Research article

Anti-cancer effects of ginsenoside CK on acute myeloid leukemia in vitro and in vivo

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ABSTRACT

Objectives: Acute myeloid leukemia (AML) is a malignant disease characterized by clonal proliferation of myeloid cells, and its treatment continues to be a challenge due to high morbidity and mortality. Ginsenoside compound K, a major active metabolite of the protopanaxadiol-type ginsenosides, exhibits biological activities in various cancer cells and animal models. Here, we investigated the role of CK in anticancer potential in AML both in vitro and in vivo.

Materials and methods: To investigate the inhibitory effects of CK in AML cells, in vitro experiments, including cell viability assays, colony forming assays, and cell cycle and apoptosis assays were performed. AML animal experiment was established and quantitative analysis of lung tumor growth nodules and spleen weight and H&E staining were carried out to further determine the effects of CK on AML. In addition, the potential key genes induced and influenced by CK during treatment was identification by RNA-seq and qRT-PCR.

Results: CK suppressed AML cell activity and induced apoptosis and G1 cell cycle arrest based on the experiment results. Moreover, significantly down-regulated expression genes of BCL2, KIT, DNMT3A, MYC and CSF-1 and up-regulated expression gene of TET2 in CK treatment AML cells were discovered.

Conclusion: Our results demonstrated that CK could be used as an anti-AML drug with significant therapeutic efficacy and good biosafety.

1. Introduction

Acute myeloid leukemia (AML) is a malignant disease characterized by clonal proliferation of myeloid cells [1, 2, 3]. In recent years, treatment of AML remains a challenge due to the significant disease incidence and mortality problems [4]. According to the American Cancer Society's 2022 Forecast Report, it is estimated that in 2022, the number of deaths due to cancer will reach 609,360, of which represents 24,000 patients will die of leukemia, and 11,540 (48%) will die of AML [5], AML become one of the most severe and heterogeneous blood cancers and is more frequent happened in adults [6].

Natural drugs are natural products with pharmacological activity that have various biological activities such as anti-cancer and anti-inflammatory [7]. Although a series of natural antitumor drugs have been used for clinical research and application, some of them are limited

by their serious side effects. Ginseng, a long-standing perennial herb belongs to the Araliaceae family, is mainly distributed in East Asia and North America [8]. A "drug-weary" population consuming increasingly toxic compounds has begun to find alternative medicines and conventional drug forms, leading to a growing in interest in studying the active ingredients of ginseng as a cancer treatment. Moreover, ginsenoside is the main and effective active ingredient in the anti-tumor effect of ginseng [9, 10, 11].

Ginsenosides are classified into dammarane type and oleanane type according to the glycoside structure difference, of which dammarane type is the most common type of ginsenoside [12]. CK, a non-natural ginsenoside belonging to the tetracyclic triterpene dammarane type, is the main metabolite of protopanaxadiol saponins in intestinal bacteria. It has been reported that CK has a protective effect on hepatocytes [13] and induces apoptosis of various cancer cells through different targets or

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signaling pathways [14, 15]. However, its therapeutic effect in AML is rarely reported.

Here, we investigated the inhibitory ability of CK as an anti-tumor agent against leukemia proliferation in vivo and in vitro. Therefore, our results revealed that CK is a low-toxicity anti-AML drug with significant therapeutic efficacy and good biosafety.

2. Materials and methods

2.1. Cells lines and culture

U937, THP-1, MV4-11 and C1498 were procured from American Type Culture Collection (Manassas, VA, USA). MOLM-13 and NB4 cell lines were obtained from FuHeng BioLogy (Shanghai, China) and MOLM14 came from Yaji BioLogical (Shanghai, China). LO-2 was obtained from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). All cells were cultured in the appropriate growth medium containing 10% fetal bovine serum (FBS: Life Technology, Shanghai, China), penicillin (100 unit/mL, Life Technology, Shanghai, China) and streptomycin (100 μg/mL, Life Technology, Shanghai, China) at 37 °C in an incubator with 5% CO_2.

2.2. Cell viability assay

Cell activity of CK (Shanghai yuanye Bio-Technology, Shanghai, China) was evaluated according to the cell counting kit-8 (CCK-8; BioSS, Beijing, China). Finally, the absorbance (abs) at 450 nm was recorded on a microplate reader (Thermo Scientific, Waltham, USA). The experiment was repeated three times.

2.3. Colony forming assays

Colony forming assay was carried out using the MethoCult mixture (Stem Cell Technologies, Vancouver, Canada). Colony counts were completed within 9–14 and photographed.

2.4. Cell cycle and apoptosis assays

The cell cycle and apoptosis analysis kit (Bestbio, Shanghai, China) is used to perform cell cycle assay. The cells were gently washed two times using pre-cooled PBS and then fixed with 70% ethanol in PBS at -20 °C for 12 h. Subsequently simple centrifugation, the cells were continued to be washed using pre-cooled PBS. Cells were resuspended with 200–500 μL of cold PBS, add 20 μL of RNase A solution, and 30 min in a 37 °C water bath. Then, discard the supernatant and add 400 μL PI staining solution. The mix was gently mixed and incubated at 4 °C for 1 h in dark. Using Annexin V-FITC/PI Apoptosis Detection Kit (BestBio, Shanghai, China) according to the manufacturer's instruction to detect cell apoptosis assays. The final analysis was carried out using a BD LSR flow cytometer (BD Biosciences, Shanghai, China). The experiment was repeated three times.

2.5. RNA-seq

RNA-seq was carried out according to previous reports [16]. In short, total RNA samples were extracted with miRNeasy Kit (PR1202, BIO-TEKE, Beijing, China) in CK treated for 48 h or untreated MOLM13 cells. Libraries were built on the Illumina Hiseq system. Differential gene expression was analyzed according to the standard Illumina sequence analysis pipeline.

