NLRP3 participates in the differentiation and apoptosis of PMA-treated leukemia cells

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Abstract. Acute myeloid leukemia (AML) is a clonal malignant proliferative disease. In recent years, with the use of all-trans retinoic acid to induce cancer cell differentiation in acute promyelocytic leukemia, and its advantages of high efficacy and low toxic side effects, tumor differentiation therapy has become a research hotspot; however, the mechanisms underlying its role remain to be fully established. Nod-like receptor family pyridine domain containing 3 (NLRP3) is the most extensively studied and well-characterized inflammasome, is involved in a variety of inflammation-related diseases, including cancer, and is a very attractive potential target for the study of novel therapeutic agents. Activation of the NLRP3 inflammasome is a double-edged sword in tumor therapy, with evidence of protective anti-tumor and pro-tumor effects in different types of cancer. Whether the NLRP3 inflammasome promotes disease progression or exerts a protective anti-tumor effect in hematological malignancies remains contested. In the present study, the protective anti-tumor effects of NLRP3 on leukemia cells during their differentiation and maturation were investigated. It was found that the upregulation of NLRP3 expression induced using Phorbol 12-Myristate 13-Acetate played a role in promoting the differentiation and maturation of leukemia cells into monocytes/macrophages, and it was directly involved in the apoptosis of leukemia cells and the differentiation and maturation of CD11b$^+$ cells. These results provide novel theoretical evidence for exploring the mechanism of differentiation therapy in leukemia and improves our understanding of the role of NLRP3 in hematologic tumors.

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Introduction

Acute myeloid leukemia (AML) is a clonal malignant proliferative disease that is caused by the blocking of hematopoietic stem cells at a specific stage of directed differentiation and the accumulation of immature blood cells. The current standard treatment regimen can achieve complete remission (CR) in 50-70% of patients with AML, but ~76% of patients relapse or die due to the development of resistance (1,2). In recent years, with the use of all-trans retinoic acid to induce the differentiation of cancer cells in acute promyelocytic leukemia, with its advantages of high efficacy and low side effects, tumor differentiation therapy has become a research hotspot; however, the mechanism of its action remains unclear (1). Therefore, clarifying the mechanisms of differentiation in the treatment of leukemia may assist in identifying low toxicity and efficacious treatments, to eventually reduce the recurrence of AML in patients (2).

Nod-like receptor (NLR) family pyridine domain containing 3 (NLRP3) is an important pattern recognition receptor (PRR) in the cytoplasm, and its tripartite domain organization consists of a carboxyterminal leucine-rich repeat (LRR) domain with self-inhibitory function and signal recognition ability, a central nucleotide-binding domain (NACHT) with ATPase activity and mediating self-oligomerization, and an amino-terminal pyridines (PYD) domain recruiting apoptosis-associated dot-like proteins containing CARD (ASC) (3). For innate immune defense and maintenance of intracellular environmental homeostasis in the face of microbial infection, endogenous danger signals, and environmental stimuli including antitumor drugs, NLRP3 acts as a sensor that recruits ASC and caspase-1 to form a cytoplasmic multiprotein complex, known as the NLRP3 inflammasome. The NLRP3 inflammasome can activate protease caspase-1 to promote the release of active IL-1β and IL-18 and participate in the body's immune response (4). Recent studies have shown that NLRP3

inflammasome activation leads not only to pyroptosis, but also to other types of cell death, including apoptosis, necrosis, and ferroptosis. In addition, various cell death effectors have been reported to regulate NLRP3 inflammasome activation, suggesting that cell death is closely related to NLRP3 inflammasome activation (5-7).

Aberrant activation of the NLRP3 inflammasome has been implicated in the pathogenesis of various inflammatory diseases, such as diabetes, cancer, and Alzheimer's disease. There is evidence that NLRP3 has protective anti-tumor effects as well as pro-tumor effects in different types of tumors. In leukemia, the NLRP3 inflammasome can cause bone marrow hyperplasia, cytopenia, and splenomegaly amongst other diseases (8). However, whether the activation of the NLRP3 inflammasome plays a malignant role in the progression of leukemia remains contested. It has been found that bone marrow dendritic cells (DC) activate NLRP3, playing an anti-leukemic role in AML through the IL-1β/Th1/IFN-γ pathway (9). In addition, the activation of the NLRP3 inflammasomes by cancer chemotherapy drugs has been confirmed in the treatment of malignant mesothelioma, hematological, and other solid tumors. The mechanism primarily involves the activation of IL-1β by NLRP3 inflammasomes to induce a burst of inflammation (10,11). However, to the best of our knowledge, whether NLRP3 activation is involved in the apoptosis of leukemic cells induced by chemotherapeutic drugs has not been reported.

In the present study, it was shown that NLRP3 activation played an anti-cancer role in the treatment of AML through *in vitro* experiments; NLRP3 was involved in the differentiation and apoptosis of cells in the differentiation treatment of leukemia.

