTTGAAAGTTGCAAAA
ITGAM	AGAACAACATGCCCAGAACC	GCGGTCCCATATGACAGTCT
CD14	CTGCAACTTCTCCGAACCTC	CCAGTAGCTGAGCAGGAACC
Lyz	GCCAAATGGGAGAGTGGTTA	ATCACGGACAACCCTCTTTG
MMP9	TTGACAGCGACAAGAAGTGG	GCCATTCACGTCGTCCTTAT
Myb	GGCAGAAATCGCAAAGCTAC	GCAGGGAGTTGAGCTGTAGG
Myc	TTCGGGTAGTGGAAAACCAG	CAGCAGCTCGAATTTCTTCC
TBP	TATAATCCCAAGCGGTTTGCTGCG	AATTGTTGGTGGGTGAGCACAAGG

CSF1R, colony stimulating factor 1 receptor; MAFB, V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; ITGAM, integrin αM; Lyz, Lysozyme; Myb, v-myb avian myeloblastosis viral oncogene homolog; TBP, TATA box-binding protein.

was performed using TOPreal qPCR PreMIX SYBR Green with low ROX (Enzynomics Co., Ltd.; cat. no. RT500M). The thermocycling conditions were as follows: 95˚C for 15 min followed by 40 cycles of 95˚C for 15 sec, 59˚C for 30 sec and 72˚C for 30 sec. Table I lists the primers used. Relative gene expression was analyzed using the 2-ΔΔCq method (41).

Cell proliferation assay. Cell proliferation rates were determined using trypan blue exclusion assay (42). The cells ($2x10^5$ cells/well) were seeded into a 24-well plate and treated with CIP (0, 10 or 20 μg/ml), norchelerythrine (0, 5 or 10 μM) or peltatoside (0, 500 nM, 1, 2 or 5 μM) at 37˚C for 24-120 h. Cells were stained with 0.4% Trypan blue (Gibco; Thermo Fisher Scientific, Inc.; cat. no. 15250061) for 3 min at room temperature every day, and the number of viable cells was counted using a hemocytometer.

Measurement of cell viability. Cell viability was assessed using CellTiter 96 AQueous MTS assay. The cytotoxicity of CIP and *Corydalis speciosa* Maxim. extract in human AML cell lines (HL-60 and THP-1) was tested by seeding cells in a six-well plate at a density of $4x10^5$/well followed by treatment with CIP or *Corydalis speciosa* Maxim. extracts (20 μg/ml) at 37˚C for 5-8 days. The cytotoxicity of CIP was identified by plating cells in a 96-well plate at a density of $3x10^4$ AML cells (HL-60 and U937) or $5x10^5$ normal bone marrow cells/well and treating them with CIP (0, 50, 100, 200 or 300 μg/ml) at 37˚C for 24 h. The cytotoxic effect of norchelerythrine and peltatoside was examined by treating HL-60, U937 and THP-1 cells with norchelerythrine (0 or 10 μM for 72 or 96 h) or peltatoside (0, 500 nM, 1, 2 or 5 μM for 48, 72 h or 96 h) at 37˚C. MTS reagent (Promega Corporation; cat. no. G1112) was added for 4 h at 37˚C. The absorbance at 450 nm was measured using a GloMax Microplate multi-mode reader (Promega Corporation) (43).

Trypan blue staining. Trypan blue stain was used to determine the cell apoptosis rate. HL-60 and THP-1 cells were plated in six-well plates at a density of $4x10^5$ cells/well and treated with *Corydalis incisa* (Thunb.) Pers. or *Corydalis speciosa* Maxim. extract (20 μg/ml) at 37˚C for 5-8 days. The cells were stained with 0.4% trypan blue (Gibco, Thermo Fisher Scientific, Inc.; cat. no. 15250061) for 3 min at room temperature. The number of positively stained cells was counted using a hemocytometer (44).

Colony forming assay. HL-60 cells ($1x10^4$ cells/well) were seeded in a 12-well plate in methylcellulose (Methocult H4100, StemCell Technologies) supplemented with 10% FBS and 1% penicillin/streptomycin, as previously described (43) and treated with CIP (2 μg/ml) at 37˚C for 11 days. The colonies containing >50 cells were counted manually 11 days after plating using an Olympus CX31microscope (Olympus Corporation) at 400x magnification.

Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) analysis of CIP. CIP was analyzed using Waters AQUITYTM UPLC system equipped with an XEVO-QTOF mass detector. ACQUITY UPLC BEH C18 column was used, with two mobile phases containing water with 0.1% formic acid (solvent A) and acetonitrile with formic acid (solvent B). The mobile phase was delivered at a flow rate of 0.4 ml/min, and a 1 μl injection volume was used. The elution gradient was as follows: 1, B 8%; 13, B 8-40%; 2.5, B 40%; 0.5, B 40-100%; 2.5, B 100%; 0.5, B 100-8%; 2 min B 8%. XEVO-QTOF mass detector featured electrospray ionization. The analysis was conducted in negative and positive ion modes in the range of 100-1,500 m/z. N_2 was used as the desolvation gas. The desolvation temperature was set to 350˚C at a flow rate of 800 l/h with a source temperature of 110˚C. The capillary and cone voltages were set to 300 and 40 V, respectively. CIP was prepared (3 mg/ml) in methanol.

Measurement of intracellular ROS. Intracellular ROS were detected by MitoSOX Red staining. Following treatment with 10 μM norchelerythrine at 37˚C for 48 h, cells were harvested and washed with PBS, followed by staining with MitoSOX Red (MedChemExpress, cat. no. HY-D1055) for 20 min at 37˚C in the dark. The cells were washed with PBS and analyzed using a FACSDIVA fusion Flow Cytometer (BD Biosciences). At least 10,000 cells were analyzed for each data point. Data

analysis was performed using FlowJo software version 10.8.1 (Treestar Inc.).

Western blot analysis. HL-60 and U937 cells were treated with norchelerythrine (5 or 10 μM at 37˚C for 6, 12 or 48 h) and washed in PBS. The cells lysed in RIPA buffer (Elpis Biotechnology, cat. no. EBA-1149) containing 1 Na-vanadate, 50 β-glycerophosphate disodium salt (both Sigma-Aldrich; Merck KGaA; cat. no. G9422), 142 β-mercaptomethanol (Bioworld Technology, Inc.; cat. no. 41300000-1) and 5 mM EDTA and ProteaseArrest (Thermo Scientific, Inc.; cat. no. 87786). Protein concentrations were measured using BCA assay kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The samples were boiled at 100˚C for 10 min, 25 μg proteins loaded on 6 or 15% polyacrylamide gels and transferred to Immobilon-P transfer membrane. The membranes were blocked for 1 h at room temperature in 0.1% TBST with 1% BSA (MP Biomedicals, LLC; cat. no. 160069) and incubated with primary antibodies overnight at 4˚C, washed three times for 5 min in TBST, and incubated with HRP-conjugated anti-mouse or anti-rabbit secondary antibodies for 1 h at room temperature. The membranes were washed three times for 10 min in TBST. The protein bands were detected using chemiluminescent substrate (EzWestLumi plus; ATTO corporation) and visualized using the Luminograph II (ATTO Corporation). The proteins were quantified using ImageJ 1.54 g (National Institutes of Health). The primary antibodies against p21 (cat. no. #2947S), γH2AX (cat. no. #9718S), Ataxia telangiectasia mutated (ATM) (cat. no. #2873S) and phosphorylated ATM (all 1:1,000; #5883S) were purchased from Cell Signaling Technology, Inc. The primary antibody against β-actin (1:5,000; cat. no. sc-47778) and the anti-mouse secondary antibody (cat. no. sc-516102, 1:5,000) was obtained from Santa Cruz Biotechnology, Inc. The anti-rabbit secondary antibody (cat. no. A120-101P, 1:5,000) was purchased from Bethyl Laboratories, Inc. (45).

Patient samples. Pusan National University Hospital (Busan, South Korea) provided the bone marrow samples from 4 male patients with AML, collected between August 2023 and November 2023, after obtaining informed written consent from patients and approval from the institutional review board of Pusan National University Hospital (approval no. IRB 2403-010-137). The mean age of the patients was 55 years (range, 21-80 years). Table SII lists the mutation profiles of patients.

Generation of CIP-resistant cells. CIP-resistant cell line was generated by seeding HL-60 cells ($2x10^5$ cells/well) in 12-well plates followed by treatment with DMSO or 2 μg/ml CIP at 37˚C for 96 h. Medium was removed, and the cells were cultured for an additional 48 h in RPMI-1640 medium supplemented with 10% FBS, 1% HEPES, 1% penicillin/streptomycin and 1% L-glutamine. Subsequently, the cells were treated with 4, 6, 8, 10, 12, 15, 18 and 20 μg/ml CIP, respectively, in the same manner. This cycle was repeated for over 6 months. Resistance was confirmed by FACS analysis of CD11b and cell counting.

