Role of miR-let-7c-5p/c-myc signaling axis in the committed differentiation of leukemic THP-1 cells into monocytes/macrophages

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Received January 25, 2023; Accepted July 12, 2023

DOI: 10.3892/ol.2023.13989

Abstract. In a preliminary experiment, it was found that c-myc expression was decreased following the differentiation of THP-1 cells into monocytes/macrophages induced by phorbol 12-myristate 13 acetate (PMA) + lipopolysaccharide (LPS) + interferon (IFN)-γ. The expression of miR-let-7c-5p was then found to be elevated by cross-sectional analysis using TargetScan and PubMed and differential microarray analysis. The present study aimed to investigate the role of the miR-let-7c-5p/c-myc signaling axis in the committed differentiation of THP-1 leukemic cells into monocytes/macrophages induced by PMA + LPS + IFN-γ. Human THP-1 leukemic cells were induced to differentiate into monocytes/macrophages by PMA + LPS + IFN-γ. Following induction for 48 h, the growth density of the THP-1 cells was observed directly under an inverted microscope, cell proliferation was measured using Cell Counting Kit-8 assay and the cell cycle and the expression of differentiation-related antigens (CD11b and CD14) were measured using flow cytometry. The mRNA expression of miR-let-7c-5p and c-myc was detected using reverse transcription-quantitative PCR and the protein expression of c-myc was detected using western blot analysis. Dual luciferase reporter gene analysis was used to detect the targeted binding of miR-let-7c-5p on the 3'UTR of c-myc. The relative expression of miR-let-7c-5p and c-myc genes in THP-1 cells induced by PMA + LPS + IFN-γ was found to be up- and downregulated respectively, and expression of miR-let-7c-5p was negatively correlated with the expression of c-myc gene. Dual luciferase reporter gene assays confirmed that miR-let-7c-5p targeted the 3'UTR of c-myc and inhibited luciferase activity. Following transfection with miR-let-7c-5p mimics, the expression of c-myc was markedly downregulated and the proliferative ability of the THP-1 cells was decreased, while the expression rate of CD11b and CD14 was significantly increased. The rescue experiment revealed that the effects of miR-let-7c-5p mimics on the proliferation and differentiation of THP-1 cells were attenuated by transfection with c-myc overexpression vector. Together, the findings of the present study demonstrated that miR-let-7c-5p can target the 3'UTR region of c-myc and that the miR-let-7c-5p/c-myc signaling axis is one of the critical pathways involved in the directional differentiation of leukemic cells into monocytes/macrophages.

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Key words: leukemic cells, induced differentiation, macrophage, microRNA-let-7c-5p, c-myc

Introduction

Normal hematopoietic cells differentiate into mature granulocyte, monocyte-macrophage, erythroid, or megakaryocyte lineages, strictly according to the inherent procedure (1). The occurrence of leukemia is mainly due to the failure of the differentiation at a certain stage of hematopoietic cells, which enables them to retain only their hyperproliferative capacity and blocks their terminal differentiation (1,2). If this disordered differentiation of leukemia cells can be reversed using drugs, these drugs can then perhaps be used in clinical treatment (3). In terms of the committed differentiation of myeloid leukemia, in addition to the successful induction of the terminal differentiation of leukemic cells into granulocytes

in vitro using certain drugs, such as all trans retinoic acid (4), there have been some reports on the committed differentiation of leukemic cells into monocytes-macrophages. However, the majority of these processes are limited to the induction of partial differentiation to varying degrees, which limits their clinical application (5). Therefore, an in-depth investigation of the key mechanisms of the committed differentiation of leukemic cells may help to identify new targets which can be used to interfere with the directed differentiation of leukemic cells.

Macrophages are a highly heterogeneous group of cells, which can be polarized by two types of macrophages under various tissue environments and physiological and pathological conditions; for example, M1 and M2 are the two types of macrophages (6-9). Different types of macrophages often exhibit different phenotypes and functions, or even function via opposite mechanisms. For example, M1 type macrophages mainly induce Th1 type immune responses, producing potent antitumor effects (10). M2 type macrophages are divided into three subtypes, M2a, M2b and M2c (11,12). M2a macrophages mainly induce Th2 type immune response and M2b and M2c macrophages mainly exert immunomodulatory effects. The cytokines, GM-CSF and M-CSF, are also involved in the polarization process of macrophages (13); monocytes exposed to granulocyte-macrophage colony-stimulating factor (GM-CSF) exhibit characteristics of M1-type macrophages, whereas those exposed to macrophage colony-stimulating factor (M-CSF) exhibit characteristics of M2-type macrophages (14,15). It may be possible to induce leukemic cells to differentiate into mature macrophages, thus achieving directed differentiation induction therapy; at the same time, the induction of mature M1 macrophages may also play a role in anti-leukemia immunity. As regards the molecular mechanisms of the polar differentiation of macrophages, some associated transcription factors and signal transduction pathways have been found to be involved. Currently, it is well recognized that the PU.1 and C/EBP families are key transcription factors that regulate the committed differentiation of monocyte/macrophage lineages (16,17). Second, the Janus kinase (JAK)/STAT, PI3K/AKT/mTOR and Notch signaling pathways have also been reported to play a role in the polarization of macrophages (18-20). Although the proto-oncogene c-myc has been shown to induce or suppress the expression of hundreds of genes, including regulating macrophage activation functions (21,22), the specific molecular mechanisms of c-myc in the polarization and differentiation of leukemic cells into M1 type macrophages have not yet been fully reported.

The authors have previously confirmed that c-myc is involved in the committed differentiation of THP-1 leukemic cells into monocytes/macrophages (23). Following validation in a preliminary experiment that no significant apoptosis occurred during the differentiation induction process, the present study aimed to further explore the changes and roles in directed differentiation. In addition, non-coding RNAs (ncRNAs) have been found to play a critical role in cell proliferation and differentiation in recent years (24). Among these, microRNAs (miRNAs/miRs) are often involved in the post-transcriptional regulation of downstream target genes by functioning as oncogenes or tumor suppressor genes (25); they are also involved in some key pathological processes, such as the abnormal proliferation, differentiation or apoptosis of leukemic cells (26,27). Therefore, it was hypothesized that some miRNAs may regulate the committed differentiation of leukemic cells into monocytes/macrophages by targeting the c-myc gene. It was predicted that miR-let-7 family members are negatively associated with c-myc; by performing differential miRNA Chip screening, Gene Expression Omnibus (GEO) database analysis and a PubMed literature research, it was found that the possible upstream miRNA of c-myc was miR-let-7c-5p. The present study aimed to further investigate the role of the miR-let-7c-5p/c-myc signaling axis in the differentiation of leukemic cells into monocytes/macrophages.

Materials and methods

Materials. The human acute myeloid leukemia THP-1 cell line was purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Phorbol 12-myristate 13-acetate (PMA; cat. no. P1585-1MG), lipopolysaccharide (LPS; cat. no. L2880-10MG) and interferon (IFN)-γ (cat. no. C600039-0100) were purchased from MilliporeSigma. Fetal bovine serum (FBS), the BCA protein assay kit and the SDS-PAGE gel rapid preparation kit were purchased from Shanghai Biyuntian Biotechnology Co., Ltd. RPMI-1640 medium was purchased from Hyclone (Cytiva). Double antibody, RIPA protein lysis solution and protein loading buffer were purchased from Beijing Solarbio Science & Technology Co., Ltd. The inverted microscope was purchased from Olympus Corporation. Cell Counting Kit-8 (CCK-8) solution was purchased from Dojindo Laboratories, Inc. The microplate reader was purchased from Tecan Group, Ltd. Standard protein marker and the Lipofectamine 2000® transfection kit were purchased from Invitrogen (Thermo Fisher Scientific, Inc.). The ECL luminescence kit was purchased from Shandong Sparkjade Scientific Instruments Co., Ltd. Anti-c-myc (cat. no. sc-47694) antibody was purchased from Santa Cruz Biotechnology Inc; anti-β-actin antibody (cat. no. 20536-1-AP) was obtained from Cell Signaling Technology, Inc. HRP-labeled rabbit secondary antibody (cat. no. ZB-2301) was purchased from OriGene Technologies, Inc. Primer design was provided by Sangon Biotech Co., Ltd. The reverse transcription PrimeScript RT reagent kit with gDNA Eraser (Perfect Real-Time), the chimeric fluorescence detection kit and TB Green Premix Ex Taq (Tli RNaseH Plus) kit were purchased from Takara Biomedical Technology (Beijing) Co., Ltd. The cycle kit was purchased from Jiangsu KGI Biotechnology Co., Ltd. PE-CD11b (cat. no. 301306) and FITC-CD14 (cat. no. 301804) fluorescent-conjugated antibodies were purchased from BioLegend, Inc. TRIzol® reagent was purchased from Thermo Fisher Scientific, Inc. Trichloromethane was purchased from Sinopharm Chemical Reagent Co., Ltd.

The committed differentiation from THP-1 cells into monocytes/macrophages induced by PMA + LPS + IFN-γ. THP-1 cells were grown in RPMI-1640 complete medium containing 10% FBS at 37˚C and 5% CO_2 in a six-well plate. When the cells grew to the logarithmic phase, the appropriate amount of THP-1 cell suspension and PMA + LPS + IFN-γ solution were added to 96-well plates. The final concentration

of each well cell was $1x10^5$/ml and the final concentration of PMA + LPS + IFN-γ induction mixture was 10, 100 and 20 μg/l. The cells were cultured at 37˚C and 5% CO_2 for 48 h. In addition to observing the cell growth density directly under an inverted microscope, 10 μl Cell Counting Kit-8 (CCK-8) solution was added to each well followed by incubation at 37˚C for 2 h. The optical density (OD) values at 450 nm were measured using a microplate reader.