Materials and methods

Cell culture. THP-1 Human Monocyte Leukemia Cells (promonocytic cell line) were cultured in RPMI-1640 medium (cat. no. 11875176; Invitrogen; Thermo Fisher Scientific, Inc.) with 10% FBS (cat. no. 10100147C; Invitrogen; Thermo Fisher Scientific, Inc.), 0.05 mM β-mercaptoethanol (cat. no. 21985023; Invitrogen; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (cat. no. P1400; Beijing Solarbio Science & Technology Co., Ltd.) at 37˚C in a humidified incubator supplied with 5% CO_2.

Phorbol 12-Myristate 13-Acetate (PMA) treatment. Different concentrations (0, 50, 100, 200 and 400 ng/ml) of PMA (cat. no. 16561-29-8; MilliporeSigma) were added to $1x10^5$/ml cells and cells were incubated as above for 24 or 48 h prior to the following assays.

Giemsa staining. A Giemsa Stain kit (cat. no. G8220) was purchased from Beijing Solarbio Science & Technology Co., Ltd. THP-1 cell slides were prepared and fixed with methanol for 2 min at room temperature according to the manufacturer's protocol. The cell slides were stained using the Giemsa staining solution at room temperature for 40-60 min, after which they were washed three times with PBS buffer. Once they had dried, they were examined by light microscopy (Leica Microsystems GmbH) at a magnification of x20, x40 and x100.

Cell Counting Kit (CCK)-8 assay. Cells were seeded into 96 well plates according to the instructions of the CCK-8 assay (cat. no. G4103, Wuhan ServiceBio Technology Co., Ltd.). After the addition of the CCK-8 solution, cells were incubated for a further 4 h. Subsequently, the absorbance was measured at 450 nm using a microplate reader (Multiskan sky; Thermo Fisher Scientific, Inc.).

Apoptosis analysis. An Annexin V-FITC/7-AAD (cat. no. MA0428, Meilune) was used followed by flow cytometry to assess apoptosis. The cells were centrifuged at 200 x g for 5 min at room temperature, harvested, resuspended in precooled 1x PBS, and centrifuged at 200 x g for 5 min at room temperature, after which 300 µl 1x Binding Buffer was added. After the addition of 5 µl Annexin V-FITC to the mixture, the mixture was incubated for 15 min in the dark at room temperature. An additional 5 µl PI was added and cells were stained for 5 min at room temperature, after which 200 µl 1x Binding Buffer was added, and loaded onto the flow cytometer (NovoCyte, Agilent Technologies, Inc.). Apoptosis was analyzed using FlowJo 10.8.1 software (Becton, Dickinson and Company).

Detection of CD11b and CD14 by flow cytometry. After collecting the cells, the concentration was adjusted to $1x10^6$ cells/ml. Precooled 100 µl PBS was added to each flow tube and the cells were resuspended, followed by the addition of 5 µl PE-CD11b fluorescent antibody (cat. no. 301306, BioLegend, Inc.) and FITC-CD14 fluorescent antibody (cat. no. 301804, BioLegend, Inc.), respectively, and incubated at 4˚C in the dark for 30 min. Cells were subsequently resuspended in 200 µl PBS at 4˚C by centrifugation at 200 x g for 4 min and washed twice with PBS. Isotype rat IgG was used as the negative control.

Western blotting. Total cell protein was extracted using RIPA lysis buffer (cat. no. P0013B, Beyotime Institute of Biotechnology), loaded (30 µg/lane) on a 10% SDS-gel, resolved using SDS-PAGE and transferred to PVDF membranes (MilliporeSigma). The primary antibodies against NLRP3 (1:1,000; cat. no. 13158, Cell Signaling Technology, Inc.) or GAPDH (1:2,000; cat. no. TA-0A, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) were added to membranes and incubated overnight at 4˚C. The secondary antibodies used were HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H+L) (1:3,000; cat. no. SA00001-2, ProteinTech Group, Inc.) and HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L) (1:4,000; cat. no. SA00001-1, ProteinTech Group, Inc.). The bands were visualized using a Tanon 5200 using an ECL assay and assessed using ImageJ version 1.53 (National Institutes of Health). β-actin was used as the loading control.

RNA interference. shRNAs targeting NLRP3 (sh-NLRP3) and the negative control were obtained from Shanghai GenePharma Co., Ltd. The element sequence of the vector GV493 (Shanghai GenePharma Co., Ltd.) was hU6-MCS-CBh-gcGFP-IRES-puromycin and the shRNA sequence was inserted into MCS. The DNA sequence for shRNA-NLRP3 was 5'-CCGGCCGTAAGAAGTACAGAAAGTACTCGAGTACTTTCTGTACTTCTTACGGTTTTTG-3';