QuantSeq-3' mRNA-sequencing (seq). Total RNA from HL-60-DMSO and HL-60-CIP cell lines was extracted using Tri-RNA reagent (Favorgen, FATRR001). DNA was removed using Ambion AM1906 DNase Treatment and Removal Reagents (Ambion) according to the manufacturer's instructions. RNA quality was assessed by Agilent 4200 TapeStation System (Agilent Technologies, Inc.). QuantSeq-3'mRNA-seq was performed by ebiogen, Inc. Briefly, RNA library construction was performed using the Quant-Seq 3' mRNA-Seq V2 library prep kit FWD with UDI 12 nt Sets A1-A4, (UDI12A_0001-0384). 384 preps (cat. no. 193.384; Lexogen GmbH). The loading concentration of the final library was >4 nM and was measured using Qubit (Thermo Fisher Scientific Inc.). High-throughput single-end 75 bp sequencing was conducted using NextSeq 500/550 (Illumina, Inc.) using a NextSeq 500/550 High Output Kit v2.5 (75 cycles, cat. no. 20024906; Illumina, Inc.). Gene ontology (GO) and differentially expressed genes (DEG) were analyzed using ExDEGA version 5.0 (ebiogen, Inc.). Differentially expressed genes with fold change >2 and $P<0.05$ were selected. A heatmap was generated using MultiExperiment Viewer 4.9.0 (sourceforge.net/projects/mev-tm4/).

Statistical analysis. All data are presented as the mean ± SD. Statistically significant differences were calculated using non-parametric Mann-Whitney U or unpaired t-test or one-way ANOVA test with Tukey's post hoc test using Prism version 5.03 software (GraphPad Software, Inc.; Dotmatics). All experiments were repeated ≥3 times. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Extracts of CIP efficiently promote differentiation of AML. A total of 100 plant extracts native to South Korea were screened morphologically based on the presence of adherent cells, after treatment with 20 μg/ml plant extracts for 96 h, which indicated the induction of differentiated cells. Four extracts that promoted the formation of adherent cells were then analyzed for CD11b expression, a myeloid differentiation marker, following exposure to 20 μg/ml plant extracts for 96 h. Extracts of CIP were most effective in increasing CD11b expression (Table SI). Therefore, the present study focused on CIP extracts for further characterization. U937 AML cell line was exposed to CIP extracts (20 μg/ml) to confirm the ability of CIP to induce AML differentiation, which significantly increased CD11b expression (Fig. 1A). In addition, increases in size (forward scatter) and granularity (side scatter) are commonly observed as the cells differentiate (46), which was the case following CIP treatment (Fig. 1B). PMA can induce differentiation of AML cells and make them adherent and elongated (47). Co-treatment of CIP notably increased the number of adherent/elongated cells compared with PMA alone, suggesting that CIP enhances PMA-induced differentiation of THP-1 AML cells (Fig. 1C).

The present study analyzed expression of genes related to myeloid differentiation using RT-qPCR. Consistent with the data that CIP efficiently induces AML differentiation, myeloid differentiation markers (colony stimulating factor 1 receptor, integrin αM and CD14) were upregulated and negative regulators of myeloid differentiation (Myb and Myc) were downregulated following CIP treatment. Furthermore,

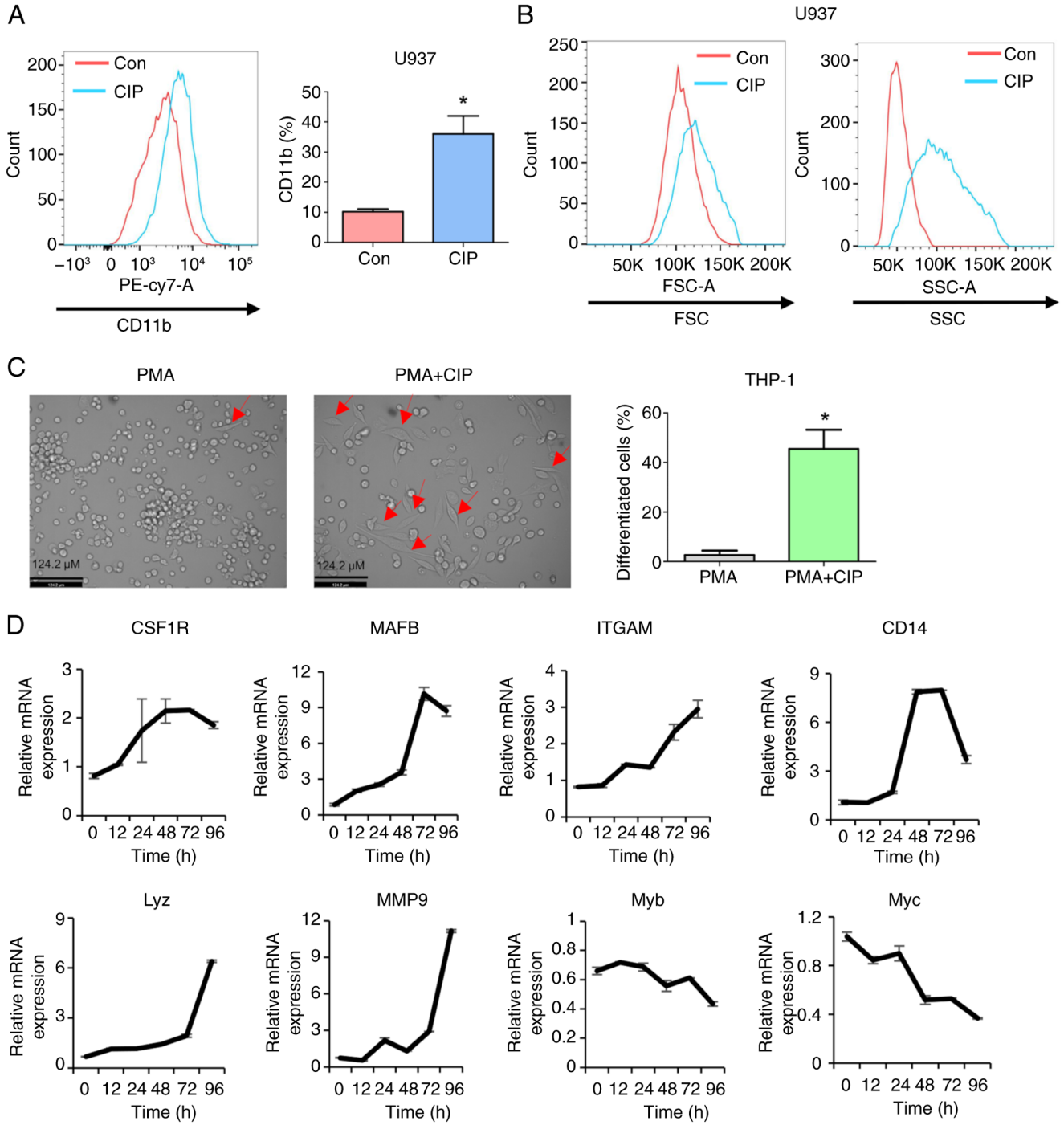

Figure 1. CIP induces differentiation in AML cells. (A) Fluorescence activated cell sorting analysis of CD11b expression levels following the CIP treatment (20 μg/ml) for 96 h. (B) FSC and SSC measured by flow cytometry. (C) THP-1 AML cells were treated with 1 ng/ml PMA in the presence or absence of CIP (20 μg/ml) for 96 h, followed by microscopic observation of adherent and elongated cells. (D) Relative expression of CSF1R, MAFB, ITGAM, CD14, Lyz, MMP9, Myb and Myc measured by reverse transcription-quantitative PCR. *P<0.05; CIP, *Corydalis incisa* (Thumb.) Pers.; AML, acute myeloid leukemia; FSC, forward scatter; SSC, side scatter; PMA, phorbol-12 myristate-13 acetate; CSF1R, colony stimulating factor 1 receptor; MAFB, V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; ITGAM, integrin alpha M; Lyz, lysozyme; Myb, v-myb avian myeloblastosis viral oncogene homolog; con, control.

MAFB, a transcription factor key for early myeloid and monocytic differentiation (48), exhibited increased expression following exposure to CIP. In addition, lysozyme and MMP9, genes associated with differentiated cell functions such as neutrophils and macrophages, showed elevated expression in response to CIP treatment. These results collectively showed that CIP effectively stimulates differentiation in human AML cells (Fig. 1D).