Detection of CD11b and CD14 differentiation antigens using flow cytometry. THP-1 cells induced by PMA + LPS + IFN-γ for 48 h were collected into flow tubes, suspended with PBS, washed twice by centrifugation at 200 x g for 3 min at 4˚C and the cell concentration was adjusted to $1x10^6$ cells/ml. Each tube was supplemented with 100 μl PBS and 5 μl PE-labeled mouse anti-human CD11b (cat. no. 301306, 1:1,000, BioLegend, Inc.) and FITC-labeled mouse anti-human CD14 fluorescent antibody (cat. no. 301804, 1:1,000, BioLegend, Inc.) were added, respectively, followed by incubation at 4˚C for 30 min in the dark. The cells were resuspended in 200 μl PBS and fixed in 1% paraformaldehyde at 4˚C for 20 min. The expression levels of CD11b and CD14 in the different treatment groups were analyzed on a FACSVerse (BD Biosciences) flow cytometer. Isotypic rat IgG (BioLegend, Inc.) was also used to examine non-specific binding. The experiment was repeated three times. According to the different detection antigens, they were divided into the CD11b PBS control group, CD11b PMA + LPS + IFN-γ experimental group, CD14 PBS control group and the CD14 PMA + LPS + IFN-γ experimental group.

Detection of protein expression of c-myc using western blot analysis. The cells in the control and experimental groups were collected and washed twice with pre-cooled PBS. Total protein was extracted and the protein concentration was determined by BCA assay; 5X protein loading buffer was added and the cells were denatured by boiling at 95˚C for 10 min. SDS-PAGE (volume fraction 10%) electrophoresis was performed with 20 μg protein. The separated proteins were transferred onto a PVDF membrane and blocked with 5% skimmed milk for 90 min at room temperature. The corresponding primary antibodies, c-myc (1:800) and β-actin (1:2,000) were then added and incubated at 4˚C overnight; this was followed by the addition of goat anti-rabbit IgG antibody (1:20,000) and incubation at room temperature for 90 min. The membranes were then washed with 1X TBST including 0.2% Tween-20 for 30 min and finally washed for 30 min for imaging observation using an ECL kit (Shandong Sparkjade Scientific Instruments Co., Ltd.). ImageJ v1.51j8 was used for densitometry (National Institutes of Health). The experiment was repeated three times.

Bioinformatics cross analysis of upstream miRNAs targeting c-myc. On the basis of confirming the reduced expression of c-myc in THP-1 cells following the induction of differentiation, the potential miR-let-7 family spliceosomes with targeted binding to c-myc were screened using TargetScan (Targetscan.org), StarBase database (starbase.sysu.edu.cn/), a literature research (PubMed) and differential microarray detection (Agilent miRNA Chip). For differential microarray detection using the Agilent miRNA Chip assay, the leukemic cells were collected before and 48 h following induction, respectively and Affymetrix 3' IVT expression profiling microarray data were analyzed to obtain miRNAs with a higher likelihood of binding to c-myc and a negative correlation with c-myc. At the same time, to further demonstrate the association between the expression of candidate miRNAs and c-myc, seven different time points during the induction of differentiation of THP-1 cells were randomly selected and the expression levels of mRNAs were detected using reverse transcription-quantitative (RT-q) PCR; correlations were analyzed using Pearson's correlation analysis.

Detection of changes in gene expression using RT-qPCR. Total RNA was extracted using TRIzol® reagent (Thermo Fisher Scientific, Inc.). The concentration of total RNA was measured using an ultramicro nucleic acid protein analyzer (Thermo Fisher Scientific, Inc.). cDNA was synthesized according to the instructions provided with the PrimeScript RT reagent kit with gDNA Eraser (Perfect Real-Time). For reverse transcription, samples were incubated in an Eppendorf PCR system at 42˚C for 30 min, then at 90˚C for 5 min and at 5˚C for 5 min. cDNA was used as a template for PCR amplification. The primers used were as follows: miR-let-7c-5p sense, 5'-CGT CATCCTGAGGTAGTAGGTTGT-3' and antisense, 5'-TAT GGTTTTGACGACTGTGTGAT-3'; U6 sense, 5'-AGAGAA GATTAGCATGGCCCCTG-3' and antisense, 5'-AGTGCA GGGTCCGAGGTATT-3'. These two primers were designed by stem-loop method. The downstream and reverse transcription primers of stem-loop method were artificially added (Sangon Biotech Co., Ltd.). c-myc sense primer 5'-CCCCTA CCCTCTCAACGACA-3, antisense 5'-CTTCTTGTTCCT CCTCAGAGTCG-3' and GAPDH sense, 5'-ACAACTTTG GTATCGTGGAAGG-3' and antisense, 5'-GCCATCACG CCAGTTTC-3'. Real-time fluorescence quantitative amplification reaction was performed according to the instructions provided with the TB Green Premix Ex Taq (Tli RNaseH Plus) kit. PCR was conducted under the following conditions: 10 sec at 95˚C; 40 cycles of 5 sec at 60˚C and 10 sec at 72˚C; 34 sec at 60˚C. Relative quantitative analysis was performed using the $2^{-\Delta\Delta Cq}$ method (28). Secondly, the transfection efficiency of miR-let-7c-5p mimics was also detected and confirmed using RT-qPCR for miR-let-7c-5p expression.

Dual-luciferase reporter gene analysis. The bioinformatics prediction website was used to predict the 3'UTR binding sequence of miR-let-7c-5p to c-myc. Dual luciferase reporter genes were used to detect the targeted binding of miR-let-7c-5p on the 3'UTR of c-myc. Briefly, the wild-type (WT) plasmid, pmirGLO-c-myc-wt and the mutant plasmid, pmirGLO-c-myc-MUT were constructed by Biosune Biotechnology (shanghai) Co.,Ltd. The WT plasmid, the mutant (MUT) plasmid and the miR-let-7c-5p mimics (UGA GGUAGUAGGUUGUAUGGUU) or NC mimics (UUCUCC GAACGUGUCACGUTT) were co-transfected into the cells by transfection reagent kit (jetPRIME; Polyplus). The activity of luciferase was determined using the Dual-Luciferase Reporter Assay System (Envision; PerkinElmer, Inc.) following 48 h of culture and *Renilla* luciferase was used as an internal control.

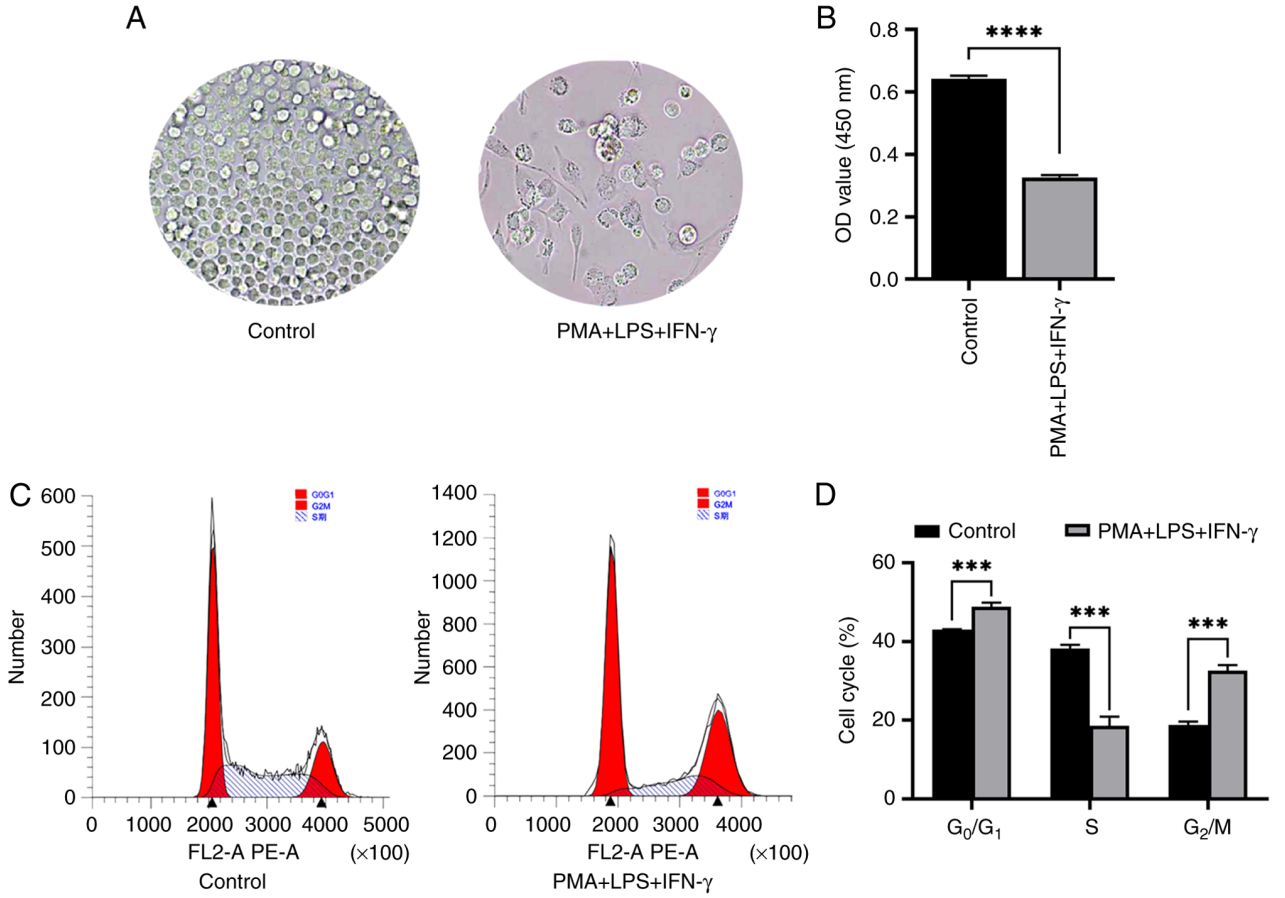

Figure 1. Changes in the proliferation of THP-1 cells following exposure to PMA + LPS + IFN-γ for 48 h. (A) Growth density of the THP-1 cells in the PBS control group and PMA + LPS + IFN-γ experimental group (magnification, x200); cell proliferation was significantly higher in the PBS control group than in the PMA + LPS + IFN-γ experimental group. (B) Results of Cell Counting Kit-8 assay in the PBS control group and PMA + LPS + IFN-γ experimental group (biological repeats). (C) Flow cytometry of the cell cycle in the PBS control group and PMA + LPS + IFN-γ experimental group. (D) THP-1 cell cycle histogram of the PBS control group and PMA + LPS + IFN-γ experimental group. ***P<0.001 and ****P<0.0001. PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; IFN, interferon.