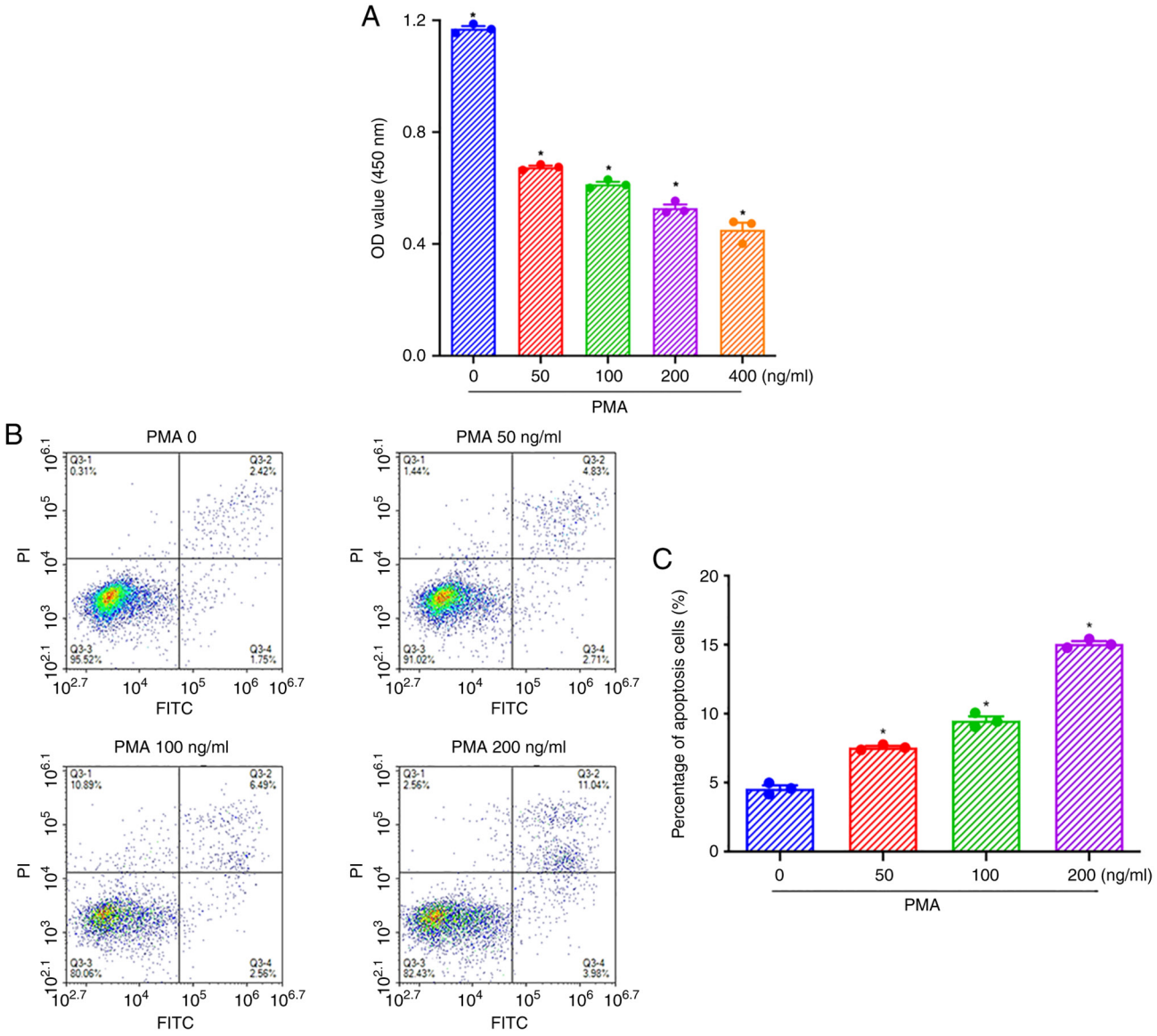

Figure 1. PMA at 100 ng/ml was the optimal concentration to stimulate apoptosis of THP-1 cells. (A) CCK-8 assays to determine the proliferation of THP-1 cells treated with 0, 50, 100, 200, or 400 ng/ml PMA for 48 h. (B) Flow cytometry was used to assess the apoptosis of THP-1 cells treated with 0, 50, 100, and 200 ng/ml PMA for 48 h. (C) Statistical analysis of the rate of apoptosis of THP-1 cells treated with different concentrations of PMA. *P<0.05 vs. control. PMA, Phorbol 12-Myristate 13-Acetate; OD, optical density.

and the sequence of the negative control was 5'-CCGGCC TTCTCCGAACGTGTCACGTCTCGAGTACTTTCTGTA CTTCTTACGGTTTTTG-3'. THP-1 cells were cultured in six-well plates ($5x10^5$ cells/well) and transfected with 1.5 μg shRNA-NLRP3 using 10 μl Lipofectamine™ 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions.

Reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from cells using a GoldHi Plasmid Mini Kit (cat. no. CW0581, Jiangsu Cowin Biotech Co., Ltd) according to the manufacturer's instructions. The mRNA levels of target genes were analyzed by qPCR using a Bio-Rad iCycler system (Bio-Rad Laboratories, Inc.). cDNA was synthesized from 0.5 ng total RNA using an Evo M-MLV RT MasterMix (cat. no. AG11728, Accurate biology) in a 10 μl reaction volume according to the manufacturer's protocol. qPCR was performed with 2 μl cDNA and gene-specific primers in a final reaction volume of 25 μl. Amplification was performed using a SYBR® Green Pro Taq HS Premix (cat. no. AG11740, Accurate biology), and the thermocycling conditions were as follows: Initial denaturation at 95˚C for 30 sec; followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. The relative mRNA expression was assessed using the $2^{-\Delta\Delta Cq}$ method (12). The sequences of the primers used were: NLRP3 forward, 5'-GCG CCTCAGTTAGAGGATGT-3' and reverse, 5'-ACCAGCTAC AAAAAGCATGGA-3'; and GAPDH forward 5'-AGAAGG CTGGGGCTCATTTG-3' and reverse, 5'-AGGGGCCATCCA CAGTCTTC-3'.

