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ORIGINAL ARTICLE

Priming with GM-CSF instead of G-CSF enhances CAG-induced apoptosis of acute monocytic leukemia cells in vitro

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Abstract

High expression of granulocyte–macrophage colony-stimulating factor (GM-CSF) receptor has been found in myelomonocytic or monocytic subtypes (M4/M5) of acute myeloid leukemia. Herein, we aimed to improve the effect of CAG [Ara-C, ACR, and G-CSF (granulocyte colony-stimulating factor)] regimen for acute monocytic leukemia by replacing G-CSF with GM-CSF. Results showed that the percentage of cells in S phase was higher with GM-CSF than with G-CSF treatment at 20 ng/mL ($P < 0.05$). When THP-1 and SHI-1 cells were primed with 20 ng/mL G-CSF or GM-CSF followed by Ara-C and ACR, cell proliferation rate in the CAGM (Ara-C, ACR, and GM-CSF) regimen was lower than in the CAG regimen ($P < 0.05$). Furthermore, CAGM regimen induced more obvious cell apoptosis than CAG regimen probably by reducing Bcl-2/Bax ratio ($P < 0.05$). Similar results were seen in primary cells from M5 patients. Collectively, our study suggests that priming with GM-CSF may be more effective than G-CSF in CAG regimen in acute monocytic leukemia.

Keywords Acute monocytic leukemia · Cell lines · Primary cells · CAG · CAGM · Cytotoxicity

Introduction

The previous studies have demonstrated that hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3) can potentiate the anti-leukemia effects of cytarabine (Ara-C) [1, 2]. Based on this rationale, CAG regimen [low-dose Ara-C, aclarubicin (ACR), and G-CSF] has been widely used to treat acute leukemia and myelodysplastic syndrome [3–5]. G-CSF promotes quiescent leukemic cells into S phase by binding with G-CSF receptor (G-CSFR) on leukemia cells, thus enhancing their susceptibility to Ara-C and anthracyclines [6, 7]. Although CAG regimen is associated with a relatively higher complete remission (CR) rate in relapsed/refractory (R/R) acute myeloid leukemia (AML, excluding acute promyelocytic leukemia) patients, the expression levels of G-CSFR in different French–American–British (FAB) subtypes are different, with higher expression in the M2/M3 subtypes than in the M4/M5 subtypes [8]. Therefore, it is reasonable to speculate that CAG priming regimen may produce different effects in these two subtypes of AML.

Similar to G-CSF, GM-CSF also renders leukemic cells more susceptible to chemotherapeutics by modulating cell cycle kinetics [9]. In fact, GM-CSF may exert dual effects in AML cells: stimulating cell proliferation and up-regulating pro-apoptotic proteins. GM-CSF priming also increases intracellular uptake of Ara-C in leukemic cells [10, 11]. The ALFA-9802 trial reported GM-CSF priming in combination with chemotherapy not only improved 3-year event-free survival (EFS) of younger adult AML patients, but also benefited patients with FLT3-ITD and MLL rearrangement [12, 13]. Interestingly, FLT3-ITD and MLL rearrangements

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are common in the adult M4/M5 AML patients, which are associated with poor prognosis [14, 15]. Furthermore, M4/M5 AML expresses significantly higher levels of GM-CSFR [8, 16].

Based on the above facts, we hypothesized that patients of M1/M2 subtypes and patients of M4/M5 subtypes may response differently to CAG regimen. We further hypothesized that priming with GM-CSF (i.e., CAGM regimen) instead of G-CSF may be more effective in M4/M5 AML.

Materials and methods

Patients and cell preparation

Medical records of R/R AML patients with FAB M1/M2 and M4/M5 subtypes from June 2006 to July 2015 at the First Affiliated Hospital of Soochow University were retrieved. The diagnosis and classification of the patients were based on the revised FAB classification and the 2008 World Health Organization (WHO) criteria [17, 18]. Relapse was defined as reappearance of leukemia blasts in the peripheral blood or the finding of > 5% blasts in the bone marrow, not attributable to another cause (e.g., bone marrow reconstitution after consolidation therapy), as well as extramedullary disease. Refractory AML was defined as not achieving CR after two courses of standard daunorubicin plus Ara-C or equivalent anthracycline-based induction regimen. All of the patients were treated with CAG regimen (subcutaneous Ara-C every 12 h for 14 days (10 mg/m^2); daily intravenous aclarubicin on days 1–8 (5–7 mg/m^2/day); and subcutaneous G-CSF on days 1–14 (200 μg/m^2/day). After the first course, therapeutic response was evaluated by bone marrow aspiration. CR and overall remission (OR) were defined according to the Cancer and Leukemia Group B (CALGB) criteria [19].

Thirteen consecutive newly diagnosed M4/M5 patients were enrolled for experimental study from June 2016 to March 2018. The diagnosis and classification of the M4/M5 patients were mainly based on microscopy and flow cytometry findings [17, 19]. Bone marrow sample was obtained from every patient before chemotherapy. The sample after erythrocyte lysis (QIAGEN, United States) was used to detect the expression of G-CSFR and GM-CSFR. Mononuclear cells of three M5 patients with high GM-CSFR expression were separated by Ficoll–Hypaque (Sigma-Aldrich, St Louis, MO, USA) density-gradient centrifugation and cultured in RPMI 1640 containing 10% fetal calf serum (Gibco) for cell cycle and apoptosis experiments.