Cell transfection. After using FAM fluorescence labeled si-NC as negative parallel control to determine transfection efficiency, THP-1 cells were transfected in accordance with the instructions and the appropriate amount of si-c-myc and its corresponding negative control were mixed with the transfection reagent Lipofectamine 2000® transfection kit (Invitrogen; Thermo Fisher Scientific, Inc.) to form the transfection complex, which was added to the six-well plate to inoculate the cells for 48 h. si-c-myc: sense 5'-CCACAC AUCAGCACAACUATT-3', antisense 5'-UAGUUGUGCUGA UGUGUGGTT-3' was co-selected for synthesis. At the same time, si-NC: sense 5'-UUCUCCGAACGUGUCACGUTT-3', antisense 5'-ACGUGACACGUUCGGAGAATT-3' was taken as the negative control. The two were synthesized by BioSune Biotechnology Co., Ltd.

Statistical analysis. GraphPad Prism 8 software (GraphPad Software, Inc.) was used for data processing and the Shapiro-Wilk test was used to assess normal distribution of the data. Each experiment was repeated three times and the measurement data are expressed as the mean ± standard deviation. Comparisons between two groups were performed using an independent samples t-test and one-way ANOVA was used for multiple-group comparisons. The Bonferroni test was used as the post-hoc test following one-way ANOVA. Pearson's correlation analysis was used to determine the correlation between miR-let-7c-5p and c-myc. All data were analyzed using two-tailed tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Proliferation of THP-1 cells is inhibited by PMA + LPS + IFN-γ. The changes in the growth density of THP-1 cells induced by PMA + LPS + IFN-γ for 48 h were observed under an inverted microscope. The results revealed that the cell density in the PMA + LPS + IFN-γ experimental group was significantly lower than that in the PBS control group and the cell growth gradually changed from growth in suspension to adherent growth (Fig. 1A). The results of CCK-8 assay revealed that the proliferative ability of the cells in the PMA + LPS + IFN-γ experimental group was lower compared with those in the PBS control group (Fig. 1B). Flow cytometry for cell cycle distribution demonstrated that the number of cells in the G_0/G_1 phase in the control group was lower than that in the PMA + LPS + IFN-γ group. In addition, the number of cells in the S phase in the PBS control group was higher than that in the PMA + LPS + IFN-γ group and the number of cells in

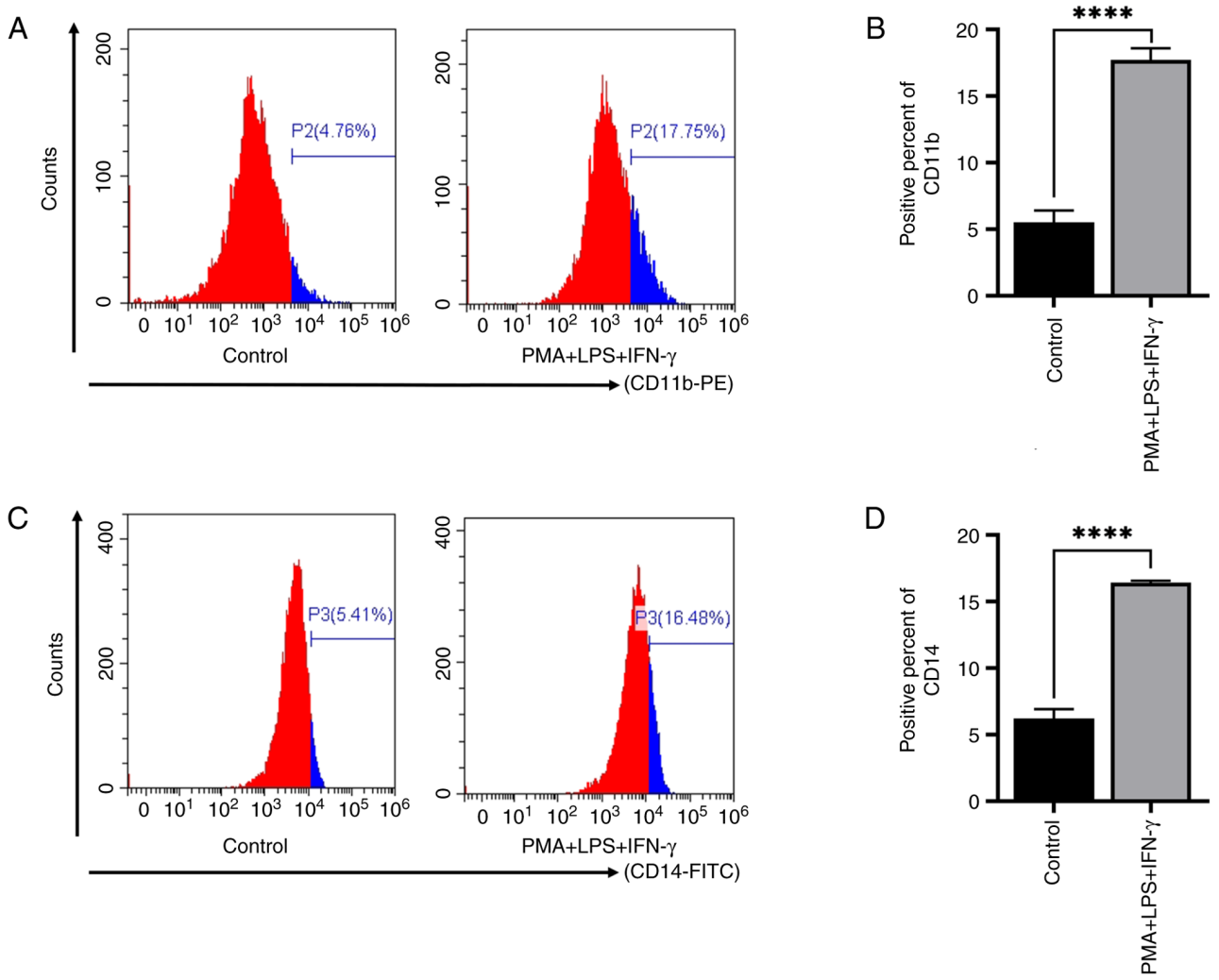

Figure 2. Effects of PMA + LPS + IFN-γ on the expression of CD11b and CD14 surface molecules in THP-1 cells. (A and B) The expression of CD11b on THP-1 cells differed significantly between the control and PMA + LPS + IFN-γ group (biological repeats). (C and D) The changes in the CD14 expression of THP-1 cells in the control and experimental group (biological repeats). ****P<0.0001. PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; IFN, interferon.

the G_2/M phase in the PBS control group was also lower than that in the PMA + LPS + IFN-γ group (Fig. 1C and D). These experimental data demonstrated that the proliferation of the THP-1 cells was inhibited by PMA + LPS + IFN-γ.

PMA + LPS + IFN-γ induces the committed differentiation of THP-1 cells into monocytes/macrophages. According to the data from the literature research and the results of the preliminary experiments, PMA + LPS + IFN-γ was used to induce the committed differentiation of THP-1 cells into monocytes/macrophages. The results of flow cytometry revealed that the average positive rates of CD11b in the PMA + LPS + IFN-γ group were markedly increased compared with those in the PBS control group (Fig. 2A and B). The expression rates of CD14 in the PMA + LPS + IFN-γ group were also increased compared with those in the PBS control group (Fig. 2C and D). These results indicated that PMA + LPS + IFN-γ induced the differentiation of THP-1 cells into monocytes/macrophages.

c-myc expression is downregulated during the monocyte/macrophage differentiation of THP-1 cells. As c-myc protein was involved in the differentiation of leukemic cells into granulocytes, the present study aimed to further investigate whether c-myc gene was also involved in the macrophage differentiation of leukemic cells and whether this type of change affects the proliferation and differentiation process of leukemic cells. The committed differentiation into monocytes/macrophages was obtained by stimulating the THP-1 cells with PMA + LPS + IFN-γ for 48 h. The results revealed that the relative expression levels of c-myc were downregulated following exposure to PMA + LPS + IFN-γ, as compared with the PBS control group (Fig. 3A and B). Furthermore, to elucidate the role of c-myc in the aforementioned committed differentiation of leukemic cells, the expression of c-myc was knocked down using siRNA (si-c-myc); FAM fluorescence labeled si-NC as a negative parallel control to observe the transfection efficiency through fluorescence observation and confirm the successful transfection (Fig 3C and D). The results indicated that si-c-myc significantly inhibited the protein expression of c-myc in THP-1 cells as compared with the si-control group (Fig. 3E and F). The results of CCK-8 assay also indicated that the proliferation of THP-1 cells was significantly inhibited following transfection with si-c-myc (Fig. 3G).