Statistical analysis. All the experiments were performed in triplicate; all data were analyzed using GraphPad Prism version 9 (GraphPad Software, Inc.) and are presented as the mean ± standard error of the mean. Differences in mean values

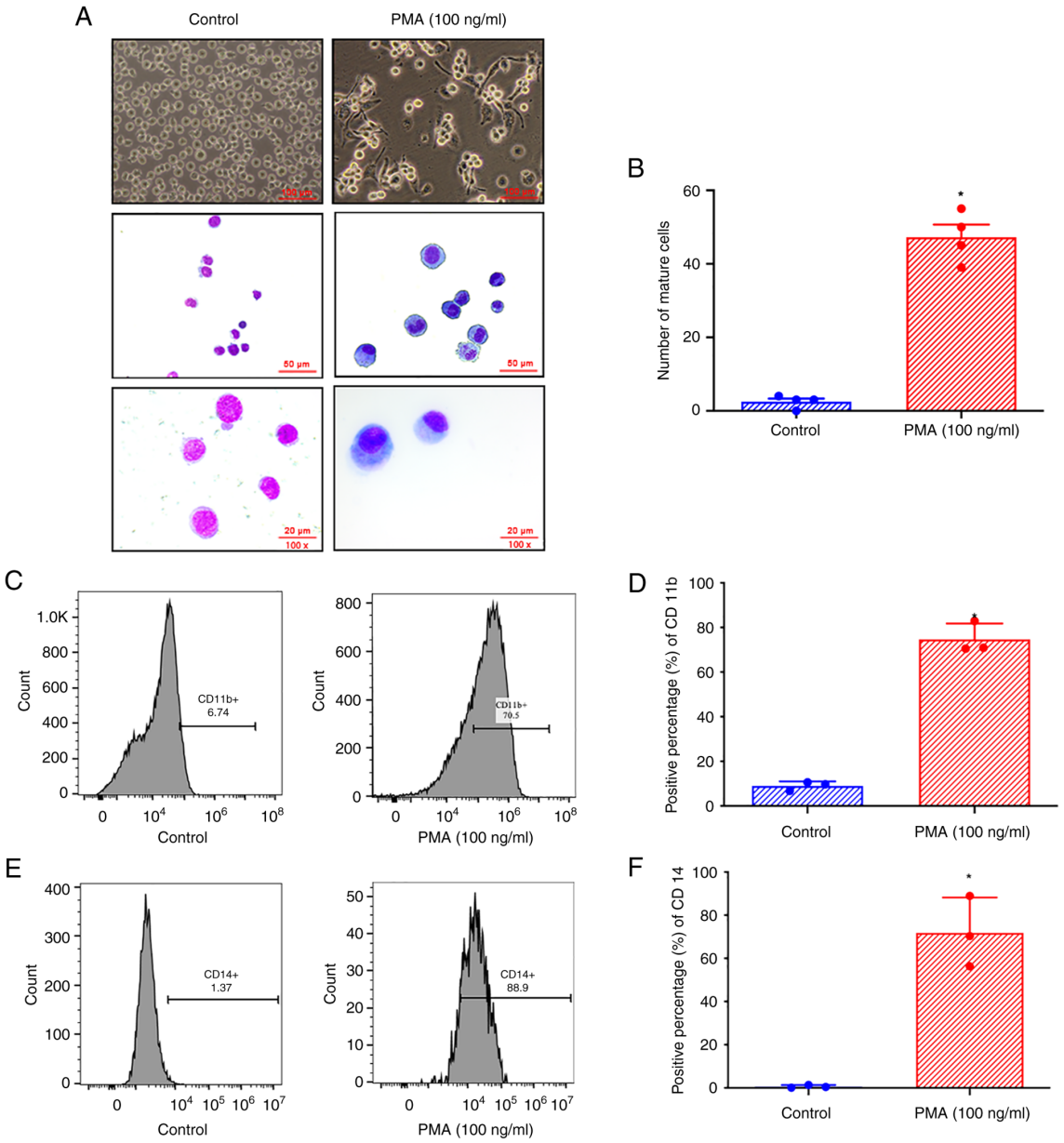

Figure 2. PMA altered the morphology of THP-1 cells and induced cell differentiation of THP-1 cells. (A) Giemsa staining was used to show the morphology of THP-1 cells. Cells were treated with 100 ng/ml PMA for 48 h. Representative images of the stained cells. Scale bars: Upper, 100 μm; middle, 50 μm; and lower, 20 μm. Magnification, x100. (B) Statistical analysis of percentage of immature and mature cells. (C and D) Flow cytometry analysis was used to determine CD11b expression in PMA treated THP-1 cells. (E and F) Flow cytometry analysis was used to determine CD14 expression in PMA treated THP-1 cells. *P<0.05 vs. control. NLRP3, Nod-like receptor family pyridine domain containing 3; PMA, Phorbol 12-Myristate 13-Acetate.