2.6. qRT-PCR

Total RNA was extracted from MOLM13, MOLM14, THP-1 and U937 cells using miRNAeasy Kit (PR1202, BIOTEKE, Beijing, China), and the Super RT Kit First-Strand Synthesis System (PR6601, BIOTEKE, Beijing,

China) was used to synthesize cDNA. The qRT-PCR was implemented with SYBR-Green master (4309155 Applied Biosystems, Shanghai, China) mix on an ABI Prism 7000 sequence detection system (Applied Biosystems, Shanghai, China). Relative gene expression levels were quantified using $2^{-\triangle\triangle CT}$ method [17]. Supplementary Table S7 is the sequence of gene specific primers.

2.7. Animal experiments

Animal study was performed according to previous reports [18, 19]. C57BL/6N mice (male, 4–6 weeks old) were purchased from Beijing Charles River Company. All animals were managed as per the Guide for the Care and Use of Laboratory Animals. All animal experiments were approved by the Animal Care and Use Committee of Changchun University of Chinese Medicine. Briefly, cells were seeded in 6-well plates for 24 h before transfection. FLT3 plasmids were transferred into cells by using Lipofectamine™ 2000 Reagent (Life Technologies, Carlsbad, CA). Two leukemia models were successfully established on the seventh day after 0.5×10^6 C1498 cells and FLT3+ cells were injected into C57BL/6N mice through the tail vein. The mice used in the experiment were inbred and immunocompetent. The cell lines used for injection are from the same inbred mice, and the bone marrow cells were transfected with the FLT3 plasmid (n = 8). Leukemia onset was confirmed based on white blood cell (WBC) counts, and the mice were intraperitoneally injected with 1 mg/kg or 5 mg/kg of CK every 2 days for 30 consecutive days. For the WBC count, 2 μL of blood was taken from the tail vein of mice, and mixed with 38 μL of Turk blood diluent (Ricca Chemical), then 10 μL mixture was taken out for WBCs counting under a microscope. At the end of the experiment, mice were euthanized, the spleen weight and the number of lung metastatic nodules were measured, then subsequently hematoxylin and eosin staining (H&E) experiments were performed.

2.8. Histopathological staining

The histopathological staining was described previously [20]. In simple terms, the collected spleen and lungs were immersed in 10% neutral buffer formalin and then a paraffin tissue embedding experiment was performed. Cut into 5 μM thin slices and stain with hematoxylin (Beyotime Biotechnology, Shanghai, China) and eosin (Beyotime Biotechnology, Shanghai, China). The stained slides were observed and photographed with a Nikon confocal fluorescence microscope and analyzed with Image-Pro Plus software.

2.9. Statistical analysis

In vitro quantitative experiments were carried out at least three replicates and independent experiments, and expressed as mean ± standard deviation (SD). The statistical analysis was carried out using student's t-test. Survival curves were estimated using the Kaplan-Meier method, and the Log-rank test was carried out to assess significant differences between survival curves. All analyses were conducted using GraphPad Prism 7 software. P < 0.05 was considered on statistically significant.

3. Results

3.1. In vitro cytotoxicity and anti-proliferation effect of CK

To elucidate the inhibitory effect of CK on AML cells, using 6 human leukemia cell lines (MOLM13, MOLM14, U937, THP-1, NB4, MV4-11). The chemical structure of CK has been shown in Figure 1a [21]. Six human leukemia cell lines were treated with CK respectively, to detect the cytotoxicity of CK on these cells using CCK-8 assay. The results showed that CK inhibited AML cell viability (Figure 1b). The results showed that CK inhibited AML cell viability (Figure 1b). In addition, the effect of the CK on normal cells LO2 was tested, the results in Figure 1b
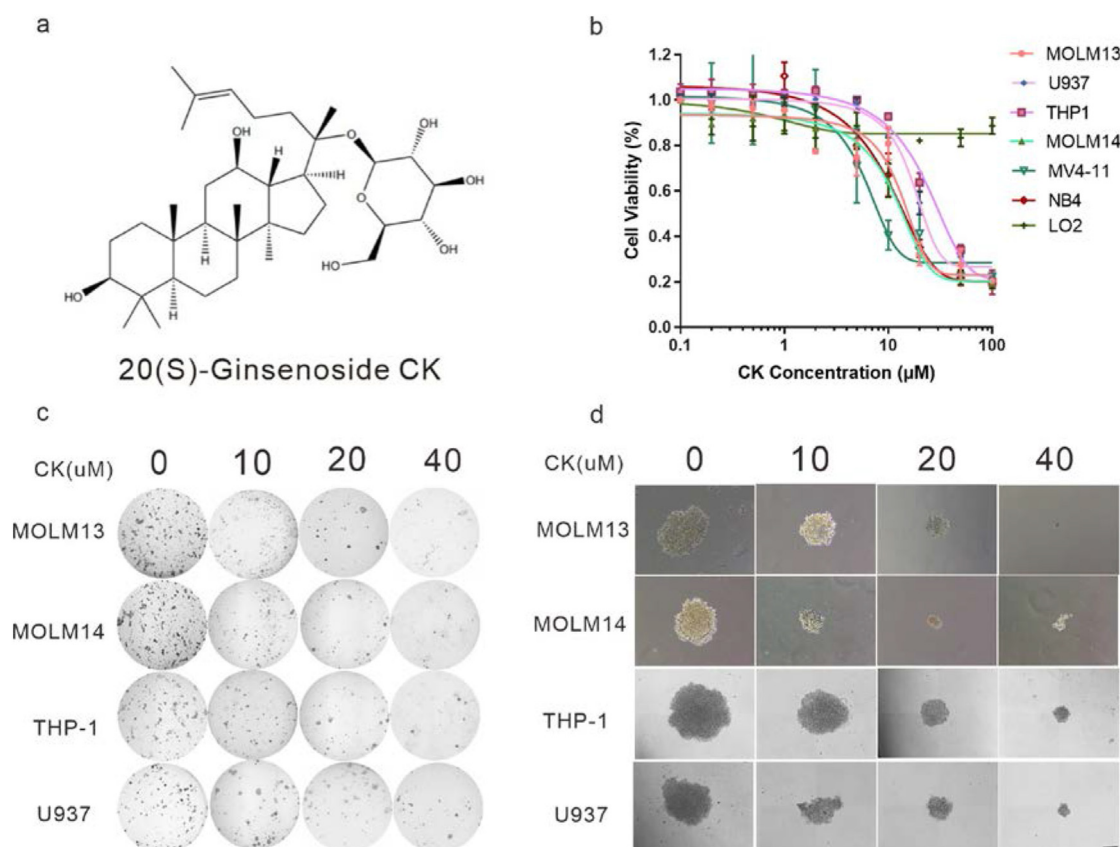
Figure 1. CK exhibits anti-proliferation activity. (a) The chemical structure of CK. (b) CCK-8 assay after 48 h of treatment with a series of concentrations of CK in AML cells and LO2 cell. (c) Cell colony formation assay of MOLM13, MOLM14, U937, and THP-1 cells treated with CK. (d) Pictures of representative colony formation experiment clones (scale bars, 50 μm).