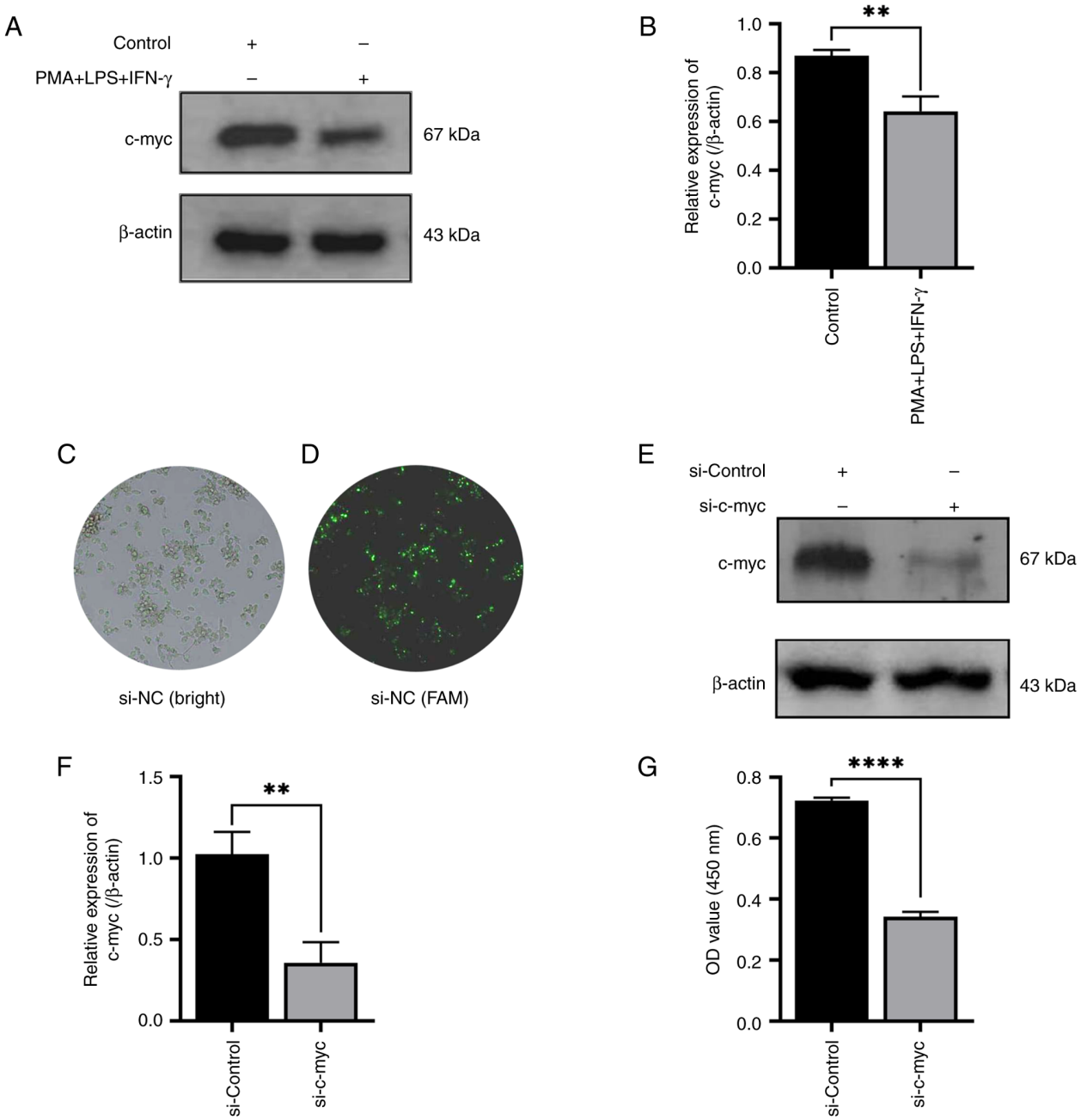

Figure 3. Expression of c-myc in THP-1 cells during macrophage differentiation. (A and B) c-myc relative expression levels in the PBS control vs. PMA + LPS + IFN-γ group (biological repeats). (C and D) The transfection efficiency through fluorescence observation of si-NC. (E and F) c-myc protein expression levels in the si-control and si-c-myc groups. (G) Cell Counting Kit-8 assay of cell proliferation (OD values) in the si-control and si-c-myc group (biological repeats). **P<0.01 and ****P<0.0001. PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; IFN, interferon; si, short interfering; NC, negative control.

c-myc upstream miRNA bioinformatics cross analysis. As it was confirmed that the expression of c-myc was reduced in THP-1 cells following the induction of differentiation, the potential miR-let-7 family spliceosomes with targeted binding to c-myc were screened using TargetScan, a PubMed literature review and differential microarray detection. Following the cross-sectional analysis of the aforementioned three data sources (Fig. 4A), it was found that miR-let-7a-5p and miR-let-7b-5p expression was decreased, whereas miR-let-7c-5p expression was elevated (Fig. 4B). miR-let-7c-5p was identified as a possible candidate miRNA for the targeted regulation of c-myc, based on the fact that miRNAs target downstream genes to suppress their expression. A total of seven different time points at which PMA + LPS + IFN-γ induced the differentiation of THP-1 cells were randomly selected and the expression levels of miR-let-7c-5p and c-myc were detected using RT-qPCR. Pearson's correlation analysis was performed to confirm the negative correlation between miR-let-7c-5p and c-myc (Fig. 4C); the correlation was statistically significant. Following target prediction using TargetScan, the c-myc gene 3'UTR region was found to have a sequence basis for miR-let-7c-5p targeted binding (Fig. 4D).

c-myc is a direct target gene of miR-let-7c-5p. A binding region between miR-let-7c-5p and c-myc was identified using the StarBase database; the paired sites were then predicted

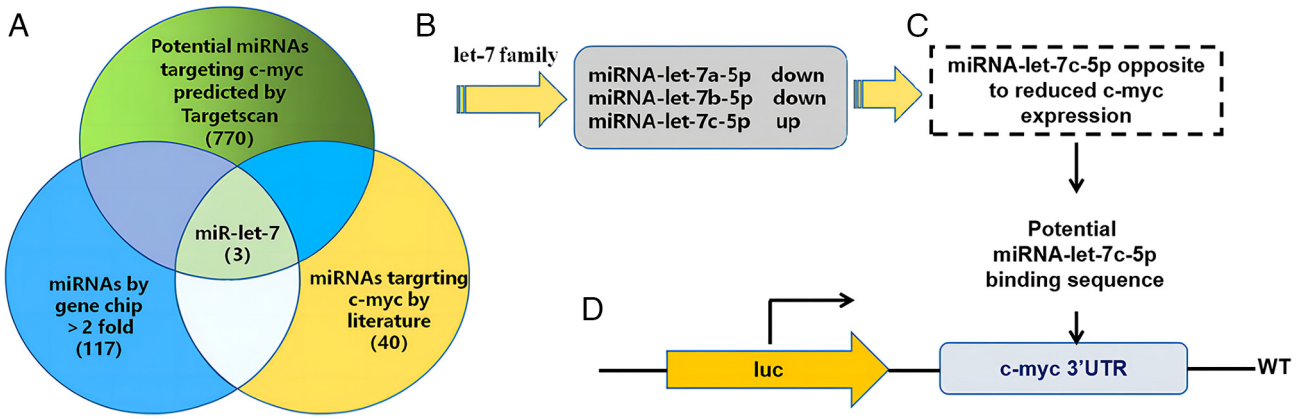

Figure 4. Results of bioinformatics cross analysis for c-myc upstream miRNA. (A) TargetScan, PubMed and Agilent miRNA Chip detection for cross-analysis of miRNAs targeting c-myc binding. (B) Cross-analysis of mir-let-7 spliceosomes for changes in expression levels following the induction of differentiation. (C) Spliceosome miR-let-7c-5p in contrast to a decreased c-myc expression. (D) miR-let-7c-5p targets the 3'UTR -binding region of c-myc. miRNA/miR, microRNA; luc, luciferase assay; WT, wild-type; MUT, mutant.

using the TargetScan database (Fig. 5A). To examine the direct binding of miR-let-7c-5p with c-myc, plasmids with WT or MUT 3'UTR of c-myc were constructed, which were co-transfected with miR-let-7c-5p mimics or NC for dual-luciferase reporter assay. The results of dual luciferase reporter gene assay revealed that the luciferase activity of the miR-let-7c-5p mimics + c-myc-WT group was significantly lower than that of the mimics NC + c-myc-WT group. There was no significant difference in the luciferase activity of the miR-let-7c-5p mimics + c-myc-MUT group and the mimics NC + c-myc-MUT group. It was found that miR-let-7c-5p mimics significantly decreased the luciferase reporter activity of the WT 3'UTR plasmid of c-myc, although it did not affect that of the MUT 3'UTR plasmid. These data indicated that c-myc was a direct target gene of miR-let-7c-5p (Fig. 5B). At 48 h following the induction of the differentiation of THP-1 cells *in vitro*, the relative expression levels of miR-let-7c-5p were examined using RT-qPCR. It was found that the expression of miR-let-7c-5p in the PMA + LPS + IFN-γ group was upregulated compared with that in the control group (Fig. 5C). However, the relative expression levels of the c-myc gene in the PMA + LPS + IFN-γ group were lower than those in the control group (Fig. 5D). Following the random selection of seven time points for miR-let-7c-5p vs. c-myc gene expression, Pearson's correlation analysis indicated that the two presented a negative correlation (Fig. 5E).