between groups were examined using a one-way ANOVA followed by a Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

PMA at 100 ng/ml is the optimal concentration to stimulate the apoptosis of THP-1 cells. To determine the optimal concentration of PMA, THP-1 cells were stimulated with 50, 100, 200, or 400 ng/ml PMA for 48 h. The results of the CCK-8 assays showed that all concentrations of PMA could significantly inhibit the proliferation of THP-1 cells, and the degree of inhibition of cell proliferation increased in a concentration-dependent manner (Fig. 1A). The results of flow cytometry showed that treatment with 50, 100, and 200 ng/ml PMA for 48 h significantly induces apoptosis of THP-1 cells (Fig. 1B). Statistical analysis showed that the apoptosis of THP-1 cells increased significantly in a dose-dependent manner (Fig. 1C). Thus, PMA at 100 ng/ml was chosen as the optimal concentration, as this concentration was also used in previous studies (13,14).

PMA promotes the maturation and differentiation of THP-1 cells into monocytes/macrophages. Giemsa staining showed that after 48 h of treatment with 100 ng/ml PMA, the morphology of THP-1 cells was notably altered; the cells became significantly larger, accompanied by an increase in the volume of cytoplasm, had a decreased nucleus-cytoplasm

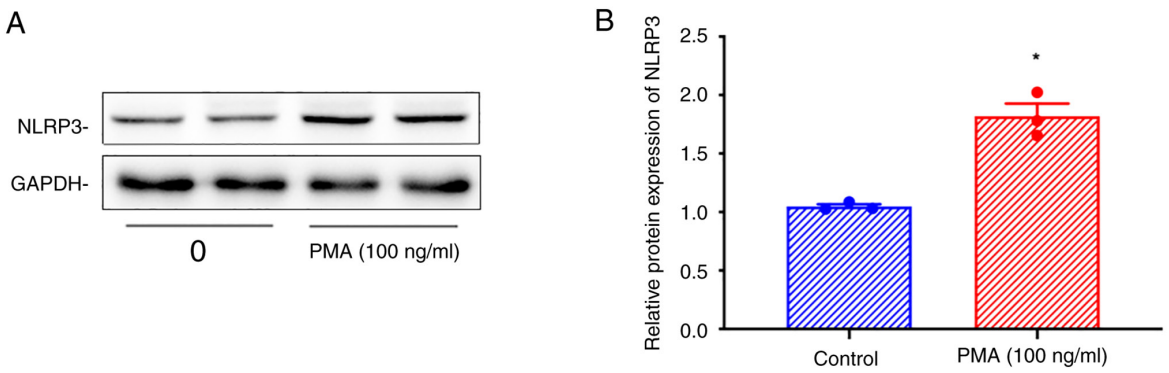

Figure 3. NLRP3 is upregulated following PMA treatment. (A) Western blotting was used to determine the protein expression levels of NLRP3 in THP-1 cells treated with PMA. (B) Statistical analysis of NLRP3 expression in THP-1 cells treated with PMA. *P<0.05 vs. control. NLRP3, Nod-like receptor family pyridine domain containing 3; PMA, Phorbol 12-Myristate 13-Acetate.

ratio, and exhibited a decrease in the size of the nucleus (Fig. 2A). Cell enlargement was accompanied by increased cytoplasmic volume, a decreased nucleus-cytoplasm ratio, and a decrease in the size of the nucleus. Compared with the control group, THP-1 cells treated with 100 ng/ml PMA for 48 h had a statistically significant increase in the number of mature cells per high-power field of view (Fig. 2B), indicating that PMA promoted the maturation of THP-1 cells. Flow cytometry was used to determine the expression of CD11b in THP-1 cells after 48 h of treatment with 100 ng/ml PMA (Fig. 2C). Analysis showed that compared with the control group, the expression of CD11b in THP-1 cells treated with 100 ng/ml PMA for 48 h was significantly increased (Fig. 2D). Flow cytometry was used to determine the expression of CD14 in THP-1 cells treated with 100 ng/ml PMA for 48 h (Fig. 2E). Analysis showed that compared with the control group, the expression of CD14 in THP-1 cells treated with 100 ng/ml PMA for 48 h was significantly increased (Fig. 2F), which indicated that PMA promoted the differentiation of THP-1 cells into monocytes/macrophages.

PMA treatment increases NLRP3 expression in THP-1 cells. Western blotting showed that the expression of NLRP3 was increased in THP-1 cells treated with 100 ng/ml PMA for 48 h (Fig. 3A and B).