showed that CK exhibited no toxic side or small effects on normal cells compared to leukaemia cell lines. See Supplementary Table S1 for the half-maximal inhibitory concentration (IC50) in AML cells treated with different CK concentrations. Colony formation assays were explored to further test the anti-proliferation effect of CK on AML cells. The results are shown in Figure 1c and d. The number and size of colonies were significantly reduced with the CK concentration increased. The number and size of clones were also quantified (Figures S1–S2). In summary, CK inhibits the growth of AML cells and has anti-proliferative effects at relatively low CK concentrations of 20–40 μM.

3.2. CK induces apoptosis and cell cycle arrest of AML cell

We further analyzed the effects of CK on apoptosis and cycle arrest of AML cells. According to the results of cell apoptosis detection, the apoptosis rates after the treatment of CK were significantly increased (Figure 2a and Figure S3). Next, the cell-cycle distribution was assessed. We found that CK induced cell-cycle arrest at G1 stage in AML cells, as shown in Figure 2b and Figure S4. Consequently, these results further suggest that the inhibition viability of AML cells may be caused by cell cycle arrest in G1 phase and induced apoptosis.

3.3. Identification of CK response-associated genes and pathways

We further identified potential genes and pathways that may be involved in CK promoting leukemia cell death by RNA sequencing (RNA-seq) of MOLM13 cell lines treated with CK. 737 upregulated genes and 1216 downregulated genes were identified by the volcano map (Figure 3a and See Supplementary Table S2). KEGG analysis revealed that the IL-17 signaling pathway, ferrotosis, pathway in cancer, and signaling pathways regulating pluriotenc had the highest proportion of

genes (Figure 3b and see Supplementary Table S3). Then, GO enrichment analysis (biological process) indicated that differentially expressed genes play a biological role through transcription factor complex in this title (Figure 3c and see Supplementary Table S4). The different genes clustering in the heat map were further analyzed, and the expression of different genes is shown by color (Figure 3d and see Supplementary Table S5). More importantly, the up-regulation or down-regulation genes of CK in the treatment of leukemia were further mapped (Figure 3e and see Supplementary Table S6). We analyzed the RNA expression levels of these genes in MOLM 13, MOLM 14, U937 and THP-1 cells treated with CK by qRT-PCR assay (Figure 3f). As anticipated, gene expression of TET2 was significantly up-regulated, and gene expression of BCL2, KIT, DNMT3A, MYC and CSF-1 was significantly down-regulated with the increase of CK concentration. These findings suggest that CK effectively inhibits the activity and interferes with the growth of AML cells through the efficient expression of these genes.