miR-let-7c-5p is involved in the induction of the differentiation of THP-1 cells and in regulating the expression of c-myc. To demonstrate whether miR-let-7c-5p is involved in the committed differentiation of THP-1 cells into monocytes/macrophages and in regulating the expression of c-myc, miR-let-7c-5p mimics were transfected into the THP-1 cells and the transfection efficiency of miR-let-7c-5p mimics was confirmed using RT-qPCR assay as indicated in Fig. 6A. The expression level of c-myc was found to be decreased in the miR-let-7c-5p mimics group compared with the miR-let-7c-5p mimics NC group (Fig. 6B and C). The proliferation of the THP-1 cells transfected with miR-let-7c-5p mimics was decreased compared with the cells transfected with miR-let-7c-5p mimics NC (Fig. 6D). The positive rates of CD11b expression in the miR-let-7c-5p mimics groups were higher than those in the miR-let-7c-5p mimics NC group (Fig. 6E). The positive rates of CD14 expression in the miR-let-7c-5p mimics group were also higher than those in the miR-let-7c-5p mimics NC group (Fig. 6F).

The rescue experiment of miR-let-7c-5p on differentiation of THP-1 cells and reversed by transfection of c-myc vector. As it was demonstrated that miR-let-7c-5p mimics play a role in promoting the differentiation of THP-1 cells by reducing c-myc expression, the present study aimed to further demonstrate whether miR-let-7c-5p promotes the committed differentiation leukemic cells into monocytes/macrophages through the c-myc pathway. Thus, a rescue experiment was performed. Firstly, parallel transfection was performed using c-myc vector and its empty vector and western blotting was used to detect the expression level of c-myc protein after transfection, verifying the success and efficiency of transfection (Fig. 7A and B). miR-let-7c-5p mimics and c-myc overexpression plasmid were co-transfected into THP-1 cells. It was found that the expression of c-myc in the mimics NC group was significantly higher than that in the miR-let-7c-5p mimics group and its expression in the miR-let-7c-5p mimics + c-myc vector group was significantly higher than that in the miR-let-7c-5p mimics group. No significant difference in c-myc expression was found between the miR-let-7c-5p mimics NC group and the miR-let-7c-5p mimics + c-myc vector group (Fig. 7C and D). As regards the proliferation of THP-1 cells, cell proliferation in the miR-let-7c-5p mimics NC group was higher compared with miR-let-7c-5p mimics group and that in the miR-let-7c-5p mimics group was lower compared with the miR-let-7c-5p mimics + c-myc vector group. There was no significant difference in cell proliferation between the miR-let-7c-5p mimics NC group and the miR-let-7c-5p mimics + c-myc vector group (Fig. 7E). The positive rates of CD11b expression in the miR-let-7c-5p mimics NC group were lower than those in the miR-let-7c-5p mimics group and those in the miR-let-7c-5p mimics group were higher than those in the miR-let-7c-5p mimics + c-myc vector group; in addition, the positive rates of CD11b expression in the miR-let-7c-5p mimics NC group

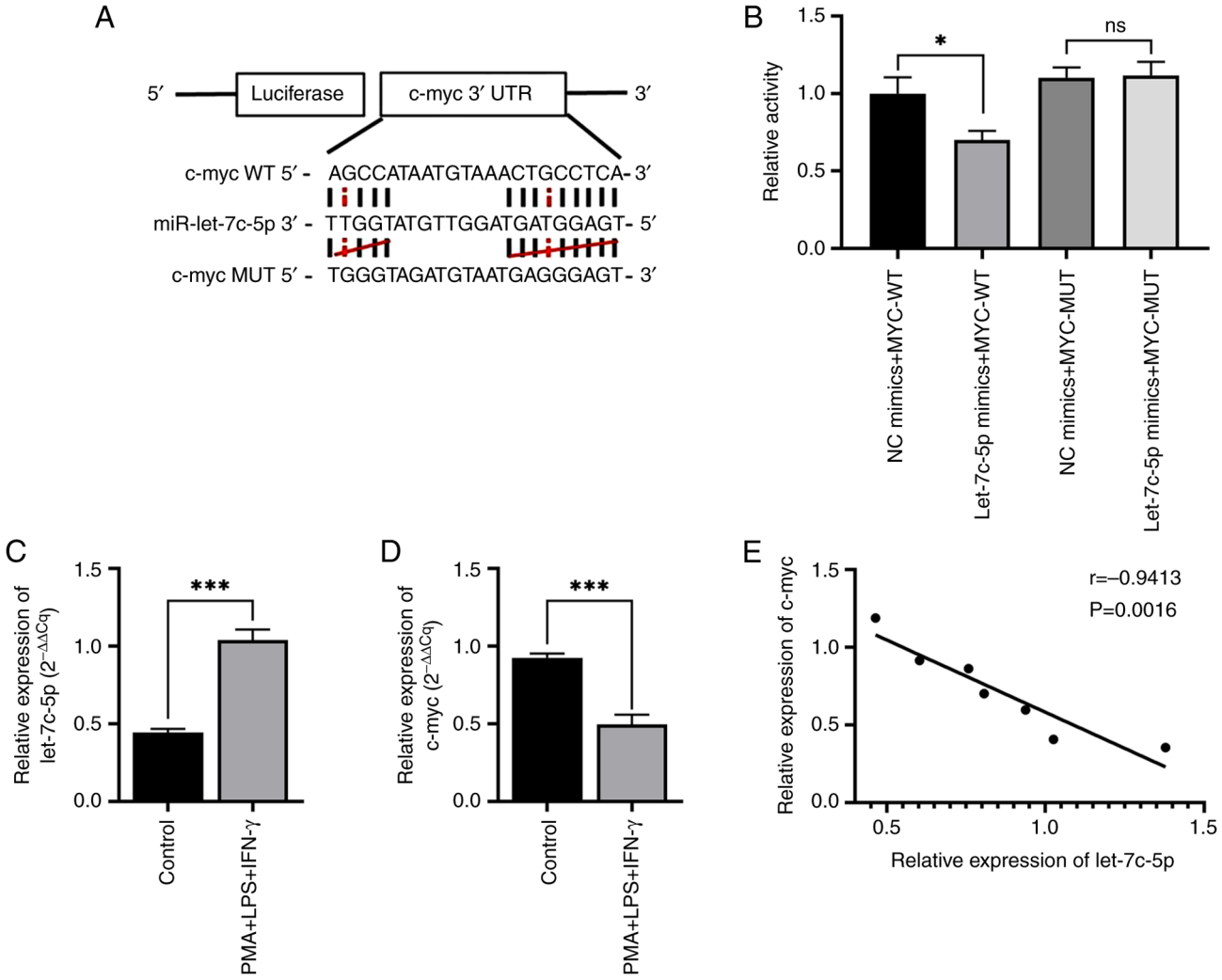

Figure 5. Targeted binding of miR-let-7c-5p to the c-myc 3'UTR in THP-1 cells. (A) 3'UTR -binding regions of miR-let-7c-5p and c-myc. (B) Results of luciferase activity assay (biological repeats). (C) Relative expression level of miR-let-7c-5p gene in the control group and PMA + LPS + IFN-γ experimental group (biological repeats). (D) Relative expression level of c-myc gene in the control group and PMA + LPS + IFN-γ experimental group (biological repeats). (E) Pearson's correlation analysis of the correlation between miR-let-7c-5p and c-myc gene expression. *P<0.05 and ***P<0.001. miRNA/miR, microRNA; WT, wild-type; MUT, mutant; ns, not significant; PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; IFN, interferon.

were slightly lower than those in the miR-let-7c-5p mimics + c-myc vector group (Fig. 7F). The positive rates of CD14 expression in the miR-let-7c-5p mimics NC group were lower than those in the miR-let-7c-5p mimics group and those in the miR-let-7c-5p mimics group were higher than those in the miR-let-7c-5p mimics + c-myc vector group; in addition, the positive rates of CD14 expression in the miR-let-7c-5p mimics NC group were slightly lower than those in the miR-let-7c-5p mimics + c-myc vector group (Fig. 7G). These results demonstrated that the effects of miR-let-7c-5p mimics on the proliferation and differentiation of THP-1 cells were markedly reversed by the overexpression of c-myc. These data indicated that miR-let-7c-5p promoted the differentiation of monocytes/macrophages by suppressing c-myc.

