

ARTICLE

Open Access

DDX5-targeting fully human monoclonal autoantibody inhibits proliferation and promotes differentiation of acute promyelocytic leukemia cells by increasing ROS production

Jing Wu¹, Yan-Qiu You², Yan-Xiu Ma¹, Yan-Hua Kang¹, Tian Wu³, Xiang-Ji Wu¹, Xiao-Xiao Hu¹, Qiao-Hong Meng¹, Yin Huang¹, Na Zhang¹ and Xiao-Ben Pan¹

Abstract

Acute promyelocytic leukemia (APL) therapy involves the compounds cytotoxic to both malignant tumor and normal cells. Relapsed APL is resistant to subsequent chemotherapy. Novel agents are in need to kill APL cells selectively with minimal toxicity. DDX5 has been recognized to be a novel target to suppress acute myeloid leukemia (AML). However, the role of DDX5 remains elusive in APL. Here a DDX5-targeting fully human monoclonal autoantibody named after 2F5 was prepared. It is demonstrated that 2F5 selectively inhibited APL cell proliferation without toxicity to normal neutrophil and tissues. Moreover, 2F5 was confirmed to induce G0/G1 phase arrest in APL cells, and promote APL cell differentiation combined with decreased DDX5 expression and increased reactive oxygen species (ROS) production. Knockdown of DDX5 by siRNA also inhibited proliferation, promoted cell differentiation and enhanced ROS production in APL cells. However, the ROS inhibitor reversed the effects of 2F5 on DDX5 and ROS in APL cells. Thus, we conclude that DDX5-targeting 2F5 inhibits APL cell proliferation, and promotes cell differentiation via induction of ROS. 2F5 showed the therapeutic value of fully human monoclonal autoantibody in APL, which provides a novel and valid approach for treatment of relapse/refractory APL.

Introduction

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by specific biological and clinical features. APL is distinguished by t(15; 17) chromosomal translocation¹, which causes the production of a fusion protein known as promyelocytic

leukemia–retinoic acid receptor (PML-RAR α)². APL has been characterized by early onset of clinical signs, disseminated intravascular coagulation and poor response to chemotherapy. Though previously marked by high mortality, it is nowadays the most curable form of AML³. AML therapy is comprised of therapeutic agents that induce apoptosis or promote the differentiation of cancer cells. At present, APL is treated by all-trans retinoic acid (ATRA) in combination with arsenic trioxide (ATO) or by ATRA and chemotherapy^{4–6}. However, the resistant to ATRA and ATO of relapse/refractory APL patients is recognized as a critical problem in clinical practice⁷. Therefore, finding alternative targeting drugs with low toxicity may bring prospective solution to the treatment of relapse/refractory APL.

Correspondence: Na Zhang (zhangna@hznu.edu.cn) or Xiao-Ben Pan (panxiaoben@hznu.edu.cn)

¹Department of Basic Medicine of Medical School, Department of Infectious Diseases of Affiliated Hospital, Institute of Liver and Metabolic Diseases, Key Laboratory of Aging and Cancer Biology of Zhejiang Province, and Key Laboratory of Inflammation and Immunoregulation of Hangzhou, Hangzhou Normal University, 310000 Hangzhou, Zhejiang, P.R. China

²Department of Laboratory Medicine, The Second Affiliated Hospital of Harbin Medical University, 150001 Harbin, Heilongjiang, P.R. China

Full list of author information is available at the end of the article

These authors contributed equally: Jing Wu, Yan-Qiu You, Yan-Xiu Ma

Edited by M. Diederich

© The Author(s) 2020



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

It has been demonstrated that AML patients had a complex karyotype which is marked by aberration expression of dead-box helicases⁸. Dead-box helicase 5 (DDX5) is a member of this family. Experimental depletion of DDX5 inhibits proliferation of AML cells and induces apoptosis by promoting the production of ROS⁹. Similarly, DDX5 is required in T-cell acute lymphoblastic leukemia (T-ALL) pathogenesis, which is evidenced by the decreased survival rate and inhibited proliferation following depletion of DDX5¹⁰. All these findings indicated that DDX5 may be a potential drug target in the treatment of APL.

Herein, a DDX5-targeting fully human monoclonal autoantibody named after 2F5 was prepared. And then the application potential of 2F5 in the therapy of APL was assessed. Results showed that 2F5 not only markedly inhibited the proliferation of APL cells, but also promoted APL cell differentiation by increasing ROS production. Considering the nontoxicity of 2F5 in cell viability, this study could provide a basis for the potential use of 2F5 in relapse/refractory APL therapy.