NLRP3 knockdown inhibits the pro-apoptotic effects of PMA on THP-1 cells and the increased expression of CD11b. After shRNA-NLRP3 transfection, the expression of NLRP3 in THP-1 cells was significantly decreased (Fig. 4A). Flow cytometry showed that the levels of apoptosis of THP-1 cells increased after 48 h of treatment with 100 ng/ml PMA, and this apoptosis decreased in shNLRP3 transfected cells (Fig. 4B). Statistical analysis showed that compared with the control group, THP-1 cells treated with 100 ng/ml PMA for 48 h exhibited increased apoptosis, and shNLRP3 transfection reduced apoptosis significantly, indicating that NLRP3 was involved in the regulation of PMA-induced THP-1 cell apoptosis (Fig. 4C). Flow cytometry showed that the expression of CD11b in THP-1 cells increased after treatment with 100 ng/ml PMA for 48 h, and the expression of CD11b decreased after transfection with shNLRP3 (Fig. 4B). Statistical analysis found that compared with the control group, the apoptosis of THP-1 cells treated with 100 ng/ml PMA for 48 h increased, and the expression of CD11b in cells transfected with shNLRP3 decreased significantly, indicating that NLRP3 was involved in the regulation of PMA-induced expression of CD11b in THP-1 cells (Fig. 4C).

Discussion

AML is a differentiating system (15). For >40 years, although combination therapy with cytarabine and anthracyclines, such as daunorubicin, has been the mainstay of AML treatment, the 5-year survival rate for adult AML patients is <30% (16). The overall long-term survival rate of the combined treatment with all-trans retinoic acid and arsenic trioxide for acute promyelocytic leukemia (APL) is >90%, suggesting that treatment based on differentiation of AML may have a promising future (17). Therefore, exploring the molecular mechanism of differentiation therapy and identifying novel targets for differentiation therapy has become a topic of increased interest in the treatment of AML. However, due to the high heterogeneity of AML, relevant targets and drugs have remained elusive. In the present study, the molecular mechanisms identified showed that NLRP3 promoted the apoptosis and differentiation of leukemia cells, through the upregulation of $CD11b^+$ and $CD14^+$ cells in the process of THP-1 differentiation following PMA treatment (Fig. 5).

The inflammasome is a polymeric protein complex sensitive to stimuli such as microbial motifs, endogenous danger signals and environmental irritants, and this is part of the innate immune response (18). NLRP3 is the most extensively studied and characterized inflammatory body, and present as a nucleotide-binding and oligomerization (NACHT) domain and C-terminal leucine-rich repeat (LRR), and N-terminal pyrin domain (PYD). When NLRP3 detects a danger signal, the NACHT domains homo-oligomerize. Subsequently, the PYD domain of NLRP3 recruits the adaptor apoptosis speckprotein (ASC). Subsequently, ASC recruits the CARD of pro-caspase-1, promotes its self-cleavage and formation of active caspase-1, and finally cleaves pro-IL-1β and pro-IL-18 into active IL-1β and IL-18 (4). In short, activation of the NLRP3 inflammasome has two primary roles, one is a pro-inflammatory response to the release of the inflammatory cytokines IL-1β and IL-18, and the other is the induction of