Discussion

As regards the development of normal hematopoietic cells, they can undergo committed differentiation and eventually differentiate into mature granulocytic, monocytic, erythroid, or megakaryocytic cells in strict accordance with the inherent procedure (1). It has been proved that a number of transcription factors are involved in this process of differentiation and determine the genetic program of each mature phenotype (29). Among them, increasing evidence suggests that c-myc is one of the main transcription factors involved. c-myc is a necessary factor for maintaining a balance between self-renewal and differentiation of hematopoietic stem cells. It is widely hypothesized that overexpression of c-myc is mainly beneficial for promoting cell proliferation and has an inhibitory effect on the directed differentiation of hematopoietic cells (30). In various malignant tumor cells, the expression level of myc gene often shows an overexpression state, which is one of the important causes of the occurrence and development of malignant tumors (31). In acute lymphocytic leukemia and myeloid leukemia and other hematological tumors, c-myc also exhibits overexpression and is closely related to disease progression. The results of a number of *in vitro* studies also confirm that c-myc mainly manifests as an oncogene, participating in the proliferation, differentiation and apoptosis processes of

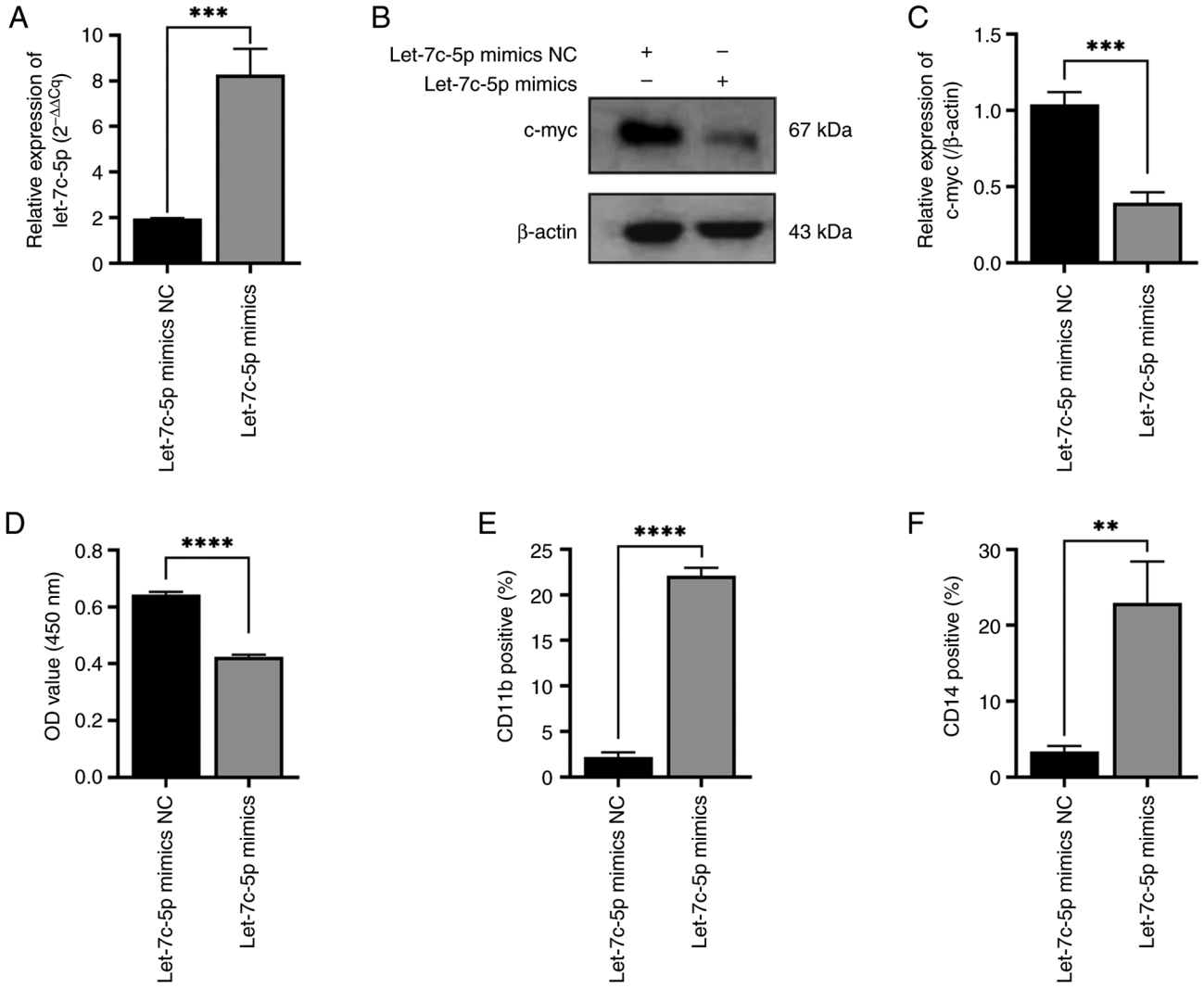

Figure 6. Effects of miR-let-7c-5p mimics on c-myc expression and on the proliferation and differentiation of THP-1 cells. (A) Relative expression level of miR-let-7c-5p gene in the miR-let-7c-5p mimics NC group and miR-let-7c-5p mimics group (biological repeats). (B) Western blot analysis of c-myc expression following transfection with miR-let-7c-5p mimics. (C) Effects of miR-let-7c-5p mimics on the relative protein expression of c-myc (biological repeats). (D) Effects of miR-let-7c-5p mimics on THP-1 cell proliferation (biological repeats). (E) Effects of miR-let-7c-5p mimics on the expression of the CD11b differentiation antigens in THP-1 cells (biological repeats). (F) Effects of miR-let-7c-5p mimics on the expression of the CD14 differentiation antigen in THP-1 cells (biological repeats). **P<0.01, ***P<0.001 and ****P<0.001. miRNA/miR, microRNA; NC, negative control.

leukemia cells (32,33). Therefore, controlling the expression of c-myc gene can interfere with the proliferation and differentiation process of leukemia cells. Among them, macrophage is an important immune effector cell, a group of highly heterogeneous cells with two types of macrophages, such as M1 and M2 macrophages (6-10). For patients with tumor and leukemia, it is more important to induce macrophages to differentiate into M1 macrophages, which are associated with anti-tumor activity (34,35). As aforementioned, c-myc plays an important role in modulating the differentiation of leukemia cells, therefore it was hypothesized that c-myc may also be involved in the maturation and differentiation of leukemia cells into monocytes and macrophages. However, the specific molecular mechanism of c-myc this committed differentiation remains to be elucidated. In the present study, to demonstrate the role of c-myc in macrophage polarization, THP-1 cells were induced to differentiate into more matured macrophages and found the expression of c-myc in THP-1 leukemia cells was downregulated after being induced to differentiate into macrophages. These results suggested that the downregulation of c-myc contributed to the inhibition of proliferation and induction of differentiation of THP-1 cells.

In recent years, ncRNAs have been found to be closely associated with development of leukemia. Among them, miRNAs are often involved in regulation of target genes by acting as oncogenes or tumor suppressor genes (25), including pathological processes such as abnormal proliferation, differentiation, or apoptosis of leukemic cells (26,27). For example, about 50% of miRNAs are located near or within cancer translocation genes and can inhibit or promote tumorigenesis by downregulating the expression of oncogenes or tumor suppressor gene (27). miRNAs also play an important role in the pathogenesis of leukemia and it has been demonstrated that miRNA expression profiling can be used as a biomarker for the diagnosis, prognosis and efficacy of leukemia patients (27,36-38). In recent years, the regulatory relationship between ncRNA and c-myc has attracted more attention: 25 miRNAs of 20 different families so far have been found to