CIP inhibits cell proliferation in AML. HL-60 and U937 AML cells were exposed to CIP (0, 10 or 20 μg/ml), followed by cell counting every 24 h to determine differentiation by CIP would affect proliferation in AML. CIP significantly decreased the cell number in a dose-dependent manner in HL-60 and U937 cells (Fig. 2A). Consistently, CIP decreased viability and increased the apoptosis rates in HL-60 and THP-1 cells (Fig. 2B and C). CIP extracts (2 μg/ml) had an inhibitory effect on the colony

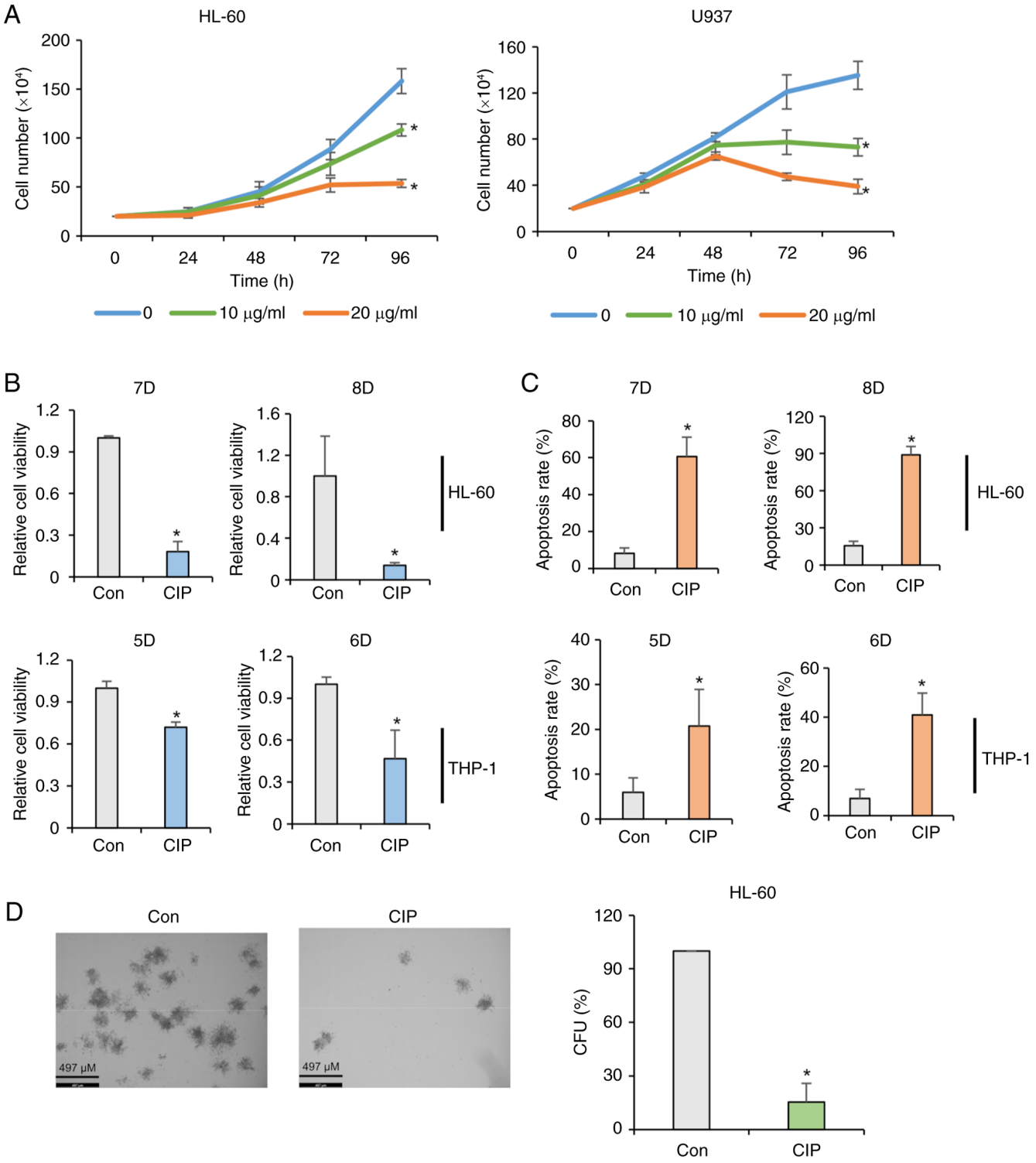

Figure 2. CIP inhibits cell proliferation in acute myeloid leukemia. (A) HL-60 and U937 cells were treated with CIP and counted every 24 h. (B) HL-60 and THP-1 cell viability by MTS assay. (C) Cell apoptosis rate was analyzed by trypan blue exclusion assays. (D) Methylcellulose-based colony-forming assay. *P<0.05; CIP, Corydalis incisa (Thumb.) Pers.; con, control; CFU, colony forming units.

formation in AML (Fig. 2D). In addition, CIP exhibited more toxicity in HL-60 and U937 AML cells than normal bone marrow cells (Fig. S1), suggesting that the cytotoxic effect of this extract may be specific to AML cells with minimal impact on normal cells. These data collectively indicated that CIP extracts have anti-proliferative and pro-apoptotic effects by triggering differentiation in AML cells.

Genome-wide profiling of genes involved in CIP-induced AML differentiation. CIP-resistant cells were generated to understand CIP-induced differentiation and identify potential targets of CIP (49). Human AML cell line HL-60 was treated with DMSO or increasing CIP concentrations (2-20 μg/ml) for 6 months. Following exposure to CIP (20 μg/ml), CD11b expression notably increased in HL-60-DMSO but increased