Cell lines and culture

Human acute monocytic leukemia cell lines (THP-1 and SHI-1) were obtained from Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University (Suzhou, China). Cells were incubated in RPMI-1640 medium (Hyclone, USA) containing 10% FCS in an incubator (Thermo Fisher Scientific, Rockford, IL, USA) with a humidified atmosphere of 5% CO_2 at 37 °C.

Flow cytometry analysis (FCAS) for G-CSFR and GM-CSFR

The number of cell was quantified by microscopy and adjusted to 1×10^6 in each tube. The cells were, respectively, labeled with phycoerythrin (PE)-conjugated anti-G-CSFR mAb (BD Bioscience) and fluorescein isothiocyanate (FITC)-conjugated anti-GM-CSFR mAb (BD Bioscience). The isotype immunoglobulin (Ig) G and IgM conjugated with the above fluorescence served as negative control. In addition, primary cells were labeled with phycoerythrin–cyanine 7 (PC7)-conjugated anti-CD45 mAb (Beckman Coulter, California, USA). The positive threshold was 20% for G-CSFR and GM-CSFR. Data acquisition was performed on a Beckman Coulter Flow Cytometer (FC500) and analyzed with the Flowjo 7.6.1 software.

Cell cycle analysis

Cells were adjusted to 2×10^5/mL and cultured in 24-well plates (1 mL/well) with 10 ng/mL, 20 ng/mL, and 40 ng/mL G-CSF (Qilu Pharmaceutical Co., Ltd, China) or 10 ng/mL, 20 ng/mL, and 40 ng/mL CM-CSF (Xiamen Amoy Bioengineering Co., Ltd, China), respectively for 24 h. Neither G-CSF nor CM-CSF was added in the control wells. After culture for 24 h, cells were harvested and fixed in 70% ethanol for more than 12 h at 4 °C, and washed with PBS and resuspended in 535 μL dye solution (Beyotime Biotechnology) for 30 min at 37 °C. Cells were acquired using the Beckman Coulter flow cytometer (FC500) and FCAS data were analyzed using the DNA cell cycle analysis software (MultiCycle).

For M5 primary cells, cells were cultured for 12 h before being harvested for FCAS.

Determination of proliferation rate

THP-1 and SHI-1 cells (2×10^5/mL) were seeded into 96-well plates (90 μL/well) containing 20 ng/mL G-CSF or GM-CSF in triplicate, respectively. After 24 h, Ara-C (4×10^{-6} mol/L, Actavis Italy S.p.A) and ACR (either 1×10^{-8} mol/L, 2×10^{-8} mol/L, 4×10^{-8} mol/L, or 8×10^{-8} mol/L, Yangzhou Pharmaceutical Co., Ltd, China) were added in different experimental groups for additional 48 h. Other groups included blank control (medium only), negative control (cells only), and positive control (cells plus G-CSF/GM-CSF). At the end of culture, 10 μL Cell Counting Kit-8 was added into each well.

Two hours later, the optical density (OD) at 450 nm was read using an automatic microplate reader. OD value was linearly related to the number of viable cells. Leukemic cell proliferation rate was calculated as follows: $(OD_{\text{treated well}} - OD_{\text{blank}})/(OD_{\text{negative control}} - OD_{\text{blank}}) \times 100\%$.

Assessment of apoptosis by FCAS

At the end of culture as described above, the percentages of cell apoptosis were determined by an Annexin V-FITC/PI double staining kit (Multisciences, Lianke). Cells were collected, washed with cold PBS solution, resuspended in binding buffer, and stained by 5 µL Annexin V-FITC (10 min) and 10 µL PI (5 min) at room temperature in the dark. Cells were finally subjected to analysis by FC500 flow cytometer.

Western blot analysis

At the end of culture as described above, THP-1 and SHI-1 cells were harvested and lysed by SDS lysis buffer (Beyotime) for protein analysis. Briefly, approximately 30 µg of protein extracts was subjected to 12% SDS-PAGE and subsequently transferred to polyvinylidene difluoride membranes (PVDF; Bio-Rad). The membranes were blocked with 5% milk and incubated with primary antibodies against Bcl-2, Bax, cleaved caspase 3, caspase 8, or caspase 9 (all from Cell Signaling Technology) overnight at 4 °C followed by incubation with HRP-linked secondary antibody (Beyotime 1:3000). The γ-tubulin antibody and the secondary antibody were also purchased from Cell Signaling Technology. Band detection via enzyme-linked chemiluminescence was performed according to the manufacturer's protocol (ECL; Pierce biotechnology Inc., Rockford, IL, USA). The protein bands were quantified using Image J 1.33 software (NIH), and data were normalized to γ-tubulin.

Statistical analysis

Statistical analysis was performed by SPSS v19.0. The difference between patient subsets was analyzed by Chi-squared test for categorical variables. Nonparametric test (Mann–Whitney *U* test) was applied to analyze non-normality data. Student's t test was performed for comparisons between the two groups in cell experiments. Probability values ($P<0.05$) were considered to be significant.

Results

Patient characteristic and response to chemotherapy

A total of 73 M1/M2 and 53 M4/M5 patients were included. Baseline demographic and disease characteristics are shown in Table 1. There were no significant differences in terms of sex, age, complex karyotype, and gene mutation (FLT-ITD mutation, MLL rearrangements) between the two groups ($P>0.05$). After induction treatment with CAG, the CR rate and OR rate of the M1/M2 group were both significantly higher than those of the M4/M5 group (64.4 vs. 34.0%, $P=0.001$; 76.4 vs. 50.9%, $P=0.003$, respectively). There were no differences in terms of myelosuppression and infection between the two groups.