3.4. In vivo suppression of leukemic growth by CK

To further explore the therapeutic effect of CK in vivo, C1498 and FLT3+ leukemia mouse models were established to evaluate the therapeutic effect of CK (Figure 4a). Two different concentrations of CK (1 mg/kg and 5 mg/kg) and PBS were injected into mice of AML model every two days as two treatment groups and control group respectively. WBC counts experiments were explored to specify the inhibitory effect of CK on WBC. The C1498 WBC result of the control group was $250 \pm 33 \times 10^9$/L, while the C1498 WBC result of 1 mg/kg CK treatment group was $163 \pm 20 \times 10^9$/L and 5 mg/kg CK treatment group was $158 \pm 28 \times 10^9$/L; the FLT3+ WBC result of control group was $242 \pm 18 \times 10^9$/L, while the FLT3+ WBC results of 1 mg/kg and 5 mg/kg CK treatment groups were $116 \pm 26 \times 10^9$/L and $116 \pm 20 \times 10^9$/L respectively (Figure 4b). In
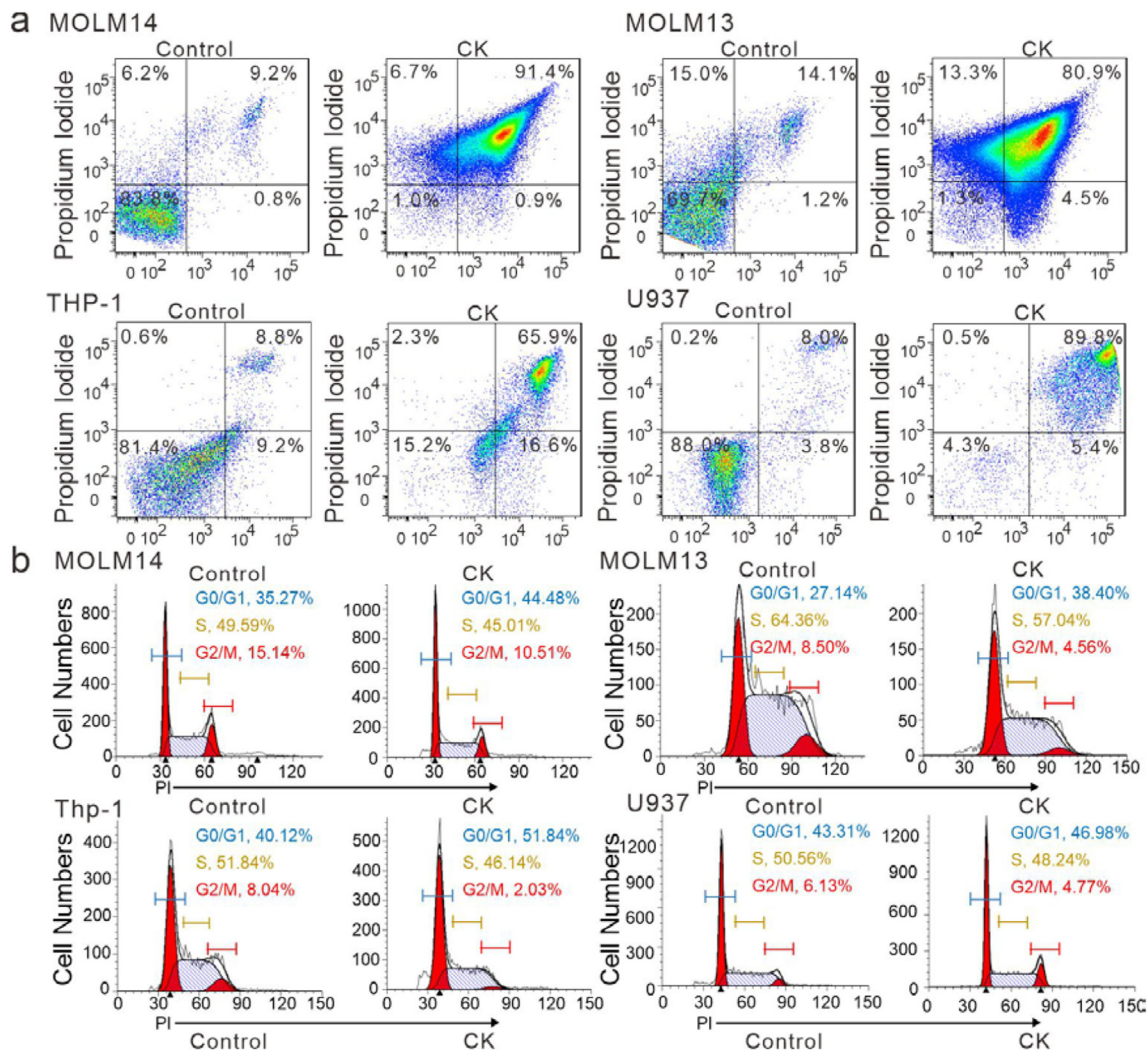
Figure 2. CK promotes cell cycle arrest and apoptosis in AML cells. (a) The effect of CK on apoptosis in MOLM14, MOLM13, THP-1, and U937 cells after 48 h of treatment was analyzed. (b) The cell cycle phase was analyzed.

addition, the numbers of lung metastatic nodules in the two mice models were also significantly lower in the CK treatment groups (Figure 4c, 1 mg/kg: 8.5 ± 3.0 for C1498 mice, 9.2 ± 2.7 for FLT3+; 5 mg/kg: 3.4 ± 1.7 for C1498 mice, 4.8 ± 2.3 for FLT3+ mice) versus the control groups (13.6 ± 2.1 for C1498 mice, 13.2 ± 3.4 for FLT3+ mice).

Importantly, mice treated with CK exhibited a significant reduction in leukemic burden than the control group, and these results were validated by a significantly decreased number of metastatic leukemic cells in the spleen and reversed splenomegaly (Figure 4d, e, C1498 mice: control, 346 ± 34 mg; 1 mg/kg, 256 ± 52 mg; 5 mg/kg, 211 ± 17 mg; FLT3+ mice: control, 363 ± 57 mg; 1 mg/kg, 278 ± 53 mg; 5 mg/kg, 213 ± 59 mg). The extended overall survival and increased body weight (Figure 4f, g) after mice modulated by CK indicated that they showed fewer signs of distress. In summary, CK significantly inhibited leukemia progression in the treatment group mice, as indicated by lower WBC counts, reduced splenomegaly, and lead to a lack of lung metastasis, which corresponded to prolonged survival, indicating that CK has a significant inhibitory effect on AML in vivo.

4. Discussion

Acute myeloid leukemia (AML) is a malignancy of the bone marrow caused by normal hematopoietic failure in which abnormal myeloid cell over-proliferation occurs [22]. Although some achievements have been made in the treatment of AML, novel treatments are urgently needed [23]. Ginsenoside CK is a non-natural ginsenoside, which can be converted from other protopanaxadiol-type ginsenosides. CK has been shown a promising anti-tumor effect on all kinds of cancers, such as CK synergized with TRAIL to inhibit colon cancer at a concentration of 25 μM [24]. Nanogels loaded with CK enhanced the inhibition of lung cancer cells at range of 20–60 μg/mL [25]. CK induced apoptosis in prostate cancer cells by activating miR193a-5p and inhibiting PD-L1 and STAT3 signaling pathways at a concentration of 10 μM [26], and we found through cellular experiments that CK had a significant inhibitory effect on AML at lower concentrations of 20–40 μM [27].

