Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dihydromyricetin sensitizes human acute myeloid leukemia cells to retinoic acid-induced myeloid differentiation by activating STAT1

Ming-hui He, Qiang Zhang, Gang Shu, Ju-chun Lin, Ling Zhao, Xiao-xia Liang, Lizi Yin, Fei Shi, Hua-lin Fu*, Zhi-xiang Yuan**

Department of Pharmacy, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China

ARTICLE INFO

Article history:
Received 22 November 2017
Accepted 6 December 2017
Available online xxx

Keywords:
Myeloid differentiation
Acute myeloid leukemia
Dihydromyricetin
ATRA
STAT1

ABSTRACT

The success of all-trans retinoic acid (ATRA) in differentiation therapy for patients with acute promyelocytic leukemia (APL) highly encourages researches to apply a new combination therapy based on ATRA. Therefore, research strategies to further sensitize cells to retinoids are urgently needed. In this study, we showed that Dihydromyricetin (DMY), a 2,3-dihydroflavonol compound, exhibited a strong synergy with ATRA to promote APL NB4 cell differentiation. We observed that DMY sensitized the NB4 cells to ATRA-induced cell growth inhibition, CD11b expression, NBT reduction and myeloid regulator expression. PML-RARα might not be essential for DMY-enhanced differentiation when combined with ATRA, while the enhanced differentiation was dependent on the activation of p38-STAT1 signaling pathway. Taken together, our study is the first to evaluate the synergy of DMY and ATRA in NB4 cell differentiation and to assess new opportunities for the combination of DMY and ATRA as a promising approach for future differentiation therapy.

1. Introduction

Because all-trans retinoic acid (ATRA) (Fig. 1A) was successfully employed for the treatment of acute promyelocytic leukemia (APL), which is a distinct subtype of acute myeloid leukemia (AML), it has opened new perspectives for differentiation therapy [1,2]. However, early death rate remains high despite the wide availability of all-trans retinoic acid [3]. Therefore, a new differentiation therapy that improves the effectiveness of ATRA is urgently needed. One possible means for overcoming these problems might be the use of a combination of ATRA with other agents, which was tested in leukemic by different groups during the past decades. Some studies showed that, Bortezomib [4], TAK165 [5], Emodin [6] and Pharicin B [7] could effectively sensitize AML cells to ATRA differentiation, which suggested that it was a promising way to find another agent works with ATRA.

Dihydromyricetin (DMY, $C_{15}H_{12}O_8$, PubChem CID: 161557), a 2,3-dihydroflavonol compound (Fig. 1B), is the main bioactive component extracted from Ampelopsis grossedentata, is one kind of flavonoids that has many biologic effects, including antialcohol intoxication, reducing blood pressure, antibacterial and antioxidant [8–10]. Recently, it has been shown in some cancer cells that DMY possesses antitumor effects, such as antiproliferation, cell-cycle arrest, induction of apoptosis, and increased sensitivity to chemotherapeutic drugs [11]. Previous studies have reported significant inhibitory activity of DMY against breast cancer MCF-7 cells [12], liver cancer Bel-7402 cells [13] and lung cancer H1299 cells [14]. However, very little is known about its effects on cell differentiation and the underlying mechanisms of DMY's anticancer effects are still under investigation.

In the present study, we observed significant synergy between DMY and ATRA when they were used in combination against AML cells. We demonstrate that the enhanced differentiation might be associated with the STAT1 activation rather than PML-RARα degradation. STAT1 knockdown significantly decreased the differentiating effect of DMY and ATRA. Collectively, this study evaluated the capacity of DMY to synergize with ATRA on AML cell differentiation, suggesting that this combination therapy is a promising approach as a future differentiation therapy for AML patients.

* Corresponding author.
** Corresponding author.
E-mail addresses: fuhl2005@sohu.com (H.-l. Fu), zhixiang-yuan@hotmail.com (Z.-x. Yuan).

https://doi.org/10.1016/j.bbrc.2017.12.030
0006-291X/

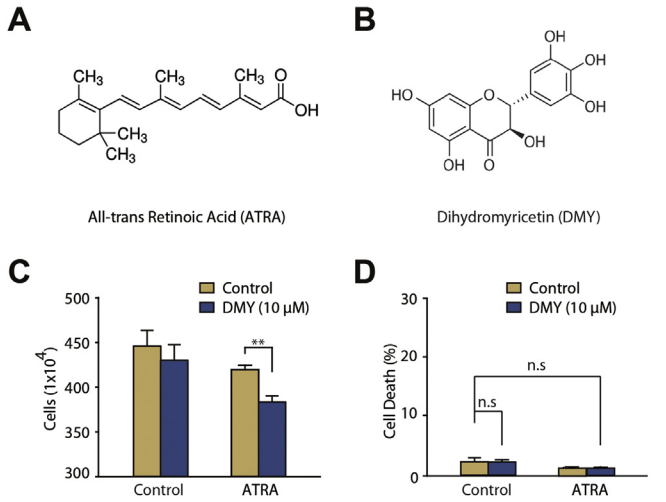

Fig. 1. Effect of DMY and ATRA on NB4 cell proliferation and apoptosis. (A–B) The chemical structures of ATRA and DMY. (C) NB4 cell proliferation assay. The cells were treated with the indicated concentrations of DMY in the presence of 3 nM ATRA, and the number of cells was counted on day 7. (D) PI staining followed by flow cytometric assay in NB4 cell. The cells were treated with the indicated concentrations of DMY in the presence of 3 nM ATRA for 3 days. In (C) and (D), the data represent the mean ± SD of 3 independent experiments. $^{**}p < 0.01$; n.s, $p > 0.05$.