G-CSFR and GM-CSFR profile in cell lines and primary cells

G-CSFR and GM-CSFR were both detected on THP-1 and SHI-1 cells, but GM-CSFR expression level was higher. Similarly higher GM-CSFR expression was found on M5 primary cells (Fig. 1). According to FAB type, 13 AML patients were classified into M4 ($n=5$) and M5 ($n=8$). For M4 patients, 80% and 60% were positive for G-CSFR and GM-CSFR, respectively. For M5 patients, 25% and 87.5% were positive for G-CSFR and GM-CSFR, respectively. Bone marrow samples of three M5 patients with high GM-CSFR expression were collected for subsequent experiments.

Effect of G-CSF/GM-CSF on cell cycle

For THP-1 cells, despite no differences in the percentages of S phase between 10 and 40 ng/ml G-CSF and GM-CSF groups were found ($P>0.05$), there was significant difference in the percentage of S phase between 20 ng/mL G-CSF and GM-CSF groups ($P<0.05$) (Fig. 2a, b). For SHI-1 cells, there were significant differences in the percentage of S phase between 10, 20, and 40 ng/mL G-CSF groups and the corresponding GM-CSF groups ($P<0.05$) (Fig. 2c, d). Similar results were also seen in M5 primary cells ($P<0.05$) (Fig. 4e, f). Based on these results and reports by others, 20 ng/mL G-CSF and GM-CSF were chosen for further experiments [10].

Effect of CAG and CAGM on cell proliferation

Although G-CSF or GM-CSF alone enhanced proliferation of THP-1 and SHI-1 cells, combination of G-CSF (GM-CSF) with Ara-C and ACR induced cell death.

Table 1 Clinical characteristics of two groups

	M1/M2 group *n* (73)	M4/M5 group *n* (53)	*P* value
Before reinduction			
Median age (years, range)	39 (25–51)	43 (29–52)	0.352
Gender, *n* (%)			0.501
Male	47 (64.4)	31 (58.5)	
Female	26 (35.6)	22 (41.5)	
Disease status, *n* (%)			0.558
Relapse	16 (21.9)	14 (26.4)	
Refractory	57 (78.1)	39 (73.6)	
Complex karyotype *n* (%)	5 (6.8)	6 (11.3)	0.525
FLT3-ITD mutation (±)	3/70	4/49	0.453
MLL rearrangements (±)	3/70	6/47	0.165
After reinduction			
CR rate, *n* (%)	47 (64.4)	18 (34.0)	**0.001**
OR rate, *n* (%)	56 (76.4)	27 (50.9)	**0.003**
WBC decrease, *n* (%)			0.829
Grade 1 or 2	9 (11.0)	8 (12.5)	
Grade 3 or 4	73 (89.0)	58 (87.5)	
PLT decrease, *n* (%)			0.842
Grade 1 or 2	8 (9.6)	6 (8.7)	
Grade 3 or 4	75 (90.4)	63 (91.3)	
Days of WBC $<0.5\times10^9$/L			0.237
Median(range)	12 (0–20)	13 (10–27)	
Days of PLT $<50\times10^9$/L			0.590
Median (range)	15 (0–37)	16 (7–29)	
Infection rate, *n* (%)			0.644
Lung	34 (27.9)	19 (21.8)	0.776
Sepsis	2 (1.6)	2 (2.4)	
Febrile neutropenia	34 (27.9)	28 (32.2)	
Other organs	7 (5.7)	9 (10.3)	
No infection	45 (36.9)	29 (33.3)	

FAB French–American–British, *WBC* white blood cell count, *PLT* platelet, *CR* complete remission, *OR* overall remission, *FLT3-ITD* Fms-like tyrosine kinase3-internal tyrosine duplication, *MLL* mixed lineage leukemia

In particular, CAGM was more effective than CAG in inhibiting cell proliferation at the presence of either 4×10^{-8} mol/L or 8×10^{-8} mol/L ACR ($P<0.05$) (Fig. 3a, b).

CAG and CAGM induce cell apoptosis

CAG or CAGM with the lowest ACR concentration did not induce obvious apoptosis. However, with increasing ACR concentration, apoptosis in both CAG and CAGM groups increased gradually; and CAGM group had a significantly higher apoptosis rate (Annexin V-FITC +/PI − and Annexin V-FITC +/PI +) than the corresponding CAG group in THP-1 cells ($P<0.05$) (Fig. 4a, b). Similar results were seen in SHI-1 cells ($P<0.05$) (Fig. 4c, d), and in M5 primary cells ($P<0.05$) (Fig. 4e, f).

Effect of CAG and CAGM on expression of apoptosis-related proteins

At 24 h, caspase 9 down-regulation was observed with increasing concentrations of ACR in both CAG and CAGM groups. CAGM induced higher expression of cleaved caspase 3 than CAG at high concentrations of ACR ($P<0.05$) (Fig. 5a1, 2 for THP-1 and Fig. 5c1, 2 for SHI-1). However, caspase 8 expression was not detectable at 24 h or 48 h in both groups (Supplemental Fig). These results suggest that CAG and CAGM-induced apoptosis involves mitochondrial apoptotic pathway. Because this pathway is regulated by Bcl-2 family proteins, we next examined Bcl-2 and Bax expression. Results showed the expression levels of Bcl-2 decreased, while the expression of Bax increased in both THP-1 and SHI-1 cells in an ACR dose-dependent manner. As a result, CAGM