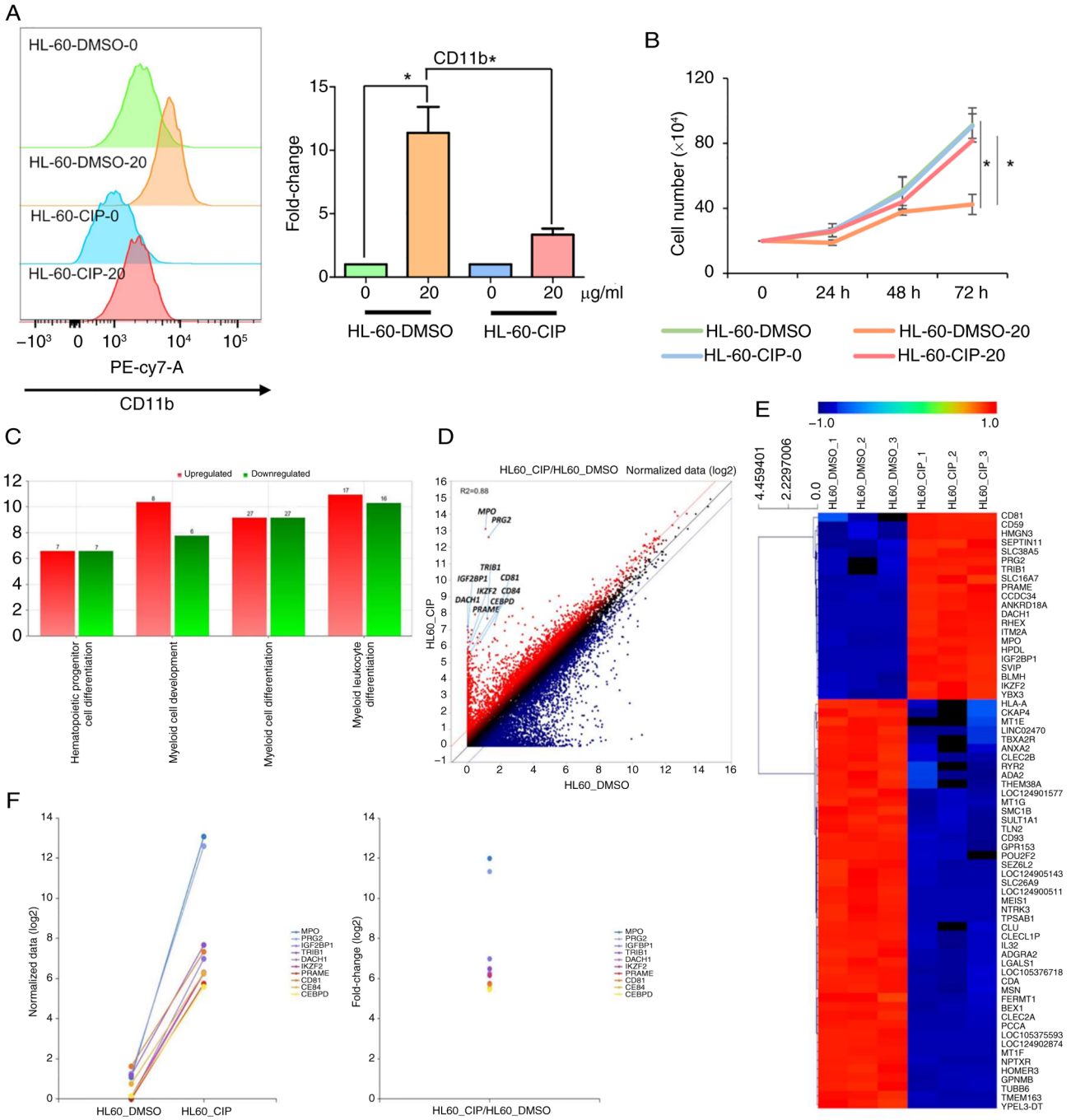

Figure 3. mRNA sequencing of genes involved in CIP-induced acute myeloid leukemia cell differentiation in CIP-resistant cells. (A) HL-60-DMSO and HL-60-CIP cells were treated with CIP (20 μg/ml) for 96 h and flow cytometry was conducted to measure CD11b expression. (B) Cells were treated with DMSO or CIP (20 μg/ml) for 72 h and counted every 24 h. (C) Gene Ontology analysis of HL-60-DMSO and HL-60-CIP cells. (D) Significantly upregulated genes in HL-60-CIP compared with HL-60-DMSO cells. (E) Heatmap from hierarchical clustering of significant genes between HL-60-DMSO and HL-60-CIP cells. (F) Expression of differentially expressed genes between HL-60-DMSO and HL-60-CIP cells. *P<0.05; CIP, *Corydalis incisa* (Thumb.) Pers.

to a lesser extent in HL-60-CIP cells (Fig. 3A and B). HL-60-DMSO cells were sensitive to CIP treatment, leading to a decrease in cell number, while HL-60-CIP cells were resistant to CIP, showing no decrease in cell number. These data showed that HL-60-CIP cells are resistant to CIP-induced differentiation and cell cycle arrest (Fig. 3A and B). Furthermore, when HL-60-CIP cells were cultured in the absence of CIP, they remained resistant to CIP for 3 weeks, suggesting that resistance was achieved through stable genetic alteration (Fig. S2).

The genes involved in CIP-induced AML differentiation were identified at the genome level using Quantseq 3'mRNA-seq using HL-60-DMSO and HL-60-CIP cells. The genes regulated by CIP were enriched in 'hematopoietic progenitor cell differentiation', 'myeloid cell development' and 'myeloid cell differentiation' (Fig. 3C). RNA-seq analysis showed that genes that promote leukemogenesis and inhibit myeloid differentiation, such as myeloperoxidase (50), proteoglycan 2 (51), insulin-like growth factor 2 mRNA-binding protein 1 (52), tribbles homolog 1 (53),

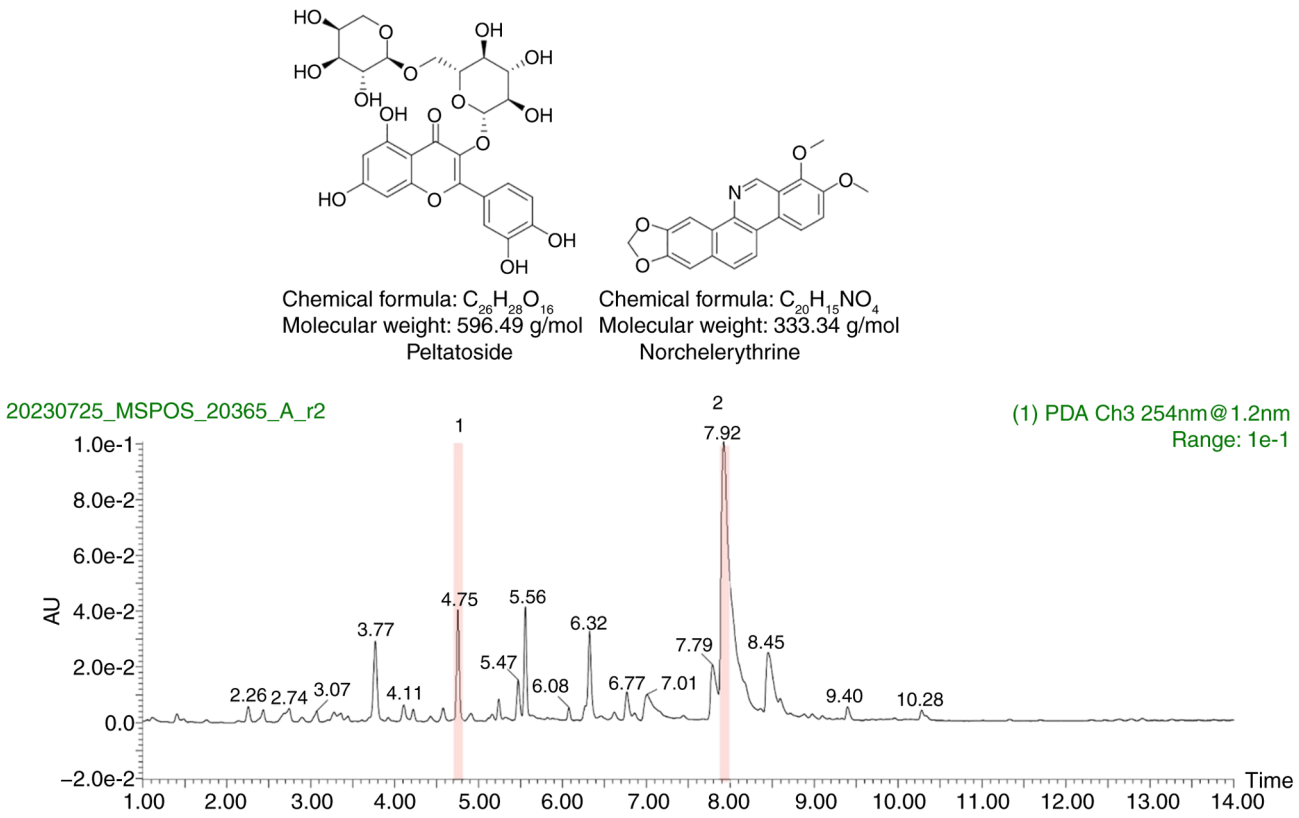

Figure 4. Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry analysis of *Corydalis incisa* (Thumb.) Pers. AU, arbitrary units.

dachshund homolog 1 (54), ikaros family zinc finger 2 (55), preferentially expressed antigen in melanoma (56), *CD81* (57), CCAAT/enhancer binding protein δ (58) and *CD84* (59), were upregulated in CIP-resistant HL-60 cells, validating that HL-60-CIP cells are resistant to differentiation-inducing agents by regulating genes involved in modulating AML differentiation (Fig. 3D-F).

Norchelerythrine in CIP induces differentiation. The compounds in CIP that induce differentiation in AML cells were identified and quantified by UPLC-QTOF-MS on the CIP extract. Two notably peaks were identified and labeled peltatoside and norchelerythrine (Fig. 4). Peltatoside did not have any significant effect on the viability or proliferation of U937 and THP-1 cells (Fig. S3A and B). These findings suggested that peltatoside is not the differentiation-inducing compound present in CIP. Norchelerythrine significantly increased the fluorescence intensity of CD11b and CD14 in HL-60 and U937 cells (Fig. 5A). Furthermore, treatment of HL-60 and U937 with norchelerythrine resulted in significantly decreased cell viability and proliferation in a dose- dependent manner (Fig. 5B and C). Moreover, norchelerythrine decreased the number of viable cells in primary AML cells from patients harboring IDH1, ETS variant transcription factor 6, Lysine methyltransferase 2A (KMT2A) and CCAAT/enhancer binding protein α mutation (Figs. 5D and S4). Myeloid lineage cells distribution was not altered by norchelerythrine, suggesting that norchelerythrine did not have any effect on normal hematopoiesis in mice (Fig. S5).

The effects of *Corydalis* genus plant extract on AML cells were examined based on the hypothesis that plants of the same genus have comparable compound compositions and similar biological effects. *C. speciosa* Maxim. extract increased expression of the CD11b surface marker (Fig. S6A). Similarly to CIP, the extract of *C. speciosa* Maxim. decreased cell viability and increased the rate of apoptosis (Fig. S6B and C). Consistent with these results, norchelerythrine, but not peltatoside, was also observed in the UPLC-QTOF-MS chromatogram of the *C. speciosa* Maxim. extract (Fig. S7). These findings suggest that norchelerythrine may be one of the major compounds in CIP responsible for promoting cell differentiation and inhibiting cell proliferation in AML.