To discuss the influence of related genes of CK's anti-leukemia activity, we analyzed MOLM13 cells treated with CK using RNA-seq. However, in this paper, only changes in gene expression of CK during treatment were analyzed. We found different expression levels of related genes after CK treatment according to the volcano map. KEGG is a comprehensive database that integrates genomic, chemical, and system function information for systematic analysis of gene functions and linking genomic information generated by genome sequencing with higher order functional in formation [28, 29]. Here, KEGG pathway enrichment analysis was employed to identify significant signal transduction pathways in differentially expressed genes. And the results of KEGG analysis indicated the upregulated genes were closely related to IL-7 signaling pathway, Toll-like receptor signaling pathway, MAPK signaling pathway
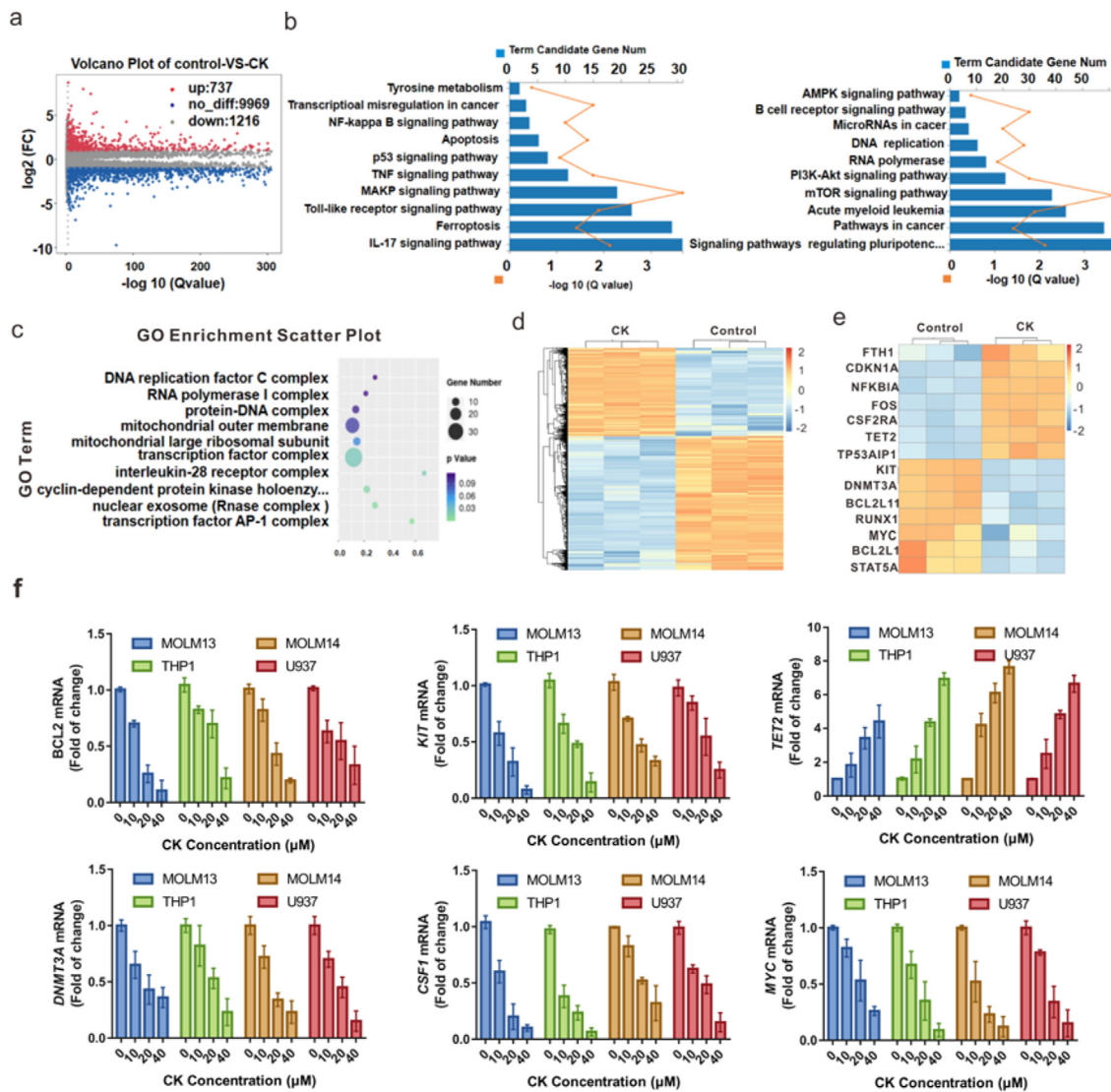
Figure 3. Identification of related genes and pathways in the treatment of CK. (a) Scatter plots of differentially expressed genes in control and k-treated MOLM13 cells, red and blue representing up-regulation and down-regulation, respectively. (b) KEGG pathway analysis was carried out for the above differentially expressed genes. (c) GO enrichment analysis of differentially expressed genes in MOLM13 cells treated with CK, revealed the enriched biological processes. (d) Analysis of the heat map of different gene clustering, and the expression of different genes are shown by color in CK treated MOLM13 cells. (e) The most significant up-regulation and down-regulation genes. (f) The expression of BCL2, KIT, TET2, DNMT3A, MYC and CSF1 genes were analyzed by qRT-PCR.

and apoptosis, whereas the down-regulated genes were related to acute myeloid leukemia, mTOR signaling pathway, PI3K-Akt signaling pathway, etc. Gene Ontology (GO) is a controlled vocabulary of terms to represent biology in a structured way. The terms are subdivided into three distinct ontologies that represent different biological aspects: molecular function, biological process, and cellular component [30]. GO enrichment was determined by submitting differentially expressed genes to the GO database to achieve classification [31]. GO term enrichment analysis was employed in this study to gain how the functional genes were enriched or related to the specific biological processes. A heat map is a data matrix that visualizes the values in cells by using color gradients. This provides a good overview of the maximum and minimum values in the matrix [32]. Finally, total of 7 up-regulated and 7 down-regulated genes of CK in the treatment of AML were shown by heat map. In conclusion, these datas is used to study the expression of genes of CK in the treatment of AML, which will further provide a theoretical basis for our subsequent studies on the molecular mechanism or therapeutic target of CK in the treatment of AML, and contribute to promote the diagnosis of AML.