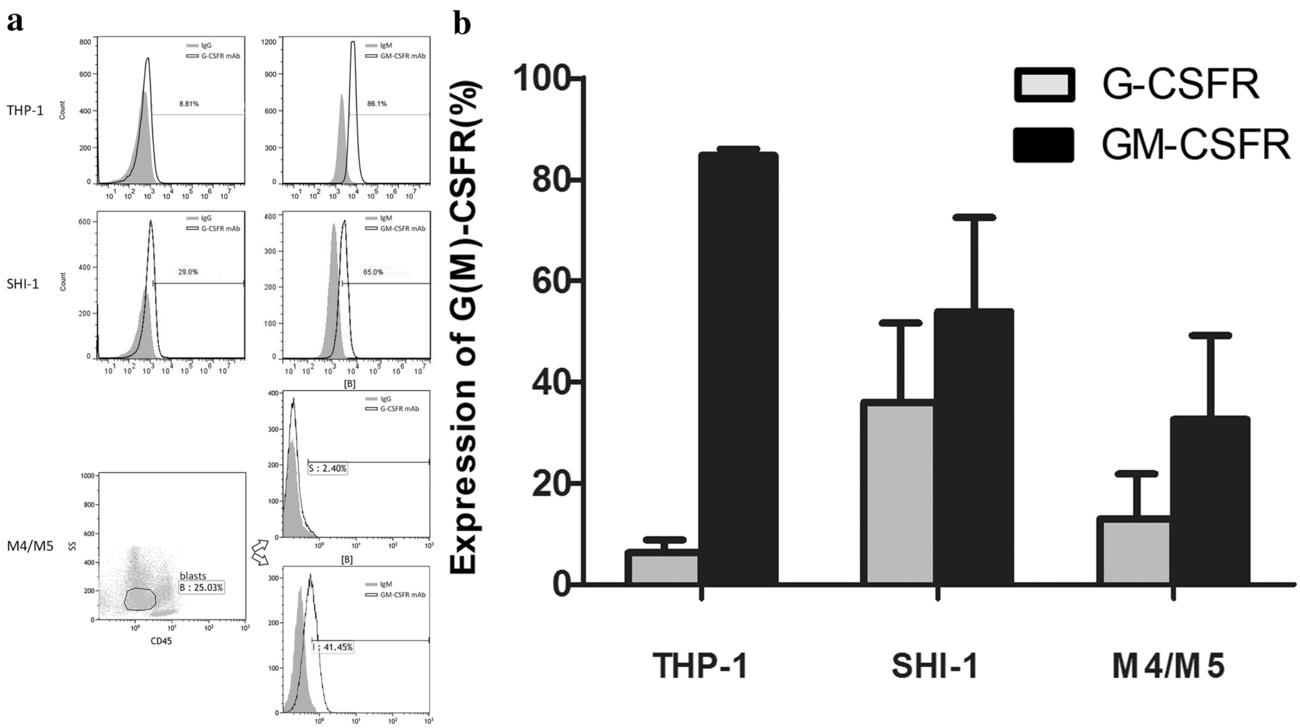

Fig. 1 G-CSFR and GM-CSFR expression in cell lines and primary cells

caused significantly lower Bcl-2/Bax ratio than CAG did ($P < 0.05$) (Figs. 5a3, 4, c3, 4).

Discussion

AML is a heterogeneous disease and different subsets of AML may response differently to the same treatment. In AML patients refractory to the first course of induction chemotherapy, those with non-M4/M 5 subtypes benefited more from the CAG regimen than those with M4/M5 subtypes, but the underlying reason is unknown [20]. The present retrospective study found that patients of M1/M2 subtypes benefited more from the CAG regimen than patients of M4/M5 subtypes, which may be due to higher expression levels of G-CSFR on M1/M2 cells [8, 21, 22]. We also found higher expression levels of GM-CSFR on M5 cell lines and primary cells. As expected, substitution of GM-CSF for G-CSF in the CAG regimen can augment the anti-leukemia effect of CAG regimen in acute monocytic leukemia in vitro.

Addition of either G-CSF or GM-CSF increased the percentage of cells in S phase, especially GM-CSF, in both SHI-1 cells and THP-1 cells, but it was worth noting that no significant differences were obtained in the percentages of S phase with 10 and 40 ng/mL for both G-CSF and GM-CSF in THP-1 cells ($P > 0.05$) (Fig. 2a, b). We think that a concentration of 10 ng/mL of either G-CSF or GM-CSF was not high enough to stimulate cell cycle conversion. At the concentration of 40 ng/mL, GM-CSF may stimulate some cells into G2 phase from S phase, which decreased the percentage of cells in S phase. That is why, the percentages of S phase of GM-CSF groups with the above two concentrations were not much higher than that in the corresponding G-CSF groups. This indicates an optimal dose of GM-CSF should be explored when GM-CSF is used for M5 patients in the clinical setting.

To confirm the different priming efficacy of G-CSF and GM-CSF on acute monocytic leukemia, we performed cytotoxic assays of these two regimens by mimicking clinical administration of CAG regimen. As expected, either G-CSF or GM-CSF alone stimulated cell proliferation [10]. Upon addition of Ara-C and ACR, cell proliferation was inhibited, with a greater inhibitory effect seen in the CAGM group. Similar results had been observed in AML blasts with G-CSF and Ara-C [23]. Apoptosis assay also confirmed that anti-leukemic effect of CAGM regimen was significantly stronger than CAG regimen. In addition, apoptosis increased in an ACR concentration-dependent manner of in both CAG and CAGM regimens, which was in line with clinical findings that increasing ACR dose in the CAG regimen increased CR rate of R/R AML patients [24]. Notably, the differences in proliferation rate were small between CAG and CAGM regimens, whereas the differences were much greater for apoptosis. The underlying reason is unknown.