ROS-mediated DNA damage response is involved in norchelerythrine-induced differentiation. ROS influence cellular signaling processes key for cell proliferation and differentiation. In particular, ROS concentration plays a regulatory role in the differentiation of cells during hematopoiesis (60,61). The mechanism of norchelerythrine-induced differentiation was examined using a fluorescent probe (MitoSOX Red) to detect ROS levels in the presence or absence of norchelerythrine. The cells treated with norchelerythrine had higher intracellular ROS levels than those treated with DMSO (Fig. 6A). Previous studies have suggested that alkaloids promote ROS accumulation, leading to DNA damage (62-64). DNA damage in human and murine myeloid leukemia cells is associated with myeloid differentiation and cell cycle arrest (65,66). Therefore, the present study

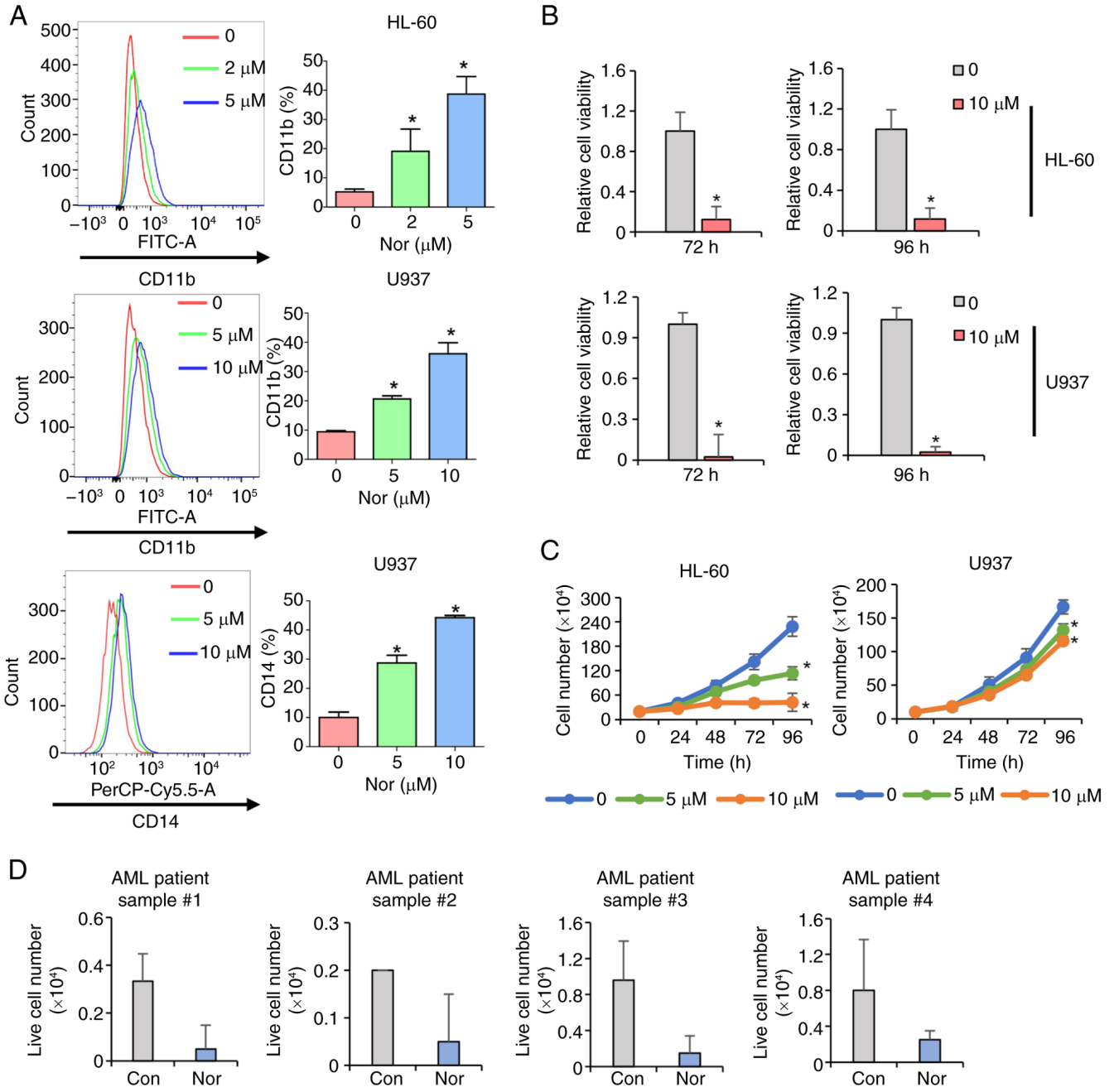

Figure 5. Norchelerythrine inhibits cell viability and proliferation in AML. (A) fluorescence activated cell sorting analysis of myeloid differentiation cell surface markers CD11b and CD14 in DMSO- or norchelerythrine-treated AML cells. (B) HL-60 and U937 cells were treated with 10 μM norchelerythrine. Relative cell viability was measured using MTS assays. (C) Cells were counted every 24 h. (D) Primary samples from patients with AML were treated with 10 μM norchelerythrine for 16 h. Viable cell number was measured. *P<0.05; AML, Acute myeloid leukemia; con, control; Nor, norchelerythrine.

examined whether norchelerythrine activates DNA damage response via ROS production. Norchelerythrine treatment led to increased levels of H2AX phosphorylation at Ser139 (γH2AX), which is a marker of DNA double-strand breaks. In addition, levels of phosphorylated ATM and p21, but not total ATM, increased following exposure to norchelerythrine (Fig. 6B). The ROS scavenger NAC was used to determine if ROS accumulation mediated differentiation of AML cells. NAC treatment reduced the differentiation induced by norchelerythrine significantly (Fig. 6C), suggesting that ROS generated by norchelerythrine were involved in AML differentiation. These findings suggest that ROS and DNA damage response are critical in norchelerythrine-induced differentiation.

Discussion

The primary chemotherapy of AML, the 7 + 3 regimen, has not changed for several decades. The 5-year survival rate is poor, particularly for patients aged >65 years. Patients with APL are successfully treated with differentiation therapy, highlighting the need to develop novel agents for treating AML (67,68). The present study showed that among 100 plant extracts, CIP extracts were the most effective in overcoming differentiation