Again, we analyzed by qRT-PCR the genes related to AML cells that affect their proliferation, apoptosis or prognosis. CSF can induce differentiation of leukemia cells, it also plays a strong role in promoting cell proliferation [33]. TET2 is frequently mutated in AML [34]. BCL-2, a key protein regulator of cell apoptosis, over-expression occurs frequently in AML with the result of defective apoptosis that causes enhanced cell survival and drug resistance [35]. DNMT3A mutation contributes to AML pathogenesis [36], leading to a worse prognosis in AML [37]. MYC is an important transcription factor that is aberrantly overexpressed in AML [38]. KIT mutations (KIT +) are common in core binding factor AML and have been associated with prognostic significance [39]. As indicated above, all these mutated genes are associated with the development and prognosis of AML and their changes can reflect the therapeutic effect of CK on AML. Therefore, these genes were selected for further investigation. The results showed that qRT-PCR was consistent with RNA-seq results.

Finally, we established animal models of AML to study the therapeutic effect of CK in vivo. The typical AML model is C1498. It has been reported that FLT3 internal tandem duplication (FLT3/ITD) is relevant to
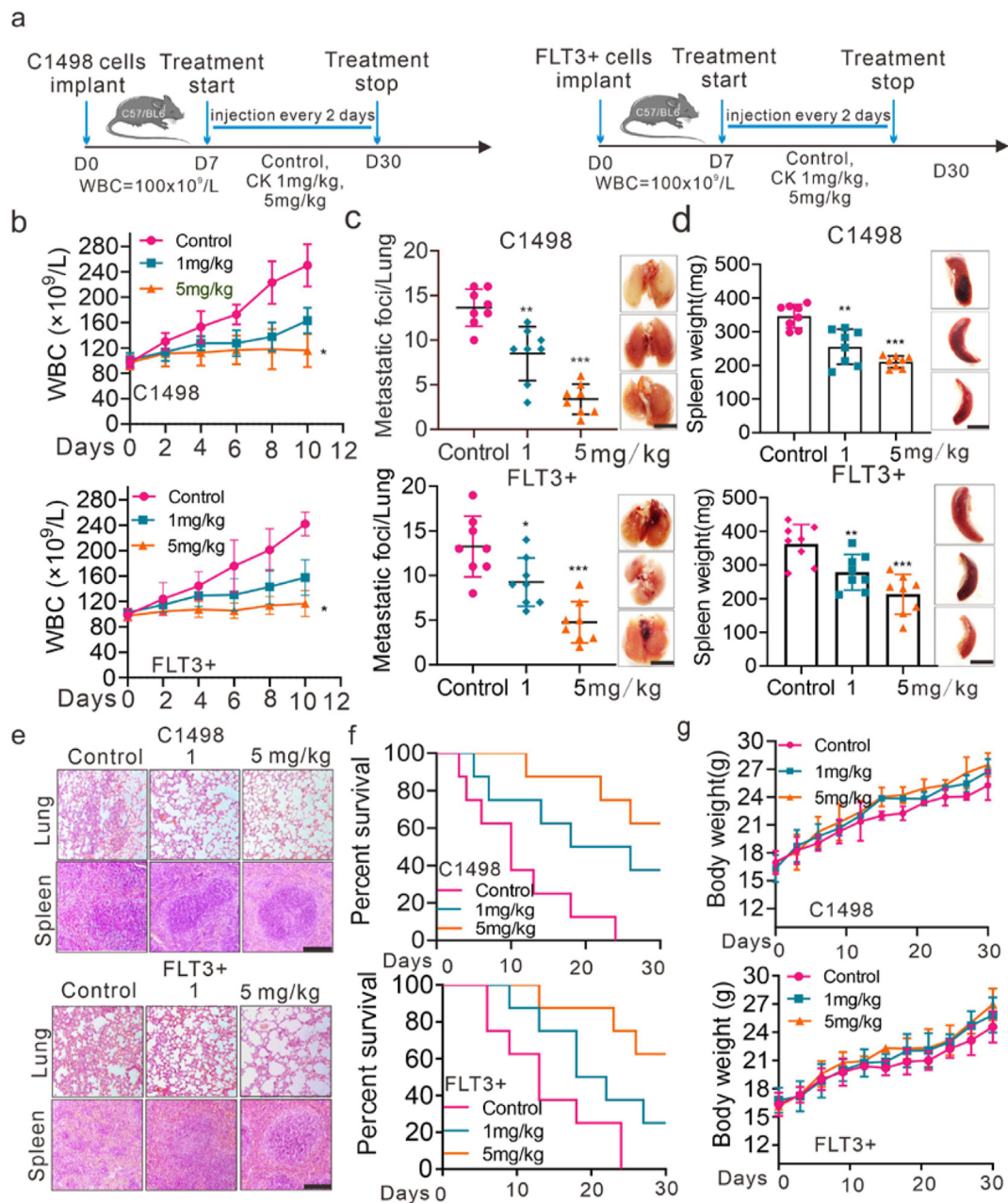
Figure 4. Effect of CK on leukemia in vivo. (a) The mouse model of C1498 and FLT3+ leukemia was established. Subsequently, CK with different concentrations was injected as the treatment group and PBS as the control group. (b) White blood cell count in mice with leukemia (n = 8). (c) Quantitative analysis of lung tumor growth nodules and display of a representative external view of the lung (Scale bars, 1 cm). (d) Quantitative analysis of spleen weight and display of a representative external view of the spleen (Scale bars, 1 cm). (e) H&E staining for spleen and lung tissue sections (Scale bars, 1 cm, lower). (f) Percent survival of leukemic mice (n = 8). (g) Body weight. Data are mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001.

poor prognosis and increased risk of recurrence in AML patients and a common mutation in AML [40]. Therefore, we also established the FLT3+ mouse model to further study the therapeutic effect of CK in vivo. Fortunately, it is known from the results of in vivo experiments that CK inhibits the further growth of WBCs, reduces pulmonary metastatic nodules and successfully reverses splenomegaly. CK also prolongs the survival rate of mice. Thus, we found that CK has significant therapeutic effects. Taken together, in vivo experiments further proved that CK has obvious efficient anti-tumor therapeutic effect on AML, and also demonstrated that CK has good biological safety.