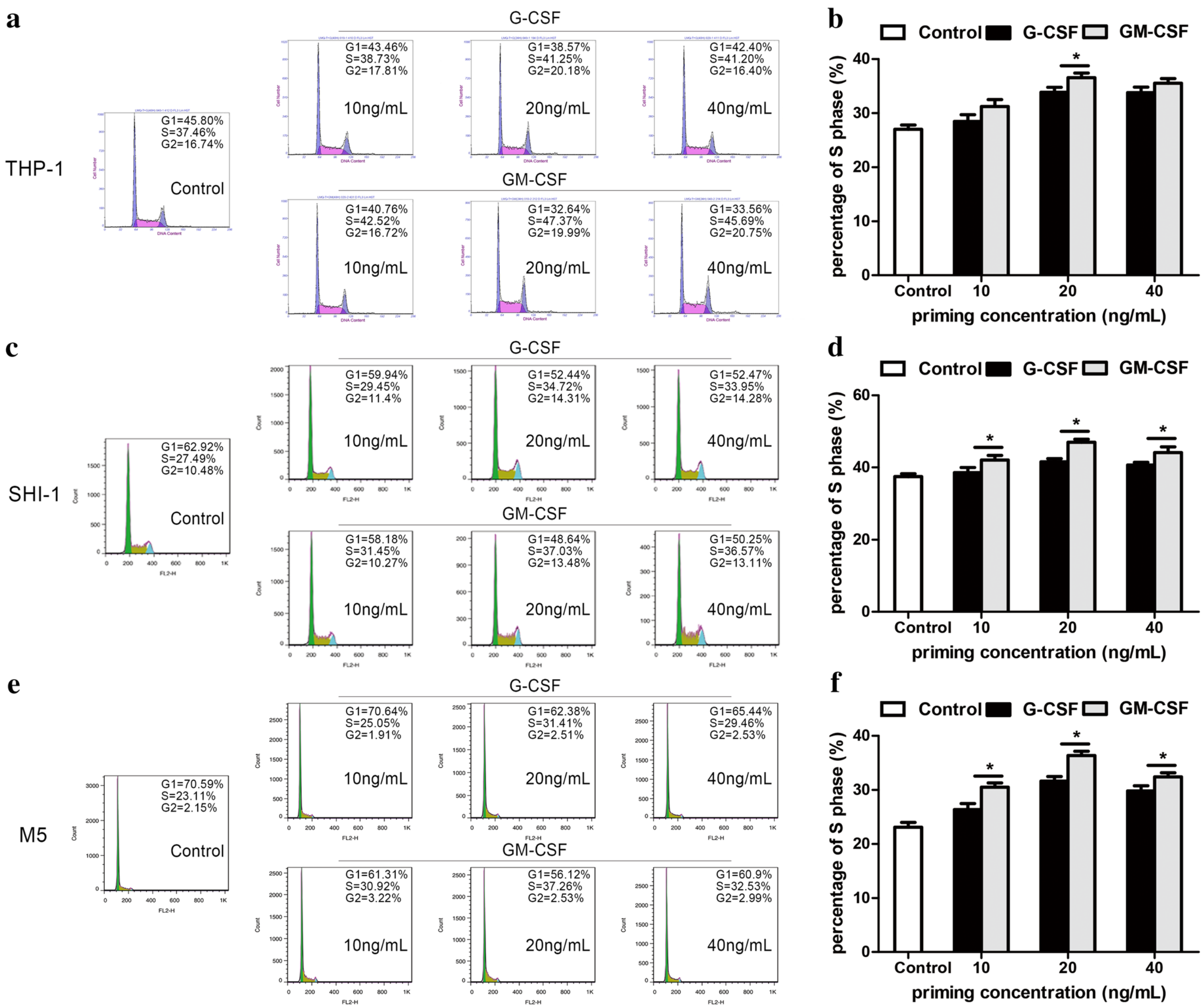

Fig. 2 Effect of G-CSF (GM-CSF) on cell cycle in acute monocytic leukemia cells

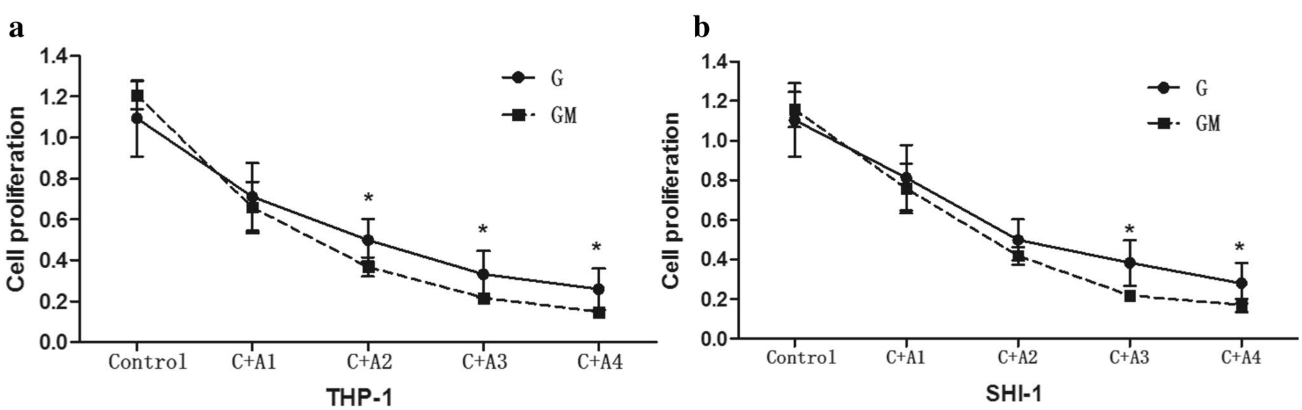

Fig. 3 Cell proliferation rate in cell lines by CCK-8 assay

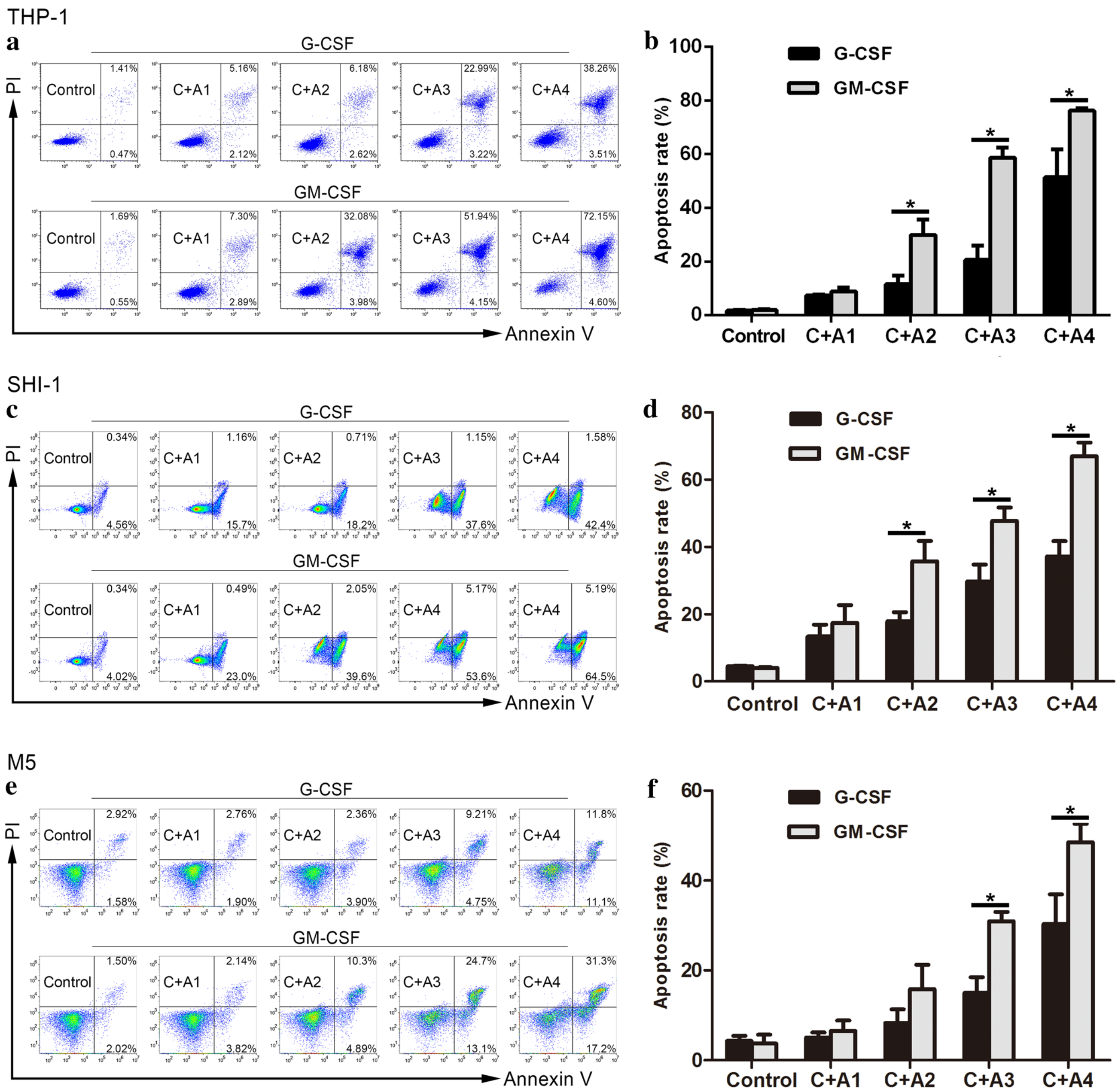

Fig. 4 Effect of CAG and CAGM on cell lines and primary cells

Apoptosis can be induced by intrinsic pathway (mitochondria) with activation of caspase 9 and extrinsic pathway with activation of caspase 8. Activated caspase 8 and caspase 9 then induces caspase 3 cleavage [25]. Our results showed that caspase 8 was not detectable in the presence of either CAGM or CAG, indicating that only mitochondrial pathway was involved in apoptosis process induced by these two regimens. As expected, caspase 9 and cleaved caspase 3 were activated more strongly by CAGM than by CAG. The previous studies have demonstrated a negative correlation between Bcl-2/Bax ratio and apoptotic sensitivity [26, 27]. In THP-1 and SHI-1 cells, the decrease of Bcl-2/Bax ratio upon CAG and CAGM treatment suggested that these two members of Bcl-2 family were involved in mitochondria-mediated apoptotic pathway.