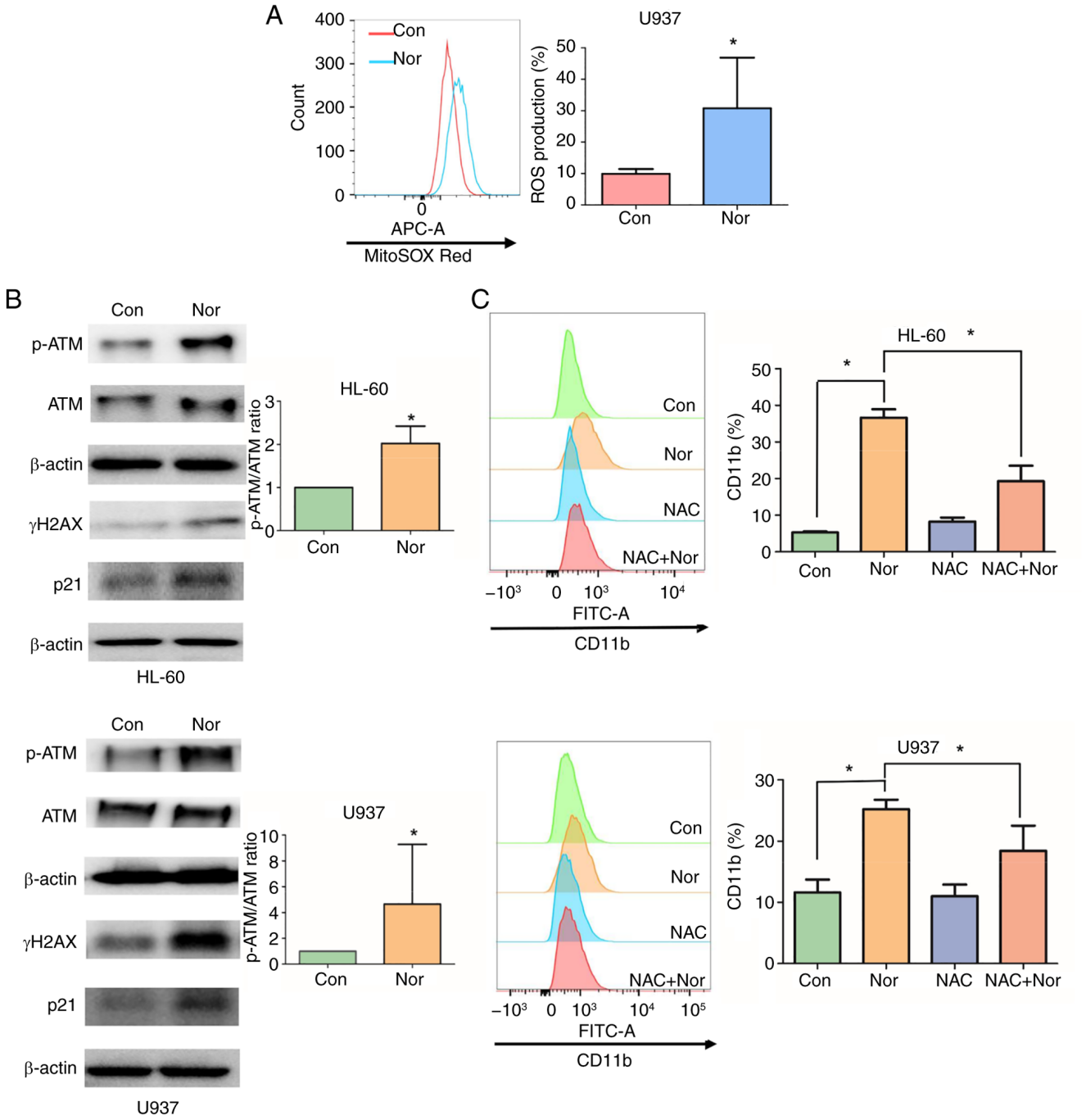

Figure 6. Norchelerythrine induces ROS generation and DNA damage signaling in acute myeloid leukemia cells. (A) ROS levels were assessed by flow cytometry following treatment with DMSO or norchelerythrine for 48 h. (B) p-ATM, ATM, γH2AX, and p21 levels were measured by western blotting. β-actin served as a loading control. The ratio of p-ATM/ATM from three independent experiments was analyzed. (C) Cells were pretreated with 5 mM NAC for 1 h and exposed to DMSO or norchelerythrine for 96 h. CD11b expression was measured by flow cytometry. *P<0.05; ROS, Reactive oxygen species; p-ATM, phosphorylated ataxia telangiectasia-mutated; NAC, N-acetylcysteine; con, control; Nor, norchelerythrine.

arrest in AML. Furthermore, UPLC-QTOF-MS analysis identified norchelerythrine as one of the key compounds in CIP, which exerted anti-leukemic efficacy *in vitro*. The present mechanistic study found that the ROS generated by norchelerythrine were responsible for AML differentiation and the inhibition of cell proliferation through DNA damage response (DDR) activation.

Our previous study demonstrated that CIP exhibits cytotoxicity in diffuse large B cell lymphoma cells (35). To the best of our knowledge, the present study is the first to reveal that CIP has anti-leukemic activity in AML. CIP contains several alkaloids, including corynoline and acetylcorynoline (69-75). To the best of our knowledge, the present study is the first to demonstrate that CIP contains norchelerythrine, a phytochemical with diverse biological activities, such as inhibitory effects against several microorganisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* (76). In addition, norchelerythrine has antifeedant activity against *Tribolium castaneum*, causing damage to stored grain

products (77). Studies have assessed its cytotoxic effects on human hematoma, cervical carcinoma and gastric cancer and murine lymphocytic leukemia cells *in vitro* (78-80). However, the impact of norchelerythrine on AML has been unexplored. To the best of our knowledge, the present study is the first to reveal the anti-AML activity of norchelerythrine and explore the underlying mechanisms.

Cells respond differently to ROS and DDR; leukemic cells differentiate but hematopoietic stem cells exit quiescence and differentiate (81). The hypothesis that ROS serves a pivotal role in norchelerythrine-mediated AML differentiation is supported by direct FACS measurements showing increased ROS levels and inhibition of these effects by NAC, a ROS scavenger. These data suggest that norchelerythrine engages the tumor-suppressive signaling pathway shared by chemotherapeutic drugs, such as cisplatin and doxorubicin.

Norchelerythrine effectively induces apoptosis in samples from patients with AML, including samples with myelodysplasia-related changes (MRCs). AML-MRCs include patients with ≥20% of blasts, prior history of myelodysplastic syndrome (MDS) or MDS/myeloproliferative neoplasm, a cytogenetic abnormality related to MDS and multilineage dysplasia. AML-MRC account for up to 48% of all adult AML cases and mainly affects elderly patients, showing a poor prognosis with lower remission rates and shorter overall survival time compared with other AML subtypes (82,83). Here, norchelerythrine decreased the number of viable cells in two samples from patients with AML-MRC, suggesting it may be an effective treatment for these patients.

The present study did not identify the direct target of norchelerythrine. Quantseq 3' mRNA-seq was conducted in CIP-resistant AML cells. Upregulated genes such as ANKRD18A, ITM2A, and BLMH may be involved in inhibiting differentiation and could be potential targets of CIP and norchelerythrine. The observation that several genes upregulated in CIP-resistant cells have no reported association with myeloid differentiation provides novel avenues for research in AML. Further studies are needed to understand their functions and potential impacts on AML.

The present results suggested that norchelerythrine exhibits anti-leukemic effects by inducing myeloid differentiation, decreasing cell viability and causing cell cycle arrest. Norchelerythrine inhibits cell proliferation in samples from patients with AML harboring various mutations. Norchelerythrine mechanistically activated the DDR by generating ROS. However, the present study was limited to *in vitro* experiments and lacks supporting *in vivo* evidence, leaving the anti-tumor activity and toxicity of norchelerythrine *in vivo* unclear. Further *in vivo* studies in leukemic mouse models will be required to evaluate the preclinical therapeutic potential of norchelerythrine in AML.

In summary, the present study showed that CIP and norchelerythrine exhibited anti-leukemic effects by generating ROS, leading to activation of DDR and the subsequent induction of terminal differentiation in AML cells. Based on these findings, CIP and norchelerythrine hold promise as novel therapeutic candidates for treating AML. In addition, considering that differentiation therapy with ATRA and other agents is being evaluated in various types of cancer, including hepatocellular carcinoma (25,84), further research is warranted to explore their full therapeutic potential and mechanisms of action in clinical settings.

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Availability of data and materials

The data generated in the present study may be found in the Gene Expression Omnibus under accession number GSE280425 or at the following URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280425).

Authors' contributions

JL designed and performed the experiments and wrote the manuscript. BJ and CK performed the experiments. JL and SWK confirm the authenticity of all the raw data. HK, JL, BJ, CK, TJK, YS, SHL, HJS and SWK analyzed the data. TJK, SHL, SWK and HJS supervised the study. YS revised the manuscript. SWK designed and conceived the study and wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of Pusan National University Hospital (approval no. 2403-010-137). Written informed consent was obtained from all participants included in the study. The animal protocol was reviewed and approved by the Pusan National University-Institutional Animal Care and Use Committee (approval no. PNU 2022-0239).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript. Subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