Materials and methods

Ethics statement

Experiments involving human and animal samples were approved by the Research Ethics Review Committee of Hangzhou Normal University. Animal procedures performed in this work followed guidelines in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals. Written informed consents were obtained from all participants.

The preparation of DDX5-targeting fully human monoclonal autoantibody

Monoclonal antibodies were generated with hybridoma technology. SPYMEG (MBL, Nagoya, Japan)^{11,12} was used as a fusion partner cell for generating human monoclonal antibody that recognizes DDX5 specifically. Peripheral blood mononuclear cells (PBMCs) were obtained from the blood sample of SLE patient, and then were fused with SPYMEG to yield hybridomas. The resulting hybridomas were screened for DDX5-specific antibody secretion and cloned by limiting dilution. One stable clone secreting anti-DDX5 human monoclonal autoantibody was obtained and named after 2F5. The specific binding and affinity between 2F5 and DDX5 (OriGene, Rockville, USA) was determined by Surface Plasmon Resonance (Biacore X100, GE, USA) (Fig. S1b).

Cell lines and culture

The human APL cell lines (HL-60 and NB4), T-ALL cell lines (Jurkat and CEM-C7), and monocytic leukemia cell line (THP-1) were purchased from Jennio Biotechnology Co., Ltd (Guangzhou, Guangdong, CHN). Blood samples

were obtained from healthy volunteer. Neutrophils were isolated with human neutrophil isolation Kit (STEM-CELL, CA, USA). PBMCs and monocytes were extracted with isolation kit (Solarbio, Beijing, China). Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco, Waltham, MA, USA) at 37 °C in a humidified incubator with 5% CO₂. Cells were cultured in culture medium (normal control), and were treated with 2F5 or IgG (negative control) with different concentrations (20, 40, and 80 μM) for 4, 8, 12, and 16 days. Every 4 days, the cultures were established by centrifugation and then the cell pellets were resuspended in fresh corresponding medium respectively.

Animal treatment and pathological examination

The 8-week-old Balb/c male mice were obtained from the Center for Laboratory Animals, Hangzhou Normal University, and were randomly divided into two groups. Mice were treated with 12 μg/kg 2F5 by intravenous injection ($n = 6$). Control mice received the same volume of saline ($n = 6$). Mice were euthanized with inhalation of anesthesia 24 h after injection. Tissue samples were fixed, dehydrated, embedded in paraffin, and sectioned into 4-μm slices for subsequent H&E staining. Wright-Giesma staining was performed using a staining kit (Solarbio, Beijing, China).

Cell proliferation assay

Cell Counting Kit-8 (CCK-8, Sigma Aldrich, St. Louis, MO, USA) was used to detect cell proliferation, according to the manufacturer's protocol. Cells were divided into three groups including 2F5 treatment group, IgG control and normal control group, and then 1×10^4 cells were seeded with 100 μL different treatment medium per well in 96-well plate. CCK-8 solution was added to each well, and incubated for 4 h in the incubator. The absorbance was measured at 450 nm using a microplate reader. The living cell number in each group was calculated by the formula from standard curve.

Western blotting

Protein lysates (50 μg/lane) were resolved on appropriate SDS-PAGE gels and transferred to PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked, incubated with the indicated primary antibodies at 4 °C overnight, and then with the appropriate horseradish peroxidase-conjugated secondary antibodies. Blots were quantified with Image J (National Institutes of Health, Bethesda, MD, USA). Primary antibodies from Abcam (Cambridge, USA) were used: DDX5 (#ab126730), cyclin A2 (#ab181591), CDK4 (#ab108357), cyclin D1 (#ab16663), PCNA (#ab92552). β-actin (#AC026, ABclonal, Wuhan, China) was used as an internal control.

NBT reduction assay

Cells were harvested in number of 1×10^6 and incubated with a 1:1 mixture of culture medium and PBS containing 2 mg/mL nitro blue tetrazolium (NBT) and 1 μ g/mL phorbol 12-myristate 13-acetate (Sigma Aldrich, St. Louis, MO, USA) at 37 °C for 1 h. The reaction was stopped by adding 200 μ L of solution of hydrochloric acid (1 M). The absorbance at 562 nm was measured by spectrophotometer (Bio-Rad, Hercules, CA, USA).