Encouraged by findings from experiments of cell lines, we further harvested bone marrow mononuclear cells from M5 subtype patients for similar investigations. Again, more notable accumulation of S phase population was observed in the GM-CSF group than in the G-CSF group ($P < 0.05$) (Fig. 2e, f). The percentage of apoptosis cell was also higher in the GM-CSF group ($P < 0.05$)

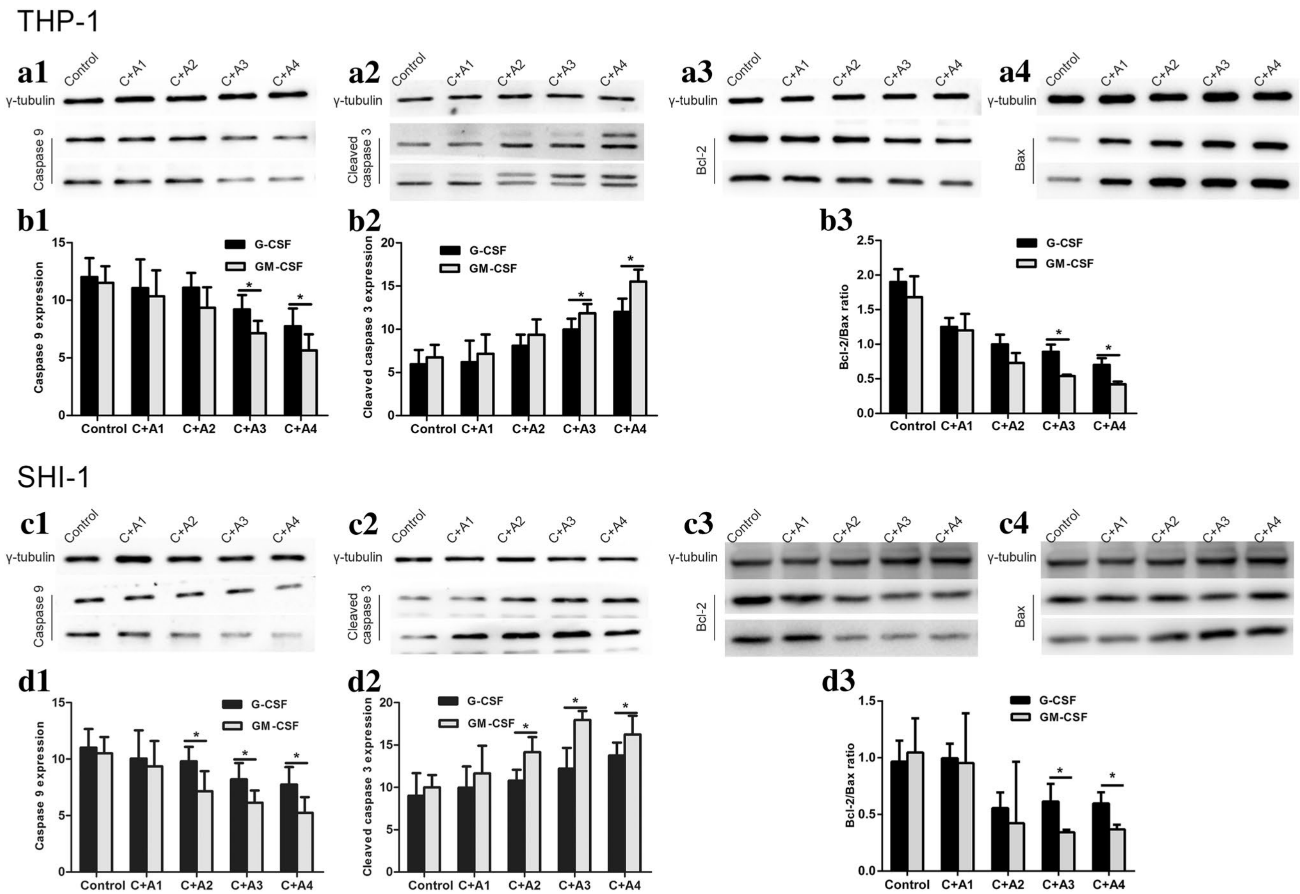

Fig. 5 Western blot analysis of level of caspase 9, cleaved caspase 3, Bcl 2, and Bax in cell lines

(Fig. 4e, f). Interestingly, we noticed a small increase in the percentage of S phase resulted in a dramatically higher sensitivity of leukemic cells to chemotherapy. This indicated that other mechanisms may be involved in addition to GM-CSFR signal pathway.

In conclusion, our study demonstrated that priming with GM-CSF instead of G-CSF can maximize the cytotoxic activity of chemotherapy in acute monocytic leukemia cells in vitro. Further clinical trials are desirable to evaluate the potential benefits of CAGM regimen on patients with acute monocytic leukemia.

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Compliance with ethical standards

Conflict of interests All authors declare that they have no conflict of interest.

References

1. Tafuri A, Andreeff M (1990) Kinetic rationale for cytokine-induced recruitment of myeloblastic leukemia followed by cycle-specific chemotherapy in vitro. Leukemia 4:826–834. http://www.ncbi.nlm.nih.gov/pubmed/2243506
2. te Boekhorst PA et al (1993) Enhanced chemosensitivity of clonogenic blasts from patients with acute myeloid leukemia by G-CSF, IL-3 or GM-CSF stimulation. Leukemia 7:1191–1198. http://www.ncbi.nlm.nih.gov/pubmed/7688839
3. Saito K et al (2000) Low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor (CAG regimen) for previously treated patients with relapsed or primary resistant acute myelogenous leukemia (AML) and previously untreated elderly patients with AML, secondary AML, and refractory anemia with excess blasts in transformation. Int J Hematol 71:238–244. http://www.ncbi.nlm.nih.gov/pubmed/10846828
4. Li X et al (2015) Efficacy of cytarabine, aclarubicin and granulocyte colony-stimulating factor (CAG) regimen compared to FLAG regimen for adult patients with relapsed/refractory Philadelphia chromosome-negative acute lymphoblastic leukemia. Leuk Res. https://doi.org/10.1016/j.leukres.2015.08.013