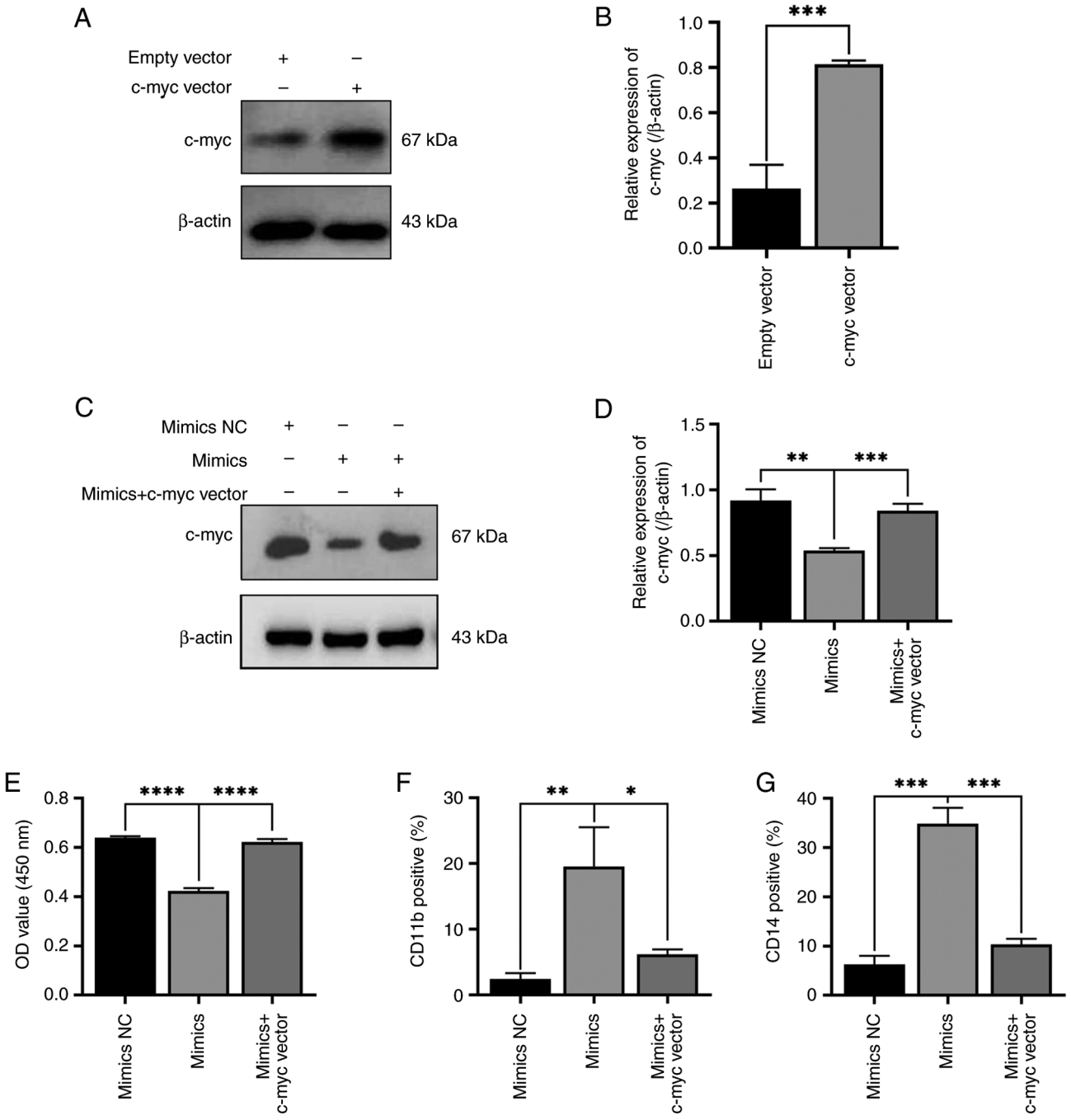

Figure 7. Effects of the overexpression of c-myc on the regulatory effects of miR-let-7c-5p mimics on THP-1 cell proliferation and differentiation. (A) Western blot analysis of c-myc protein expression in the empty vector and c-myc vector groups. (B) Relative protein expression levels of c-myc in the empty vector and c-myc vector groups. (biological repeats). (C) Western blot analysis of c-myc protein expression in the miR-let-7c-5p mimics NC , miR-let-7c-5p mimics and miR-let-7c-5p mimics + c-myc vector groups. (D) Relative protein expression levels of c-myc in the miR-let-7c-5p mimics NC, miR-let-7c-5p mimics and miR-let-7c-5p mimics + c-myc vector groups. (E) OD value of cell proliferation in the miR-let-7c-5p mimics NC, miR-let-7c-5p mimics and miR-let-7c-5p mimics + c-myc vector groups (biological repeats). (F) Positive rate of CD11b expression in the miR-let-7c-5p mimics NC, miR-let-7c-5p mimics and miR-let-7c-5p mimics + c-myc vector groups (biological repeats). (G) Positive rate of CD14 in the miR-let-7c-5p mimics NC, miR-let-7c-5p mimics and miR-let-7c-5p mimics + c-myc vector groups (biological repeats). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.001. miRNA/miR, microRNA; NC, negative control.

regulate c-myc, as well as 33 miRNAs regulated by c-myc and most of the above miRNAs tend to bind the 3'UTR of the c-myc gene in a traditional manner and exert their regulatory effects (39,40). Of which, miR-let-7 family members are also involved in the proliferation and differentiation of leukemic cells (41,42). Some studies have shown that ncRNAs play an important role in the transcription and translation regulation of this gene, with some ncRNAs directly interacting with c-myc (43), for example, as to the miRNA-let-7 family, Wong *et al* (44) demonstrated that miRNA let-7 suppresses nasopharyngeal carcinoma cells proliferation through down-regulating c-myc expression. Sampson *et al* (45) found that miRNA let-7a downregulates myc and reverts myc-induced growth in Burkitt lymphoma cells. Buechner *et al* (46) showed that tumor-suppressor miRNA let-7 could target the proto-oncogene myc and inhibit cell proliferation in

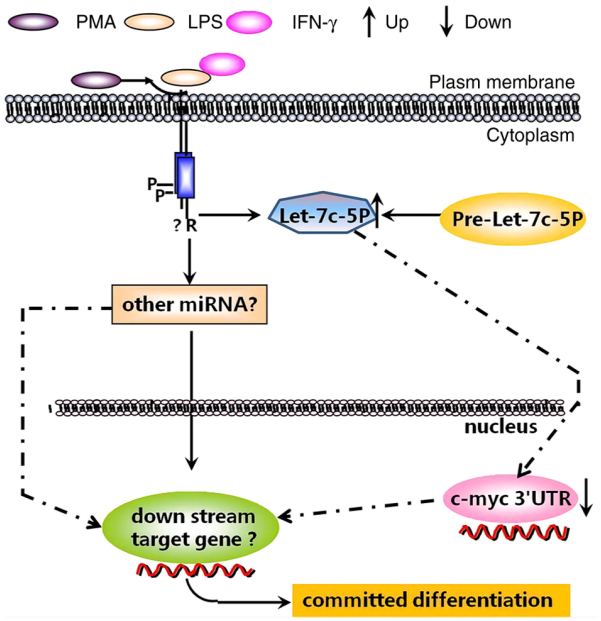

Figure 8. Schematic representation of the role of miR-let-7c-5p in the committed differentiation of THP-1 cells induced by PMA + LPS + IFN-γ. Following the induction of the committed differentiation of THP-1 cells by PMA + LPS + IFN-γ, the expression of miR-let-7c-5p was increased and the increased expression of miR-let-7c-5p decreased the expression of c-myc by directly targeting its mRNA 3'UTR. miRNA/miR, microRNA; PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; IFN, interferon; p-, phosphorylated.

MYCN-amplified neuroblastoma. Lan *et al* (47) indicated that Hsa-let-7g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-myc and upregulation of p16. He *et al* (48) demonstrated that let-7a miRNA protects against the growth of lung carcinoma by suppression of k-Ras and c-myc in nude mice. Zhang *et al* (49) found miRNA let-7g inhibited hypoxia-induced proliferation of PASMCs via G_0/G_1 cell cycle arrest by targeting c-myc. However, there are few reports on the role of c-myc in the directed differentiation of leukemia cells into macrophages.

On the basis of the aforementioned results on the downregulation of c-myc in committed differentiation of THP-1 cells towards macrophages and the role of different members of let-7 family in targeting modulation of c-myc according to the results reported in the literature, it was hypothesized that the let-7 family mediates the directed differentiation of leukemia cells into macrophages through targeted regulation of c-myc. The present study detected and predicted the upstream miR-let-7 family spliceosomes with the potential for targeting to c-myc by crosstalk analysis by TargetScan, PubMed literature research and differential microarray detection. After intersection analysis of the above three data sources, it was found that miR-let-7a-5p and miR-let-7b-5p expression was decreased, while miR-let-7c-5p expression was increased following induction of differentiation. The miR-let-7c-5p spliceosome was identified as a possible candidate miRNA for targeted regulation of c-myc, based on the fact that miRNAs often target to suppress the expression of downstream genes. At the same time, the relative expression levels of miR-let-7c-5p and c-myc gene in THP-1 cells induced to differentiate into macrophages and the dual luciferase reporter gene experiment confirmed that miR-let-7c-5p could bind 3'UTR of c-myc and regulate its promoter activity, which was involved in the committed macrophage differentiation of THP-1 cells.

In summary, the present study revealed for the first time that expression of miR-let-7c-5p was upregulated in THP-1 cells induced to differentiate into macrophages, which could downregulate the expression of c-myc by targeting c-myc 3'UTR (Fig. 8). The miR-let-7c-5p/c-myc axis may be a potential target in macrophage polarization and highlights an improved understanding for potential mechanism of pathogenesis and progression of leukemia. As the *in vitro* experimental results have preliminarily confirmed the important role of miR-let-7c-5p in targeted monocyte/macrophage differentiation of leukemia cells, the focus of the next clinical research on miR-let-7c-5p will to detect the potential role of miR-let-7c-5p in promising biomarkers for prognosis of leukemia patients, or to verify whether it can serve as a biological indicator for independent risk assessment. More importantly, will be to further explore its potential as a therapeutic target for leukemia through *in vitro* and *in vivo* experiments. Meanwhile, it should be noted that since the regulation of committed differentiation of leukemia cells is complicated and the role of miR-let-7c-5p/c-myc axis in modulation of differentiation of leukemia cells still requires further in-depth research. In addition, in-depth research on downstream target genes of miR-let-7c-5p/c-myc axis not only enriches the action pattern of this signal axis, but also has a guiding role in increasing effective targets for targeted intervention therapy.

Acknowledgements

Not applicable.

Funding

The present study was supported by the 'Twelfth Five-Year' National Science and Technology Support Program (grant no. 2013BAI07B02), Natural Science Foundation of China (grant no. 81573467), Natural Science Foundation of Shandong (grant nos. ZR2020QH160 and ZR2021MH080), The Foundation for Jinan's Clinical Science and Technology Innovation (grant no. 202134001). Cultivation Fund of the first affiliated hospital of Shandong First Medical University (Shandong Qianfoshan Hospital; grant no. QIPY2020NSFC0819).

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

GSJ, KHB, QL, RJS and YFW made substantial contributions to the conception and design and also critically reviewed the study. RJS, YFW, CZW, YHW and PCD performed the experiments. RJS, YFW, CZW and XLS analyzed the data and wrote the manuscript. GSJ, KHB and QL 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.