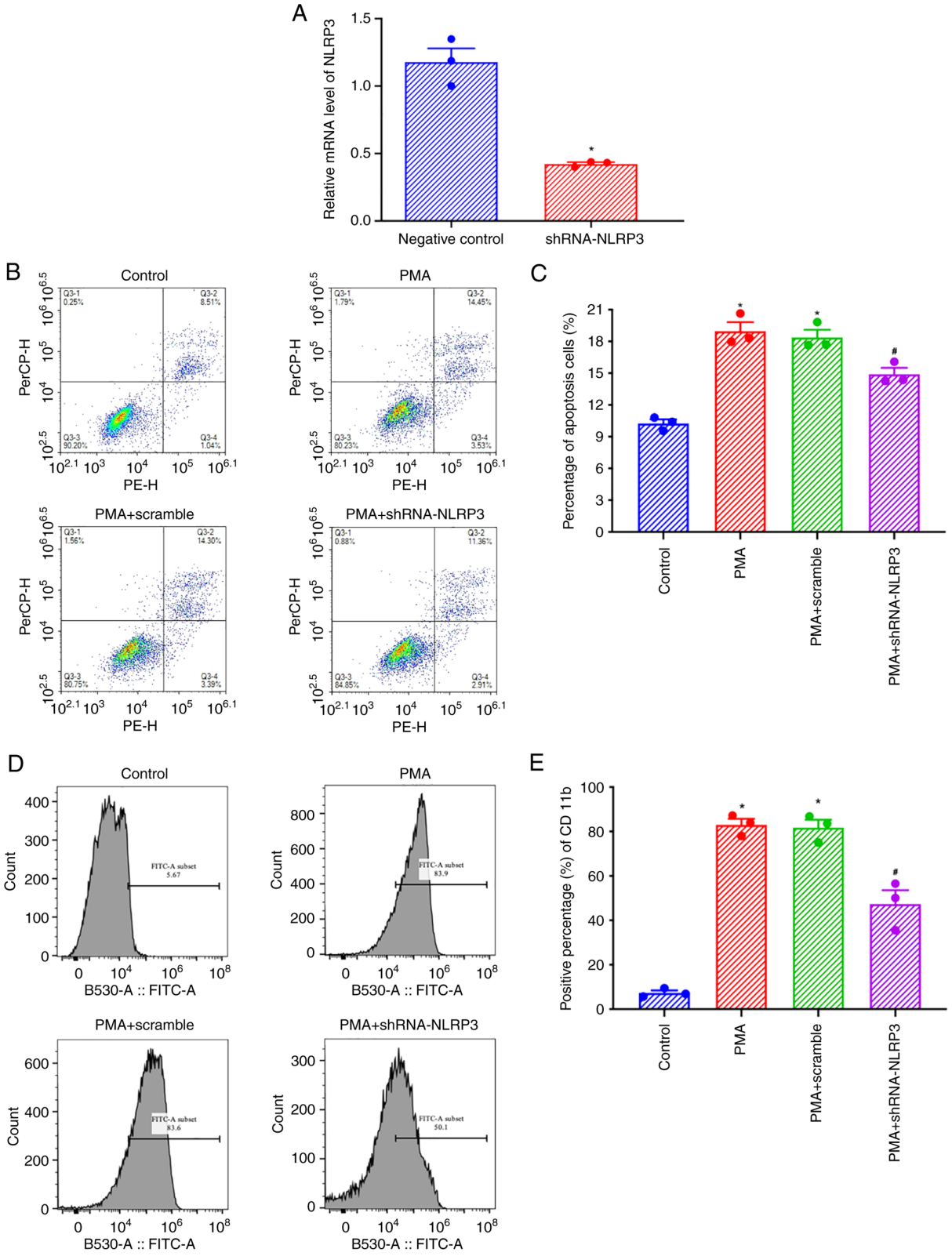

Figure 4. NLRP3 knockdown inhibited the pro-apoptotic effect of PMA on THP-1 cells and the increased expression of CD11b. (A) The knockdown efficiency of shRNA-NLRP3 was determined using reverse transcription-quantitative PCR. (B and C) Flow cytometry analysis showed that NLRP3 knockdown mitigated the pro-apoptotic effect of PMA on THP-1 cells. (D and E) Flow cytometry analysis showed that NLRP3 knockdown decreased the expression of CD11b in THP-1 cells treated with PMA. *P<0.05 vs. control. #P<0.05 vs. PMA+ scramble. NLRP3, Nod-like receptor family pyridine domain containing 3; PMA, Phorbol 12-Myristate 13-Acetate; shRNA, short hairpin RNA; PerCP-H, peridinin-chlorophyll-protein complex; PE, phycoerythrin.

programmed cell death (pyroptosis) (19). ROS generation is involved in this NLRP3 activation process (20,21). The NLRP3 inflammasome is involved in a variety of inflammation-related diseases, including cancer, and is a very attractive potential target for the study of novel therapeutic agents. At present, several small molecule compounds, such as MCC950, are

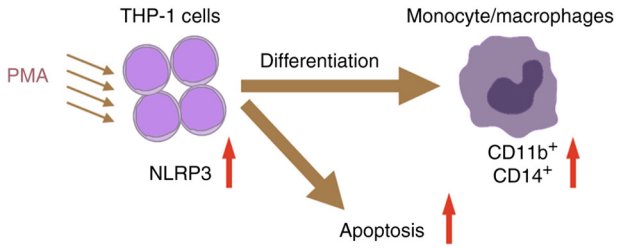

Figure 5. NLRP3 promotes the apoptosis and differentiation of leukemia cells in the process of THP-1 differentiation following PMA treatment. NLRP3, Nod-like receptor family pyridine domain containing 3; PMA, Phorbol 12-Myristate 13-Acetate.

hypothesized to possess specific inhibitory effects on NLRP3 activation (22).

Activation of the NLRP3 inflammasome is a double-edged sword in tumor therapy, with evidence of protective anti-tumor and pro-tumor effects in different types of cancer (23). NLRP3 inflammatory bodies and IL-1β production promote the infiltration of bone marrow cells, such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), providing an inflammatory microenvironment that contributes to the partial protection of breast cancer progression and skin cancer development (24,25). In addition, the role of inflammatory bodies in the pathogenesis of melanoma has also been confirmed (26). NLRP3 signaling may drive immunosuppression in pancreatic cancer by promoting tolerant T-cell differentiation and adaptive immunosuppression through IL-10 (27). Conversely, the NLRP3 inflammasome has also been shown to possess antitumor effects. Tumor cell initiation by dendritic cell-mediated IFN-γ-producing T lymphocytes requires NLRP3 inflammasomes (28). Inflammasome-related molecules play a positive protective anti-tumor role in colorectal cancer metastasis and intestinal inflammatory injury (29,30). NLRP3 plays an antitumor role in melanoma by promoting the migration of myeloid-derived suppressor cells (MDSCs) (31). NLRP3 also plays a protective anti-tumor role in liver cancer (29).