As indicated above, CK has been confirmed to inhibit the activity of AML cells, induce apoptosis and AML cell proliferation arrest, and it in vivo inhibitory effect has also been proved through animal experiments, thus successfully revealing that CK has a good therapeutic effect on AML

and is an anticancer drug with low toxicity and few side effects. Moreover, CK treatment down-regulated gene expression of BCL2, KIT, DNMT3A, MYC and CSF-1, and up-regulated gene expression of TET2. It is worth emphasizing that our work demonstrates the therapeutic effect of CK on AML, paving the way for further exploration of its molecular mechanism in AML therapy, opening a new window for the low-toxicity drug- CK to become a therapeutic agent for AML.

Declarations

Author contribution statement

Yuzhu Hou; Xiangru Meng: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

References

[1] S. Kucukyurt, A.E. Eskazan, New drugs approved for acute myeloid leukaemia in 2018, Br. J. Clin. Pharmacol. 85 (12) (2019) 2689–2693.

[2] A. Shastri, G. Choudhary, M. Teixeira, S. Gordon-Mitchell, N. Ramachandra, L. Bernard, S. Bhattacharyya, R. Lopez, K. Pradhan, O. Giricz, et al., Antisense STAT3 inhibitor decreases viability of myelodysplastic and leukemic stem cells, J. Clin. Invest. 128 (12) (2018) 5479–5488.

[3] H.-W. Chu, C.-S. Lai, J.-Y. Ko, S.G. Harroun, C.-I. Chuang, R.Y.L. Wang, B. Unnikrishnan, C.-C. Huang, Nanoparticle-based LDI-MS immunoassay for the multiple diagnosis of viral infections, ACS Sens. 4 (6) (2019) 1543–1551.

[4] X. Pei, X. Huang, New approaches in allogenic transplantation in AML, Semin. Hematol. 56 (2) (2019) 147–154.

[5] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2022, CA A Cancer J. Clin. 72 (1) (2022) 7–33.

[6] S. Baakhlagh, B. Kashani, Z. Zandi, D. Bashash, M. Moradkhani, A. Nasrollahzadeh, M. Yaghmaei, S.A. Mousavi, S.H. Ghaffari, Toll-like receptor 4 signaling pathway is correlated with pathophysiological characteristics of AML patients and its inhibition using TAK-242 suppresses AML cell proliferation, Int. Immunopharm. 90 (2021), 107202.

[7] Y. Sung, P. Jin, L. Chu, F. Hsu, M. Wang, C. Chang, S. Chiou, J. Qiu, D. Gao, C. Lin, et al., Delivery of nitric oxide with a nanocarrier promotes tumour vessel normalization and potentiates anti-cancer therapies, Nat. Nanotechnol. 14 (12) (2019) 1160–1169.

[8] Y.S. Yi, Roles of ginsenosides in inflammasome activation, J. Ginseng. Res. 43 (2) (2019) 172–178.

[9] Z. Ren, X. Chen, L. Hong, et al., Nanoparticle conjugation of ginsenoside Rg3 inhibits hepatocellular carcinoma development and metastasis, Small 16 (2) (2020).

[10] C. Hong, D. Wang, J. Liang, et al., Novel ginsenoside-based multifunctional liposomal delivery system for combination therapy of gastric cancer, Theranostics 9 (15) (2019) 4437–4449.

[11] J. Huang, D. Liu, Y. Wang, L. Liu, J. Li, J. Yuan, Z. Jiang, Z. Jiang, W.W. Hsiao, H. Liu, et al., Ginseng polysaccharides alter the gut microbiota and kynurenine/ tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy, Gut 71 (4) (2022) 734–745.

[12] X.M. Piao, Y. Huo, J.P. Kang, R. Mathiyalagan, H. Zhang, D.U. Yang, M. Kim, D.C. Yang, S.C. Kang, Y.P. Wang, Diversity of ginsenoside profiles produced by various processing Technologies, Molecules 25 (19) (2020).

[13] L. Zhou, X. Zeng, T. Rao, Z. Tan, G. Zhou, D. Ouyang, L. Chen, Evaluating the protective effects of individual or combined ginsenoside CK and the

[14] downregulation of soluble epoxide hydrolase expression against sodium valproate-induced liver cell damage, Toxicol. Appl. Pharmacol. 422 (2021), 115555.

[14] L. Chen, Y. Meng, Q. Sun, Z. Zhang, X. Guo, X. Sheng, G. Tai, H. Cheng, Y. Zhou, Ginsenoside CK sensitizes human colon cancer cells to TRAIL-induced apoptosis via autophagy-dependent and -independent DR5 upregulation, Cell Death Dis. 7 (8) (2016) e2334.

[15] E. Choi, E. Kim, J.H. Kim, K. Yoon, S. Kim, J. Lee, J.Y. Cho, AKT1-targeted proapoptotic activity of CK in human breast cancer cells, J. Ginseng. Res. 43 (4) (2019) 692–698.

[16] F. Yan, A. Al-Kali, Z. Zhang, J. Liu, J. Pang, N. Zhao, C. He, M.R. Litzow, S. Liu, A dynamic N(6)-methyladenosine methylome regulates intrinsic and acquired resistance to tyrosine kinase inhibitors, Cell Res. 28 (11) (2018) 1062–1076.

[17] K. Sun, W. Yu, B. Ji, C. Chen, H. Yang, Y. Du, M. Song, H. Cai, F. Yan, R. Su, Saikosaponin D loaded macrophage membrane-biomimetic nanoparticles target angiogenic signaling for breast cancer therapy, Appl. Mater. Today 18 (2020).