1. Estey E and Döhner H: Acute myeloid leukaemia. Lancet 368: 1894-1907, 2006.
2. Pollyea DA, Kohrt HE and Medeiros BC: Acute myeloid leukaemia in the elderly: A review. Br J Haematol 152: 524-542, 2011.
3. Saultz JN and Garzon R: Acute myeloid leukemia: A concise review. J Clin Med 5: 33, 2016.
4. Döhner H, Weisdorf DJ and Bloomfield CD: Acute myeloid leukemia. N Engl J Med 373: 1136-1152, 2015.
5. Park HJ and Gregory MA: Acute myeloid leukemia in elderly patients: New targets, new therapies. Aging Cancer 4: 51-73, 2023.
6. Robak T and Wierzbowska A: Current and emerging therapies for acute myeloid leukemia. Clin Ther 31: 2349-2370, 2009.
7. Newell LF and Cook RJ: Advances in acute myeloid leukemia. BMJ 375: n2026, 2021.
8. Kantarjian H, Kadia T, DiNardo C, Daver N, Borthakur G, Jabbour E, Garcia-Manero G, Konopleva M and Ravandi F: Acute myeloid leukemia: current progress and future directions. Blood Cancer J 11: 41, 2021.
9. Saygin C and Carraway HE: Emerging therapies for acute myeloid leukemia. J Hematol Oncol 10: 93, 2017.
10. Parisi E, Draznin J, Stoopler E, Schuster SJ, Porter D and Sollecito TP: Acute myelogenous leukemia: Advances and limitations of treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 93: 257-263, 2002.
11. Bhansali RS, Pratz KW and Lai C: Recent advances in targeted therapies in acute myeloid leukemia. J Hematol Oncol 16: 29, 2023.
12. Short NJ, Konopleva M, Kadia TM, Borthakur G, Ravandi F, DiNardo CD and Daver N: Advances in the treatment of acute myeloid leukemia: New drugs and new challenges. Cancer Discov 10: 506-525, 2020.
13. Kadia T, Ravandi F, Cortes J and Kantarjian H: New drugs in acute myeloid leukemia. Ann Oncol 27: 770-778, 2016.
14. van Dijk AD, de Bont ESJ and Kornblau SM: Targeted therapy in acute myeloid leukemia: Current status and new insights from a proteomic perspective. Expert Rev Proteomics 17: 1-10, 2020.
15. Godwin C, Gale R and Walter R: Gemtuzumab ozogamicin in acute myeloid leukemia. Leukemia 31: 1855-1868, 2017.
16. Ehninger A, Kramer M, Röllig C, Thiede C, Bornhäuser M, von Bonin M, Wermke M, Feldmann A, Bachmann M, Ehninger G and Oelschlägel U: Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. Blood Cancer J 4: e218, 2014.
17. Tabata R, Chi S, Yuda J and Minami Y: Emerging immunotherapy for acute myeloid leukemia. Int J Mol Sci 22: 1944, 2021.
18. Taussig DC, Pearce DJ, Simpson C, Rohatiner AZ, Lister TA, Kelly G, Luongo JL, Danet-Desnoyers GA and Bonnet D: Hematopoietic stem cells express multiple myeloid markers: Implications for the origin and targeted therapy of acute myeloid leukemia. Blood 106: 4086-4092, 2005.
19. Walter RB, Appelbaum FR, Estey EH and Bernstein ID: Acute myeloid leukemia stem cells and CD33-targeted immunotherapy. Blood 119: 6198-6208, 2012.
20. Damle NK and Frost P: Antibody-targeted chemotherapy with immunoconjugates of calicheamicin. Curr Opin Pharmacol 3: 386-390, 2003.
21. Castelli G, Pelosi E and Testa U: Targeted therapies in the treatment of adult acute myeloid leukemias: Current status and future perspectives. Int J Hematol Oncol 5: 143-164, 2016.
22. Yan M and Liu Q: Differentiation therapy: A promising strategy for cancer treatment. Chin J Cancer 35: 3, 2016.
23. Madan V and Koeffler HP: Differentiation therapy of myeloid leukemia: Four decades of development. Haematologica 106: 26-38, 2021.
24. Stubbins RJ and Karsan A: Differentiation therapy for myeloid malignancies: Beyond cytotoxicity. Blood Cancer J 11: 193, 2021.
25. de Thé H: Differentiation therapy revisited. Nat Rev Cancer 18: 117-127, 2018.
26. Veiga M, Costa EM, Silva S and Pintado M: Impact of plant extracts upon human health: A review. Crit Rev Food Sci Nutr 60: 873-886, 2020.
27. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG and Lightfoot DA: Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel) 6: 42, 2017.
28. Dixit S and Ali H: Anticancer activity of medicinal plant extract-A review. J Chem Cheml Sci 1: 79-85, 2010.
29. Li W, Huang H, Zhang Y, Fan T, Liu X, Xing W and Niu X: Anti-inflammatory effect of tetrahydrocoptisine from *Corydalis* impatiens is a function of possible inhibition of TNF-α, IL-6 and NO production in lipopolysaccharide-stimulated peritoneal macrophages through inhibiting NF-κB activation and MAPK pathway. Eur J Pharmacol 715: 62-71, 2013.
30. Yang C, Zhang C, Wang Z, Tang Z, Kuang H and Kong ANT: Corynoline isolated from *Corydalis* bungeana Turcz. exhibits anti-inflammatory effects via modulation of Nfr2 and MAPKs. Molecules 21: 975, 2016.
31. Zheng J, Zhao Y, Lun Q, Song Y, Shi S, Gu X, Pan B, Qu C, Li J and Tu P: *Corydalis* edulis Maxim. Promotes insulin secretion via the activation of protein kinase Cs (PKCs) in mice and pancreatic β cells. Sci Rep 7: 40454, 2017.
32. Xu Z, Chen X, Zhang Q, Chen L and Wang Y: *Corydalis* yanhusuo W.T. Wang extract inhibits MCF-7 cell proliferation by inducing cell cycle G2/M arrest. Am J Chin Med 39: 579-586, 2011.
33. Oh MT, Eom HS and Chi GY: Antiproliferative effect and apoptotic mechanism of extract of *Corydalis* yanhusuo on human hepatocarcinoma cells. J Physiol Pathol Korean Med 21: 1437-1449, 2007.
34. Lu JJ, Bao JL, Chen XP, Huang M and Wang YT: Alkaloids isolated from natural herbs as the anticancer agents. Evid Based Complement Alternat Med 2012: 485042, 2012.
35. Habli Z, Toumieh G, Fatfat M, Rahal ON and Gali-Muhtasib H: Emerging cytotoxic alkaloids in the battle against cancer: Overview of molecular mechanisms. Molecules 22: 250, 2017.
36. Spirin P, Shyrokova E, Lebedev T, Vagapova E, Smirnova P, Kantemirov A, Dyshlovoy SA, Amsberg GV, Zhidkov M and Prassolov V: Cytotoxic marine alkaloid 3,10-dibromofascaplysin induces apoptosis and synergizes with cytarabine resulting in leukemia cell death. Mar Drugs 19: 489, 2021.
37. Wang XD, Li CY, Jiang MM, Li D, Wen P, Song X, Chen JD, Guo LX, Hu XP, Li GQ, *et al*: Induction of apoptosis in human leukemia cells through an intrinsic pathway by cathachunine, a unique alkaloid isolated from Catharanthus roseus. Phytomedicine 23: 641-653, 2016.
38. Silva SLR, Dias IRSB, Rodrigues ACBDC, Costa RGA, Oliveira MS, Barbosa GADC, Soares MBP, Dias RB, Valverde LF, Rocha CAG, *et al*: Emetine induces oxidative stress, cell differentiation and NF-κB inhibition, suppressing AML stem/progenitor cells. Cell Death Discov 10: 201, 2024.
39. Gupta K, Chakrabarti A, Rana S, Ramdeo R, Roth BL, Agarwal ML, Tse W, Agarwal MK and Wald DN: Securinine, a myeloid differentiation agent with therapeutic potential for AML. PLoS One 6: e21203, 2011.
40. Wu G, Liu T, Li H, Li Y, Li D and Li W: c-MYC and reactive oxygen species play roles in tetrandrine-induced leukemia differentiation. Cell Death Dis 9: 473, 2018.
41. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
42. Woo YR, Kwon CS, Lee JE, Jeon BE, Kim TJ, Choo J, Seo YS and Kim SW: Ajania pacifica (Nakai) K. bremer and humphries extract limits MYC expression to induce apoptosis in diffuse large B cell lymphoma. Curr Issues Mol Biol 46: 4580-4594, 2024.
43. Kwon CS, Lee JE, Jeon BE, Woo YR, Kim YS, Kim JW, Park CJ, Jang SY and Kim SW: Anti-leukemic effects of Idesia polycarpa Maxim branch on human B-cell acute lymphoblastic leukemia cells. Curr Issues Mol Biol 45: 4035-4049, 2023.
44. Lee JE, Kwon CS, Jeon BE, Kim WR, Lee DH, Koh S, Kim HS and Kim SW: Genome-wide gene expression profiling defines the mechanism of anticancer effect of colorectal cancer cell-derived conditioned medium on acute myeloid leukemia. Genes (Basel) 13: 883, 2022.
45. Jeon BE, Kwon CS, Lee JE, Moon K, Cha J, Park I, Koh S, Yoon M, Kim SW and Kim JN: Anticancer activity of continentalic acid in B-cell lymphoma. Molecules 26: 6845, 2021.
46. Vidriales MB, Orfao A, López-Berges MC, González M, López-Macedo A, García MA, Galende J and San Miguel JF: Light scatter characteristics of blast cells in acute myeloid leukaemia: Association with morphology and immunophenotype. J Clin Pathol 48: 456-462, 1995.
47. Mol BA, Wasinda JJ, Xu YF, Gentle NL and Meyer V: 1,25-Dihydroxyvitamin D_3 augments low-dose PMA-based monocyte-to-macrophage differentiation in THP-1 cells. J Immunol Methods 532: 113716, 2024.