Whether the NLRP3 inflammasome exerts disease progression or protective anti-tumor effects in hematological malignancies remains contested. In chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, and other rare types of AML, NLRP3 inflammasome activation that harbor KRAS mutations play a key role in the characteristic symptoms of leukemia, such as cell reduction, splenomegalysis, and bone marrow hyperplasia (32). Conversely, NLRP3-activated bone marrow dendritic cells have been found to play an anti-leukemic role in AML through an IL-1β/Th1/IFN-γ axis. Activation of NLRP3 inflammasomes affects Th cells in AML. They induce IL-1β-dependent immunity and promote the transformation of CD4+ T cells into Th1 cells that produce tumor-specific IFN-γ allowing for recognition of leukemia cells. In recent years, a considerable number of DC treatments have achieved non-specific and antigen-specific immune effects on AML (9). However, whether each component of the NLRP3 inflammasome is involved in the process of leukemia cell differentiation, and whether it plays a protective anti-tumor or a pro-tumor role has not been determined, to the best of our knowledge. This study focused on the role of NLRP3, the core component of the NLRP3 inflammasome, in the process of THP-1 differentiation. It was found that NLRP3 expression was increased during THP-1 differentiation, and its deletion inhibited the differentiation of leukemia cells, suggesting that the changes in NLRP3 expression levels were directly involved in the differentiation of leukemia cells, which plays a protective anti-tumor role in the differentiation and maturation of leukemia cells.

PMA can promote the apoptosis of tumor cells in the process of promoting the differentiation and maturation of leukemia cells (33). In the present study, different concentrations and durations of PMA treatment were used. The CCK-8 assay showed that >50 ng/ml PMA significantly inhibited the proliferation of THP-1 cells, and the flow cytometry results also showed that >50 ng/ml PMA significantly induced the apoptosis of THP-1 cells. In a preliminary experiment (data not shown), our group also used 72 h of PMA induction. There was no significant difference between cells that underwent PMA induction for 72 h and cells that underwent PMA induction for 48 h in flow cytometry assays, and as the cell growth was fast, the state of cells after 72 h was not as good as that after 48 h of PMA induction. Meanwhile, the concentrations of PMA in other studies ranged from 100-200 nM (61.6-132 ng/ml) (13,14,34-36). Based on previous studies and the results of experiments, 100 ng/ml PMA treatment for 48 h was chosen. In addition, it was found that the effect of PMA on THP-1 cell differentiation and maturation was also affected by cell culture conditions (37). Cells were more sensitive to PMA and $CD14^+$ cells were increased in high-density culture, while cells were less responsive to PMA under low-density culture conditions (37). The present study mainly examined the effect of PMA on NLRP3 in the process of differentiation and maturation of THP-1 cells in high-density cell cultures, while the changes and effects of PMA on NLRP3 in THP-1 cells in low-density cell cultures were not investigated. Therefore, culture conditions will also be considered in future experiments.

NLRP3 knockdown inhibited the proapoptotic effects of PMA on leukemia cells, suggesting that NLRP3 was directly involved in the proapoptotic effects of PMA. Numerous studies have shown that NLRP3 is involved in inflammatory pyroptosis during tumor progression (38,39). Pyroptosis, as a type of apoptosis, plays a prominent role in tumor development and metastasis, although the inflammatory microenvironment of pyroptosis can promote tumor development and metastasis during tumor development (40). As the knockdown of NLRP3 can partially reverse the differentiation and apoptosis of THP-1 cells, NLRP3 was hypothesized to participate in the differentiation of THP-1 cells but was not solely responsible.

The present study is only a preliminary exploration of the role of NLRP3 in the differentiation of acute leukemia cells, so there were some limitations. These included the fact that the effect of NLRP3 knockout on CD14 expression and the changes of cell cycle-related proteins in leukemia cells were not assessed. Additionally, PMA was used to induce differentiation of leukemia cells towards a monocyte/macrophage-like lineage via the JNK/c-JUN pathway, which may involve inflammation. Therefore, the role played by NLRP3 in this induced monocytic lineage differentiation system may not be applicable to other models of leukemia cell differentiation

induced by other inducers, such as APL NB4 cells induced by all-trans retinoic acid, which will be assessed in a future study. Additionally, a series of experiments to further confirm the role of NLRP3 in THP-1 cells and K256 cells induced by PMA, and the effect of knockdown and overexpression of NLRP3 using vectors or ATP+LPS priming in differentiation and cell cycle progression and the role of NK cell interactions with leukemia cells together will be performed.

In conclusion, in the present study, the protective anti-tumor role of NLRP3 in the differentiation and maturation of leukemia cells was investigated. It was found that the upregulation of NLRP3 expression induced by PMA played a role in promoting the differentiation and maturation of leukemia cells, and it was directly involved in the apoptosis of leukemia cells and the differentiation and maturation into monocyte/macrophages. The results of the present study provide a novel theoretical basis for exploring the mechanism of differentiation therapy in leukemia and improves our understanding of the role of NLRP3 in hematologic tumors.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YW and CX designed the study, collected the data, performed the statistical analysis, and drafted the manuscript. XL assisted in data collection and statistical analysis. XC and RJ designed the study and wrote the manuscript. YW and CX confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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