2. Materials and methods

2.1. Cells and reagents

Human myeloid leukemia NB4 cells were kind gifts from Dr. Lingtao Wu (University of Southern California, Los Angeles). Monkey kidney COS-7 cell lines were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Human embryonic kidney 293FT cell line was supplied by Invitrogen (Grand Island, NY). Upon arrival in our laboratory, the cells were grown and were frozen as seed stocks as they became available. All cell lines were passaged for a maximum of 2 months, after which, new seed stocks were thawed. All cell lines were authenticated using DNA fingerprinting (variable number of tandem repeats), confirming that no cross-contamination occurred during this study. All cell lines were tested for mycoplasma contamination at least every month. The NB4 cell line was cultured in RPMI-1640 media (Gibco BRL). The COS7 cells were cultured in Dulbecco's Modified Eagle Medium. All of the media were supplemented with 10% fetal calf serum (Gibco BRL) and 1% penicillin/streptomycin. The cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

ATRA was purchased from Sigma and dissolved in ethanol. Nitrobluetetrazolium (NBT) was from Calbiochem (San Diego, CA). DMY was purchased from Zelang Biochemical Technology Co. Ltd.(Nanjing, China). All of them were stored at −20 °C. In all of the experiments, the final DMSO solvent concentration was ≤0.1% (v/v).

2.2. Cellular proliferation, apoptosis analysis

The total cell number and the viability were assessed by trypan blue exclusion with manual counting in Burkerchambers. Apoptosis was detected with the Annexin VFITC Apoptosis Detection Kit according to the manufacturer's instructions (BD Biosciences).

2.3. Differentiation detection

The induction of cell differentiation was determined by assessing CD11b expression, and a nitro blue tetrazolium (NBT) reduction assay.

To assess CD11b expression, the cells (1×10^6) were harvested and washed with PBS, blocked with 3% bovine serum albumin (BSA) in PBS for 30 min, and incubated with anti-human CD11b antibody (PE conjugated) for 45 min on ice. After incubation, the CD11b expression levels were analyzed with a FACS Calibur flow cytometer (BD Biosciences).

To assess NBT reduction, the cells (5×10^5) were harvested and incubated with PBS containing NBT (1 mg/mL) and freshly diluted 12-*O*-tetradecanoylphorbol-l3-acetate (TPA; 1 mg/mL) at 37 °C for 30 min. The cytospin slides were prepared and examined for cells containing precipitated for mazan particles. At least 200 cells were assessed for each experiment.

2.4. Western blotting

Protein extracts were resolved by 8%−15% SDS-PAGE. The proteins were transferred onto polyvinylidenedifluoride (PVDF) membranes; the membranes were blocked with 5% nonfat dry milk and incubated with primary antibodies. Antibodies against ERK1 (K-23), JNK (FL), MEK1/2, p38 (H-147), *p*-MEK-1 (Thr291), *p*-ERK1/2 (Thr204), and GAPDH (FL-335) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). PU.1, p-STAT1 (Ser727), p-p38, STAT1, C/EBPβ (LAP), and *p*-SAPK/JNK (Thr183/Tyr185) antibodies were purchased from Cell Signaling Technology (Danvers, MA). PML-RARa (ab43152) was purchased from Abcam. The western blot was visualized using HRP-conjugated secondary antibodies (Jackson Immuno Research Laboratories, Inc., West Grove, PA), followed by enhanced chemiluminescence detection (Biological Industries, BeitHaemek, Israel).

2.5. Real-time PCR

Total RNA was extracted from 1×10^6 cells with theTrizol reagent (Bio Basic, Inc.), and cDNA was synthesized using 2 μg of total RNA with Revert Aid M-MuLV Reverse Transcriptase (Fermentas International, Inc.). Equal amounts of cDNA were taken for transcript PCR amplification, which was carried out using QuantiTect SYBR Green PCR Kits (Qiagen, Inc.). Glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) was used as an internal standard. The primers used for PCR were as follows: PU.1: forward 5′-ATGTGCCTCCAGTACCCATC-3′, reverse 5′-TCTTCTGGTAGGTCATCTTC-3'; CEBPB: forward 5′-ACAGCGACGAGTACAAGATCC-3′, reverse 5′-GCAGCTGCTTGAACAAGTTCC-3'; CEBPE: forward 5′-CAGCCACTCGAGTTCTCAGG-3′, reverse 5 ′-TGGCTTCACGGCAAAGAGAT-3'; RARB: forward 5′-TTCAGTGCAAGGGAGATCT-3′, reverse 5′-GACGGACTCGCAGTGTAGAAATC-3'; PXN: forward 5′-CATGTACGTCCCCACGAACTG-3'; reverse 5′-CACTGCTGAAATATGAGGAAGAGATG-3'. The PCR protocol consisted of thermal cycling as follows: an initial denaturation at 95 °C for 2 min followed by 40 cycles of 95 °C for 20 s; 58 °C for 30 s; and 72 °C for 30 s using an Eppendorfep Gradient Mastercycler (Eppendorf, Hamburg, Germany). In all of the experiments, two negative controls were carried through all of the steps.

2.6. Virus production and lentiviral transduction

Recombinant lentiviruses were produced by co-transfecting the 293FT cells with STAT1 shRNA expression plasmid (RHS4533-NM_007315; Open Biosystems), pRΔ8.9 packaging plasmids and pMD.G envelope plasmids. Supernatants containing the infectious virus particles were collected 48 h after the transfection and then used to transduce the AML cells by spinoculationin the presence of polybrene (6 μg/mL). The expression ofSTAT1 was analyzed by

Please cite this article in press as: M.-h. He, et al., Dihydromyricetin sensitizes human acute myeloid leukemia cells to retinoic acid-induced myeloid differentiation by activating STAT1, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.12.030