48. Kelly LM, Englmeier U, Lafon I, Sieweke MH and Graf T: MafB is an inducer of monocytic differentiation. EMBO J 19: 1987-1997, 2000.
49. Sykes DB, Kfoury YS, Mercier FE, Wawer MJ, Law JM, Haynes MK, Lewis TA, Schajnovitz A, Jain E, Lee D, *et al*: Inhibition of dihydroorotate dehydrogenase overcomes differentiation blockade in acute myeloid leukemia. Cell 167: 171-186. e15, 2016.
50. Hosseini M, Rezvani HR, Aroua N, Bosc C, Farge T, Saland E, Guyonnet-Dupérat V, Zaghdoudi S, Jarrou L, Larrue C, *et al*: Targeting myeloperoxidase disrupts mitochondrial redox balance and overcomes cytarabine resistance in human acute myeloid leukemia. Cancer Res 79: 5191-5203, 2019.
51. Liu Q and Dong F: Gfi-1 inhibits the expression of eosinophil major basic protein (MBP) during G-CSF-induced neutrophilic differentiation. Int J Hematol 95: 640-647, 2012.
52. Zhou J, Bi C, Ching YQ, Chooi JY, Lu X, Quah JY, Toh SH, Chan ZL, Tan TZ, Chong PS and Chng WJ: Inhibition of LIN28B impairs leukemia cell growth and metabolism in acute myeloid leukemia. J Hematol Oncol 10: 138, 2017.
53. Yoshino S, Yokoyama T, Sunami Y, Takahara T, Nakamura A, Yamazaki Y, Tsutsumi S, Aburatani H and Nakamura T: Trib1 promotes acute myeloid leukemia progression by modulating the transcriptional programs of Hoxa9. Blood 137: 75-88, 2021.
54. Lee JW, Kim HS, Kim S, Hwang J, Kim YH, Lim GY, Sohn WJ, Yoon SR, Kim JY, Park TS, *et al*: DACH1 regulates cell cycle progression of myeloid cells through the control of cyclin D, Cdk 4/6 and p21Cip1. Biochem Biophys Res Commun 420: 91-95, 2012.
55. Park SM, Cho H, Thornton AM, Barlowe TS, Chou T, Chhangawala S, Fairchild L, Taggart J, Chow A, Schurer A, *et al*: IKZF2 drives leukemia stem cell self-renewal and inhibits myeloid differentiation. Cell Stem Cell 24: 153-165.e7, 2019.
56. Kirkey DC, Loeb AM, Castro S, McKay CN, Perkins L, Pardo L, Leonti AR, Tang TT, Loken MR, Brodersen LE, *et al*: Therapeutic targeting of PRAME with mTCRCAR T cells in acute myeloid leukemia. Blood Adv 7: 1178-1189, 2023.
57. Boyer T, Guihard S, Roumier C, Peyrouze P, Gonzales F, Berthon C, Quesnel B, Preudhomme C, Behal H, Duhamel A, *et al*: Tetraspanin CD81 is an adverse prognostic marker in acute myeloid leukemia. Oncotarget 7: 62377-62385, 2016.
58. Prajapati S, Meydan C, Dillon R, Dunham N, Fan H, Gandara JA, Lee T, Neelamraju Y, Sheridan C, Wang Z, *et al*: Loss of CCAAT-enhancer binding protein delta promotes acute myeloid leukemia cell proliferation and survival by upregulating cyclin D1 expression. Blood 142 (Suppl 1): S1380, 2023.
59. Zhu Y, Park M, Murtadha M, Caserta E, Nguyen LXT, Singer M, Estepa MD, Nigam L, Dona' AA, Sanchez JF, *et al*: CD84 is a therapeutically targetable driver of leukemogenesis via disruption of energy supply in acute myeloid leukemia. Blood 140 (Suppl 1): S89-S90, 2022.
60. Sauer H, Wartenberg M and Hescheler J: Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 11: 173-186, 2001.
61. Prieto-Bermejo R, Romo-González M, Pérez-Fernández A, Ijurko C and Hernández-Hernández Á: Reactive oxygen species in haematopoiesis: Leukaemic cells take a walk on the wild side. J Exp Clin Cancer Res 37: 125, 2018.
62. Murata T, Kohno S, Ogawa K, Ito C, Itoigawa M, Ito M, Hikita K and Kaneda N: Cytotoxic activity of dimeric acridone alkaloids derived from Citrus plants towards human leukaemia HL-60 cells. J Pharm Pharmacol 72: 1445-1457, 2020.
63. Long Q, Xiao X, Yi P, Liu Y, Varier KM, Rao Q, Song J, Qiu J, Wang C, Liu W, *et al*: L20, a Calothrixin B analog, induces intrinsic apoptosis on HEL cells through ROS/γ-H2AX/p38 MAPK pathway. Biomed Pharmacother 137: 111336, 2021.
64. Alhuthali HM, Bradshaw TD, Lim KH, Kam TS and Seedhouse CH: The natural alkaloid Jerantinine B has activity in acute myeloid leukemia cells through a mechanism involving c-Jun. BMC Cancer 20: 629, 2020.
65. Santos MA, Faryabi RB, Ergen AV, Day AM, Malhowski A, Canela A, Onozawa M, Lee JE, Callen E, Gutierrez-Martinez P, *et al*: DNA-damage-induced differentiation of leukaemic cells as an anti-cancer barrier. Nature 514: 107-111, 2014.
66. Nicolae CM, O'Connor MJ, Constantin D and Moldovan GL: NFκB regulates p21 expression and controls DNA damage-induced leukemic differentiation. Oncogene 37: 3647-3656, 2018.
67. De Kouchkovsky I and Abdul-Hay M: 'Acute myeloid leukemia: A comprehensive review and 2016 update'. Blood Cancer J 6: e441, 2016.
68. Johnson DE and Redner RL: An ATRActive future for differentiation therapy in AML. Blood Rev 29: 263-268, 2015.
69. Zhang Ying ZY, Qin MinJian QM and Xie GuoYong XG: Analysis of alkaloid compositions from *Corydalis incise*, 2008. https://www.cabidigitallibrary.org/doi/full/10.5555/20093000856
70. Manske RHF: The alkaloids of fumariaceous plants. XLIV. *Corydalis incisa* (Thunb.) Pers. and the constitutions of adlumidine and capnoidine. J Am Chem Soc 72: 3207-3208, 1950.
71. Kametani T, Ihara M and Honda T: Morphinandienone alkaloids from *Corydalis incisa*. Phytochemistry 10: 1881-1883, 1971.
72. Nonaka G and Nishioka I: Alkaloids of *Corydalis incisa* PERS. V. The structures of corydalispirone and corydalisol. Chem Pharm Bull 23: 294-298, 1975.
73. Nonaka G and Nishioka I: Alkaloids of *Corydalis incisa* PERS. III. The structures of corydamine hydrochloride and N-formyl corydamine. Chem Pharm Bull 21: 1410-1414, 1973.
74. Nonaka G and Nishioka I: Alkaloids of *Corydalis incisa* PERS. VI. The structures of benzo [c] phenanthridine-type alkaloids, 12-hydroxycorynoline and 11-epicorynoline. Chem Pharm Bull 23: 521-526, 1975.
75. Sulaiman M, Jannat K, Nissapatorn V, Rahmatullah M, Paul AK, de Lourdes Pereira M, Rajagopal M, Suleiman M, Butler MS, Break MKB, *et al*: Antibacterial and antifungal alkaloids from Asian angiosperms: Distribution, mechanisms of action, structure-activity, and clinical potentials. Antibiotics (Basel) 11: 1146, 2022.
76. Luo X, Pedro L, Milic V, Mulhovo S, Duarte A, Duarte N and Ferreira MJ: Antibacterial benzofuran neolignans and benzophenanthridine alkaloids from the roots of Zanthoxylum capense. Planta Med 78: 148-153, 2012.
77. Wang CF, You CX, Yang K, Guo SS, Geng ZF, Fan L, Du SS, Deng ZW and Wang YY: Antifeedant activities of methanol extracts of four Zanthoxylum species and benzophenanthridines from stem bark of Zanthoxylum schinifolium against *Tribolium castaneum*. Ind Crops Prod 74: 407-411, 2015.
78. Pang SQ, Wang GQ, Lin JS, Diao Y and Xu RA: Cytotoxic activity of the alkaloids from Broussonetia papyrifera fruits. Pharm Biol 52: 1315-1319, 2014.
79. Chang YC, Chang FR, Khalil AT, Hsieh PW and Wu YC: Cytotoxic benzophenanthridine and benzylisoquinoline alkaloids from Argemone mexicana. Z Naturforsch C J Biosci 58: 521-526, 2003.
80. Chen JJ, Fang HY, Duh CY and Chen IS: New indolopyridoquinazoline, benzo[c]phenanthridines and cytotoxic constituents from Zanthoxylum integrifoliolum. Planta Med 71: 470-475, 2005.
81. Weiss CN and Ito K: DNA damage: A sensible mediator of the differentiation decision in hematopoietic stem cells and in leukemia. Int J Mol Sci 16: 6183-6201, 2015.
82. Baer C, Walter W, Stengel A, Hutter S, Meggendorfer M, Kern W, Haferlach C and Haferlach T: Molecular classification of AML-MRC reveals a distinct profile and identifies MRC-like patients with poor overall survival. Blood 134 (Suppl 1): S2735, 2019.
83. Arber DA and Erba HP: Diagnosis and treatment of patients with acute myeloid leukemia with myelodysplasia-related changes (AML-MRC). Am J Clin Pathol 154: 731-741, 2020.
84. Zheng S, Bian H, Li J, Shen Y, Yang Y and Hu W: Differentiation therapy: Unlocking phenotypic plasticity of hepatocellular carcinoma. Crit Rev Oncol Hematol 180: 103854, 2022.