References

1. Carroll D and St Clair DK: Hematopoietic stem cells: Normal versus malignant. Antioxid Redox Signal 29: 1612-1632, 2018.
2. Khwaja A, Bjorkholm M, Gale RE, Levine RL, Jordan CT, Ehninger G, Bloomfield CD, Estey E, Burnett A, Cornelissen JJ, *et al*: Acute myeloid leukaemia. Nat Rev Dis Primers 2: 16010, 2016.
3. Bruserud O and Gjertsen BT: New strategies for the treatment of acute myelogenous leukemia: Differentiation induction-present use and future possibilities. Stem Cells 18: 157-165, 2000.
4. Stubbins RJ and Karsan A: Differentiation therapy for myeloid malignancies: Beyond cytotoxicity. Blood Cancer J 11: 193, 2021.
5. Gocek E and Marcinkowska E: Differentiation therapy of acute myeloid leukemia. Cancers (Basel) 3: 2402-2420, 2011.
6. Sica A and Mantovani A: Macrophage plasticity and polarization: In vivo veritas. J Clin Invest 122: 787-795, 2012.
7. Sica A, Erreni M, Allavena P and Porta C: Macrophage polarization in pathology. Cell Mol Life Sci 72: 4111-4126, 2015.
8. Walajtys-Rode E and Dzik JM: Monocyte/macrophage: NK cell cooperation-old tools for new functions. Results Probl Cell Differ 62: 73-145, 2017.
9. Gurski CJ and Dittel BN: Myeloperoxidase as a marker to differentiate mouse monocyte/macrophage subsets. Int J Mol Sci 23: 8246, 2022.
10. Zhao X, Di Q, Liu H, Quan J, Ling J, Zhao Z, Xiao Y, Wu H, Wu Z, Song W, *et al*: MEF2C promotes M1 macrophage polarization and Th1 responses. Cell Mol Immunol 19: 540-553, 2022.
11. Wang LX, Zhang SX, Wu HJ, Rong XL and Guo J: M2b macrophage polarization and its roles in diseases. J Leukoc Biol 106: 345-358, 2019.
12. Yunna C, Mengru H, Lei W and Weidong C: Macrophage M1/M2 polarization. Eur J Pharmacol 877: 173090, 2020.
13. Huang X, Li Y, Fu M and Xin HB: Polarizing macrophages in vitro. Methods Mol Biol 1784: 119-126, 2018.
14. Draijer C, Penke L and Peters-Golden M: Distinctive effects of GM-CSF and M-CSF on proliferation and polarization of two major pulmonary macrophage populations. J Immunol 202: 2700-2709, 2019.
15. Jaguin M, Houlbert N, Fardel O and Lecureur V: Polarization profiles of human M-CSF-generated macrophages and comparison of M1-markers in classically activated macrophages from GM-CSF and M-CSF origin. Cell Immunol 281: 51-61, 2013.
16. Qian F, Deng J, Lee YG, Zhu J, Karpurapu M, Chung S, Zheng JN, Xiao L, Park GY and Christman JW: The transcription factor PU.1 promotes alternative macrophage polarization and asthmatic airway inflammation. J Mol Cell Biol 7: 557-567, 2015.
17. Liu L, Peng S, Duan M, Liu C, Li L, Zhang X, Ren B and Tian H: The role of C/EBP homologous protein (CHOP) in regulating macrophage polarization in RAW264.7 cells. Microbiol Immunol 65: 531-541, 2021.
18. Xie J, Wu X, Zheng S, Lin K and Su J: Aligned electrospun poly(L-lactide) nanofibers facilitate wound healing by inhibiting macrophage M1 polarization via the JAK-STAT and NF-κB pathways. J Nanobiotechnology 20: 342, 2022.
19. Vergadi E, Ieronymaki E, Lyroni K, Vaporidi K and Tsatsanis C: Akt signaling pathway in macrophage activation and M1/M2 polarization. J Immunol 198: 1006-1014, 2017.
20. Juhas U, Ryba-Stanislawowska M, Szargiej P and Mysliwska J: Different pathways of macrophage activation and polarization. Postepy Hig Med Dosw (Online) 69: 496-502, 2015.
21. Pello OM, De Pizzol M, Mirolo M, Soucek L, Zammataro L, Amabile A, Doni A, Nebuloni M, Swigart LB, Evan GI, *et al*: Role of c-MYC in alternative activation of human macrophages and tumor-associated macrophage biology. Blood 119: 411-421, 2012.
22. Pello OM: Macrophages and c-MYC cross paths. Oncoimmunology 5: e1151991, 2016.
23. Jiang G, Albihn A, Tang T, Tian Z and Henriksson M: Role of Myc in differentiation and apoptosis in HL60 cells after exposure to arsenic trioxide or all-trans retinoic acid. Leuk Res 32: 297-307, 2008.
24. Zhang P, Wu W, Chen Q and Chen M: Non-coding RNAs and their integrated networks. J Integr Bioinform 16: 20190027, 2019.
25. Hill M and Tran N: MiRNA interplay: Mechanisms and consequences in cancer. Dis Model Mech 14: dmm047662, 2021.
26. Liu Y, Cheng Z, Pang Y, Cui L, Qian T, Quan L, Zhao H, Shi J, Ke X and Fu L: Role of microRNAs, circRNAs and long noncoding RNAs in acute myeloid leukemia. J Hematol Oncol 12: 51, 2019.
27. Anelli L, Zagaria A, Specchia G, Musto P and Albano F: Dysregulation of miRNA in Leukemia: Exploiting miRNA expression profiles as biomarkers. Int J Mol Sci 22: 7156, 2021.
28. 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.
29. Edginton-White B and Bonifer C: The transcriptional regulation of normal and malignant blood cell development. FEBS J 289: 1240-1255, 2022.
30. Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM and Felsher DW: The MYC oncogene-the grand orchestrator of cancer growth and immune evasion. Nat Rev Clin Oncol 19: 23-36, 2022.
31. Dang CV: MYC on the path to cancer. Cell 149: 22-35, 2012.
32. Pippa R and Odero MD: The role of MYC and PP2A in the initiation and progression of myeloid leukemias. Cells 9: 544, 2020.
33. Song G, Li Y, Zhang Z, Ren X, Li H, Zhang W, Wei R, Pan S, Shi L, Bi K and Jiang G: c-myc but not Hif-1α-dependent downregulation of VEGF influences the proliferation and differentiation of HL-60 cells induced by ATRA. Oncol Rep 29: 2378-2384, 2013.
34. Liu YC, Zou XB, Chai YF and Yao YM: Macrophage polarization in inflammatory diseases. Int J Biol Sci 10: 520-529, 2014.
35. Martinez FO and Gordon S: The M1 and M2 paradigm of macrophage activation: Time for reassessment. F1000Prime Rep 6: 13, 2014.
36. Murray PJ: Macrophage Polarization. Annu Rev Physiol 79: 541-566, 2017.
37. Mardani R, Abadi MHJ, Motieian M, Taghizadeh-Boroujeni S, Bayat A, Farsinezhad A, Hayat SM, Motieian M and Pourghadamyari H: MicroRNA in leukemia: Tumor suppressors and oncogenes with prognostic potential. J Cell Physiol 234: 8465-8486, 2019.
38. Curtale G, Renzi TA, Mirolo M, Drufuca L, Albanese M, De Luca M, Rossato M, Bazzoni F and Locati M: Multi-step regulation of the TLR4 pathway by the miR-125a~99b~let-7e cluster. Front Immunol 9: 2037, 2018.
39. Kagawa T, Watanabe M, Inoue N, Otsu H, Saeki M, Katsumata Y, Takuse Y and Iwatani Y: Increases of microRNA let-7e in peripheral blood mononuclear cells in Hashimoto's disease. Endocr J 63: 375-380, 2016.
40. Swier L, Dzikiewicz-Krawczyk A, Winkle M, van den Berg A and Kluiver J: Intricate crosstalk between MYC and non-coding RNAs regulates hallmarks of cancer. Mol Oncol 13: 26-45, 2019.
41. Tao J, Zhao X and Tao J: c-myc-miRNA circuitry: A central regulator of aggressive B-cell malignancies. Cell Cycle 13: 191-198, 2014.
42. Pelosi A, Careccia S, Lulli V, Romania P, Marziali G, Testa U, Lavorgna S, Lo-Coco F, Petti MC, Calabretta B, *et al*: miRNA let-7c promotes granulocytic differentiation in acute myeloid leukemia. Oncogene 32: 3648-3654, 2013.
43. Qi F, Wang X, Zhao S, Wang C, Sun R, Wang H, Du P, Wang J, Wang X and Jiang G: miR-let-7c-3p targeting on Egr-1 contributes to the committed differentiation of leukemia cells into monocyte/macrophages. Oncol Lett 24: 273, 2022.
44. Wong TS, Man OY, Tsang CM, Tsao SW, Tsang RK, Chan JY, Ho WK, Wei WI and To VS: MicroRNA let-7 suppresses nasopharyngeal carcinoma cells proliferation through downregulating c-Myc expression. J Cancer Res Clin Oncol 137: 415-422, 2011.

45. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, Petrelli NJ, Dunn SP and Krueger LJ: MicroRNA-let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. Cancer Res 67: 9762-9770, 2007.
46. Buechner J, Tømte E, Haug BH, Henriksen JR, Løkke C, Flægstad T and Einvik C: Tumour-suppressor microRNAs let-7 and mir-101 target the proto-oncogene MYCN and inhibit cell proliferation in MYCN-amplified neuroblastoma. Br J Cancer 105: 296-303, 2011.
47. Lan FF, Wang H, Chen YC, Chan CY, Ng SS, Li K, Xie D, He ML, Lin MC and Kung HF: Hsa-let-7g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-myc and upregulation of p16(INK4A). Int J Cancer 128: 319-331, 2011.
48. He XY, Chen JX, Zhang Z, Li CL, Peng QL and Peng HM: The let-7a microRNA protects from growth of lung carcinoma by suppression of k-Ras and c-Myc in nude mice. J Cancer Res Clin Oncol 136: 1023-1028, 2010.
49. Zhang WF, Xiong YW, Zhu TT, Xiong AZ, Bao HH and Cheng XS: MicroRNA let-7g inhibited hypoxia-induced proliferation of PASMCs via G0/G1 cell cycle arrest by targeting c-myc. Life Sci 170: 9-15, 2017.