5. Ornstein MC et al (2015) More is better: Combination therapies for myelodysplastic syndromes. Best Practice Res Clin Haematol 28:22–31. https://doi.org/10.1016/j.beha.2014.11.002
6. Bai A et al (1999) Priming with G-CSF effectively enhances low-dose Ara-C-induced in vivo apoptosis in myeloid leukemia cells. Exp Hematol 27:259–265. https://doi.org/10.1016/S0301-472X(98)00041-1
7. Dartsch DC et al (2002) Comparison of anthracycline-induced death of human leukemia cells: programmed cell death versus necrosis. Apoptosis 7:537–548. https://doi.org/10.1023/A:1020647211557
8. Graf M et al (2004) Expression and prognostic value of hemopoietic cytokine receptors in acute myeloid leukemia (AML): implications for future therapeutical strategies. Eur J Haematol 72:89–106. https://doi.org/10.1046/j.0902-4441.2003.00184.x
9. Schmetzer HM et al (1999) GM-CSF stimulates proliferation of clonal leukemic bone marrow cells in acute myeloid leukemia (AML) in vitro. Ann Hematol 78:449–455. https://doi.org/10.1007/s002770050597
10. Faderl S et al (2003) Granulocyte-macrophage colony-stimulating factor (GM-CSF) induces antiapoptotic and proapoptotic signals in acute myeloid leukemia. Blood 102:630–637. https://doi.org/10.1182/blood-2002-06-1890
11. Reuter C et al (1997) Differential effect of GM-CSF pretreatment on intracellular Ara-C metabolism in normal bone marrow mononuclear cells vs acute myeloid leukemia (AML) blasts. Leukemia 11:561–571. https://doi.org/10.1038/sj.leu.2400613
12. Thomas X et al (2010) Which AML subsets benefit from leukemic cell priming during chemotherapy? Long-term analysis of the ALFA-9802 GM-CSF study. Cancer 116:1725–1732. https://doi.org/10.1002/cncr.24943
13. Thomas X et al (2007) Effect of priming with granulocyte-macrophage colony-stimulating factor in younger adults with newly diagnosed acute myeloid leukemia: a trial by the Acute Leukemia French Association (ALFA) Group. Leukemia 21:453–461. https://doi.org/10.1038/sj.leu.2404521
14. Yang H et al (2017) The incidence and distribution characteristics of MLL rearrangements in Chinese acute myeloid leukemia patients by multiplex nested RT-PCR. Technol Health Care 25:S259–S265. https://doi.org/10.3233/Thc-171329
15. Sironi S et al (2015) Microenvironmental hypoxia regulates FLT3 expression and biology in AML. Sci Rep 5:17550. https://doi.org/10.1038/srep17550
16. Nakase K et al (2015) Prognostic relevance of cytokine receptor expression in acute myeloid leukemia: interleukin-2 receptor alpha-chain (CD25) expression predicts a poor prognosis. PLoS One 10:e0128998. https://doi.org/10.1371/journal.pone.0128998
17. Bennett JM et al (1985) Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 103:620–625. https://doi.org/10.7326/0003-4819-103-4-620
18. Vardiman JW et al (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114:937–951. https://doi.org/10.1182/blood-2009-03-209262
19. Arber DA et al (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127:2391–2405. https://doi.org/10.1182/blood-2016-03-643544
20. Zhu HH et al (2013) Cytarabine, aclarubicin and granulocyte colony-stimulating factor regimen represents an effective and safe salvage regimen for patients with acute myeloid leukemia refractory to first course of induction chemotherapy. Leuk Lymphoma 54:2452–2457. https://doi.org/10.3109/10428194.2013.776679
21. Moon HW et al (2006) Therapeutic use of granulocyte-colony stimulating factor could conceal residual malignant cells in patients with AML1/ETO+ acute myelogenous leukemia. Leukemia 20:1408–1413. https://doi.org/10.1038/sj.leu.2404286
22. Wang Y et al (2011) Salvage chemotherapy with low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor priming in patients with refractory or relapsed acute myeloid leukemia with translocation (8;21). Leuk Res 35:604–607. https://doi.org/10.1016/j.leukres.2010.11.003
23. Hubeek I et al (2004) The effect of G-CSF on the in vitro cytotoxicity of cytarabine and fludarabine in the FLAG combination in pediatric acute myeloid leukemia. Int J Oncol 25:1823–1829. https://doi.org/10.3892/ijo.25.6.1823
24. Liu L et al (2014) Increasing the dose of aclarubicin in low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor (CAG regimen) can safely and effectively treat relapsed or refractory acute myeloid leukemia. Int J Hematol 99:603–608. https://doi.org/10.1007/s12185-014-1528-8
25. Ulukaya E et al (2011) Apoptosis: why and how does it occur in biology? Cell Biochem Funct 29:468–480. https://doi.org/10.1002/cbf.1774
26. Kontos CK et al (2014) Apoptosis-related BCL2-family members: key players in chemotherapy. Anticancer Agents Med Chem 14:353–374. https://doi.org/10.2174/18715206113139990091
27. Ren X et al (2013) Enhancement of baicalin by hexamethylene bisacetamide on the induction of apoptosis contributes to simultaneous activation of the intrinsic and extrinsic apoptotic pathways in human leukemia cells. Oncol Rep 30:2071–2080. https://doi.org/10.3892/or.2013.2684