[18] R. Deng, N. Shen, Y. Yang, H. Yu, S. Xu, Y.W. Yang, S. Liu, K. Meguellati, F. Yan, Targeting epigenetic pathway with gold nanoparticles for acute myeloid leukemia therapy, Biomaterials 167 (2018) 80–90.

[19] L. Dou, F. Yan, J. Pang, D. Zheng, D. Li, L. Gao, L. Wang, Y. Xu, J. Shi, Q. Wang, et al., Protein lysine 43 methylation by EZH1 promotes AML1-ETO transcriptional repression in leukemia, Nat. Commun. 10 (1) (2019) 5051.

[20] Y. Yu, M. Song, C. Chen, Y. Du, C. Li, Y. Han, F. Yan, Z. Shi, S. Feng, Bortezomib-encapsulated CuS/carbon dot nanocomposites for enhanced photothermal therapy via stabilization of polyubiquitinated substrates in the proteasomal degradation pathway, ACS Nano 14 (8) (2020) 10688–10703.

[21] H. Luo, C.T. Vong, H. Chen, et al., Naturally occurring anti-cancer compounds: shining from Chinese herbal medicine, Chin. Med. 14 (48) (2019).

[22] K.E. Heimbruch, A.E. Meyer, P. Agrawal, A.D. Viny, S. Rao, A cohesive look at leukemogenesis: the cohesin complex and other driving mutations in AML, Neoplasia 23 (3) (2021) 337–347.

[23] H. Liu, Emerging agents and regimens for AML, J. Hematol. Oncol. 14 (1) (2021) 49.

[24] L. Chen, Y. Meng, Q. Sun, et al., Ginsenoside compound K sensitizes human colon cancer cells to TRAIL-induced apoptosis via autophagy-dependent and -independent DR5 upregulation, Cell Death Dis. 7 (8) (2016) e2334.

[25] Z. Xue, R. Fu, Z. Duan, et al., Inhibitory effect of pH-responsive nanogel encapsulating ginsenoside CK against lung cancer, Polymers 13 (11) (2021).

[26] J.H. Lee, D.Y. Lee, H.J. Lee, et al., Inhibition of STAT3/PD-L1 and activation of miR193a5p are critically involved in apoptotic effect of compound K in prostate cancer cells, Cells 10 (8) (2021).

[27] Y. Chen, Y. Xu, Y. Zhu, et al., Anti-cancer effects of ginsenoside CK on pediatric acute myeloid leukemia cells, Cancer Cell Int. 13 (1) (2013) 24.

[28] J.K. Karanja, M.M. Aslam, Z. Qian, et al., Abscisic acid mediates drought-enhanced rhizosheath formation in tomato, Front. Plant Sci. 12 (2021), 658787.

[29] M. Kanehisa, M. Araki, S. Goto, et al., KEGG for linking genomes to life and the environment, Nucleic Acids Res. 36 (2008) D480–D484.

[30] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene ontology: tool for the unification of biology. The Gene Ontology Consortium, Nat. Genet. 25 (1) (2000) 25–29.

[31] D. Zhang, Q. Du, P. Sun, J. Lou, X. Li, Q. Li, M. Wei, Physiological and transcriptomic analyses revealed the implications of abscisic acid in mediating the rate-limiting step for photosynthetic carbon dioxide utilisation in response to vapour pressure deficit in solanum lycopersicum (tomato), Front. Plant Sci. 12 (2021), 745110.

[32] T. Metsalu, J. Vilo, ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap, Nucleic Acids Res. 43 (2015) W566–W570.

[33] G. Cipriani, S.J. Gibbons, K.E. Miller, D.S. Yang, M.L. Terhaar, S.T. Eisenman, T. Ördög, D.R. Linden, G.B. Gajdos, J.H. Szurszewski, G. Farrugia, Change in populations of macrophages promotes development of delayed gastric emptying in mice, Gastroenterology 154 (8) (2018), 2122–2136.e12.

[34] Y. Lyu, J. Lou, Y. Yang, J. Feng, Y. Hao, S. Huang, L. Yin, J. Xu, D. Huang, B. Ma, D. Zou, Y. Wang, Y. Zhang, B. Zhang, P. Chen, K. Yu, E.W. Lam, X. Wang, Q. Liu, J. Yan, B. Jin, Dysfunction of the WT1-MEG3 signaling promotes AML leukemogenesis via p53-dependent and -independent pathways, Leukemia 31 (12) (2017) 2543–2551.

[35] M. Rahmani, M.M. Aust, E. Attkisson, D.C. Williams, A. Ferreira-Gonzalez, S. Grant, Dual inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism, Cancer Res. 73 (4) (2013) 1340–1351.

[36] R. Lu, P. Wang, T. Parton, et al., Epigenetic perturbations by arg882-mutated DNMT3A potentiate aberrant stem cell gene-expression program and acute leukemia development, Cancer Cell 30 (1) (2016) 92–107.

[37] G. Marcucci, K.H. Metzeler, S. Schwind, et al., Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia, J. Clin. Oncol. 30 (7) (2012) 742–750.

[38] X. Jiang, H. Huang, Z. Li, et al., Blockade of miR-150 maturation by MLL-fusion/ MYC/LIN-28 is required for MLL-associated leukemia, Cancer Cell 22 (4) (2012) 524–535.

[39] K. Tarlock, T.A. Alonzo, Y.C. Wang, et al., Functional properties of KIT mutations are associated with differential clinical outcomes and response to targeted therapeutics in CBF acute myeloid leukemia, Clin. Cancer Res. 25 (16) (2019) 5038–5048.

[40] J. Wu, Y. Xiao, J. Sun, H. Sun, H. Chen, Y. Zhu, H. Fu, C. Yu, W. E, S. Lai, et al., A single-cell survey of cellular hierarchy in acute myeloid leukemia, J. Hematol. Oncol. 13 (1) (2020) 128.