Flow cytometry

APL cells were incubated with 2F5 with ladder concentrations and 1 mM ATRA (Sigma Aldrich, St. Louis, MO, USA) for 4, 8, 12, or 16 days. For flow cytometry analysis, 5×10^5 cells were harvested and washed with PBS, and labeled for 20 min with PE-conjugated anti-human CD11b or CD14 antibody (BD Biosciences, San Jose, CA). After washing with PBS, cells were analyzed on flow cytometry (BD Biosciences, San Jose, CA).

ROS detection

The intracellular ROS was determined by ROS assay kit (Solarbio, Beijing, China). In brief, cells in different treatment groups were harvested, rinsed with PBS and incubated with DCFH-DA at 37 °C for 30 min. Then the DCF fluorescence of the cells was detected by fluorospectrophotometer (Bio-Rad, Hercules, CA, USA) or flow cytometry at an excitation wavelength of 488 nm and an emission wavelength of 535 nm. Five micromolar of N-Acetyl-L-cysteine (NAC, APEX BIO, Houston, USA) was added to culture medium for blocking the production of ROS. As a ROS positive control, cells were treated with 100 μ M H₂O₂ for 48 h as described previously¹³.

Cell cycle analysis

Cells (1×10^6 /mL) were harvested and were centrifuged. And then the cells were fixed with cold 70% ethanol overnight at 4 °C, and then were washed twice by cold PBS. Cells were resuspended with 0.5 mL PI/RNase staining solution. After gentle vortex, cells were incubated for 30 min in room temperature, protected against light. Then cell cycle was analyzed by flow cytometry. The cell cycle data was fitted and analyzed by ModFit LT (verity software house, USA).

siRNA transfection

Small interference RNA (siRNA) targeting DDX5, siDDX5 #1, 5'-GGUGCAGCAAGUAGCUGCUGAAUAU-3'; siDDX5 #2, 5'-GGAAUCUUGAUGAGCUGCCUAAAU-3'; and its negative control scrambled siRNA were synthesized from Sangon Biotech (Shanghai, China) Co., Ltd. siRNA targeting DDX5 and control siRNA were transfected to HL-60 and NB4 cells according to the manufacturer's

instructions. Briefly, cells were transfected with 50 nM siRNA in serum-free and antibiotics-free DMEM containing 5 μ L of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The medium was changed 6 h later with normal growth medium supplemented with FBS. After 96 h of transfection cells were harvested.

Surface plasmon resonance (SPR)

SPR is used to generate affinity information on specific interactions between 2F5 and DDX5 (Biacore X100, GE, USA). An anti-IgG antibody was firstly covalently immobilized onto a CM5 chip. Then 25 μ M 2F5 were captured noncovalently onto the surface via their Fc region providing an optimal analyte-binding orientation. The resulting complex was stabilized by crosslinking with EDC/NHS to avoid baseline drift during measurement and regeneration. Finally, the interaction between 2F5 and DDX5 was monitored. PBS was used as running buffer in the binding assay. DDX5 (100 nM) were injected at a flow rate of 50 μ L/min. After association and dissociation of the analyte, a regeneration step was performed in order to remove the remaining bound analyte.

Statistical analysis

Data are presented as mean \pm standard error of the mean (SEM), and all the experiments were repeated at least three times. Statistical analysis was performed using SPSS 13.0. One-way analysis of variance (ANOVA) was used to compare the differences between control and treatment groups. $p < 0.05$ was considered statistically significant.

Results

APL cell proliferation inhibited by monoclonal autoantibody 2F5

The effect of 2F5 on cell proliferation of different leukemia cell lines was detected by CCK-8 assay. Five cell lines including NB4, HL-60, Jurkat, CEM-C7, and THP-1 cells were treated with 40 μ M 2F5, respectively for 4 days. Human neutrophil, PBMC, and monocyte were used as healthy controls. The results of CCK-8 assay indicated that 2F5 could significantly inhibit cell proliferation of NB4 and HL-60 cells compared with nontargeting IgG and normal control group (Fig. 1a, b, *** $p < 0.001$). However, 2F5 had no effect on cell proliferation of other three leukemia cell lines as well as the healthy controls (Fig. 1c–h). Cyclin D1 and PCNA expression levels in NB4 and HL-60 cells were analyzed for 4 days after 2F5 treatment with three different concentrations (20, 40, and 80 μ M). Compared with both nontargeting IgG and normal control group, 2F5 induced a significant dose-dependent decrease of cyclin D1 and PCNA expression levels in NB4 and HL-60 cells (Fig. S2).

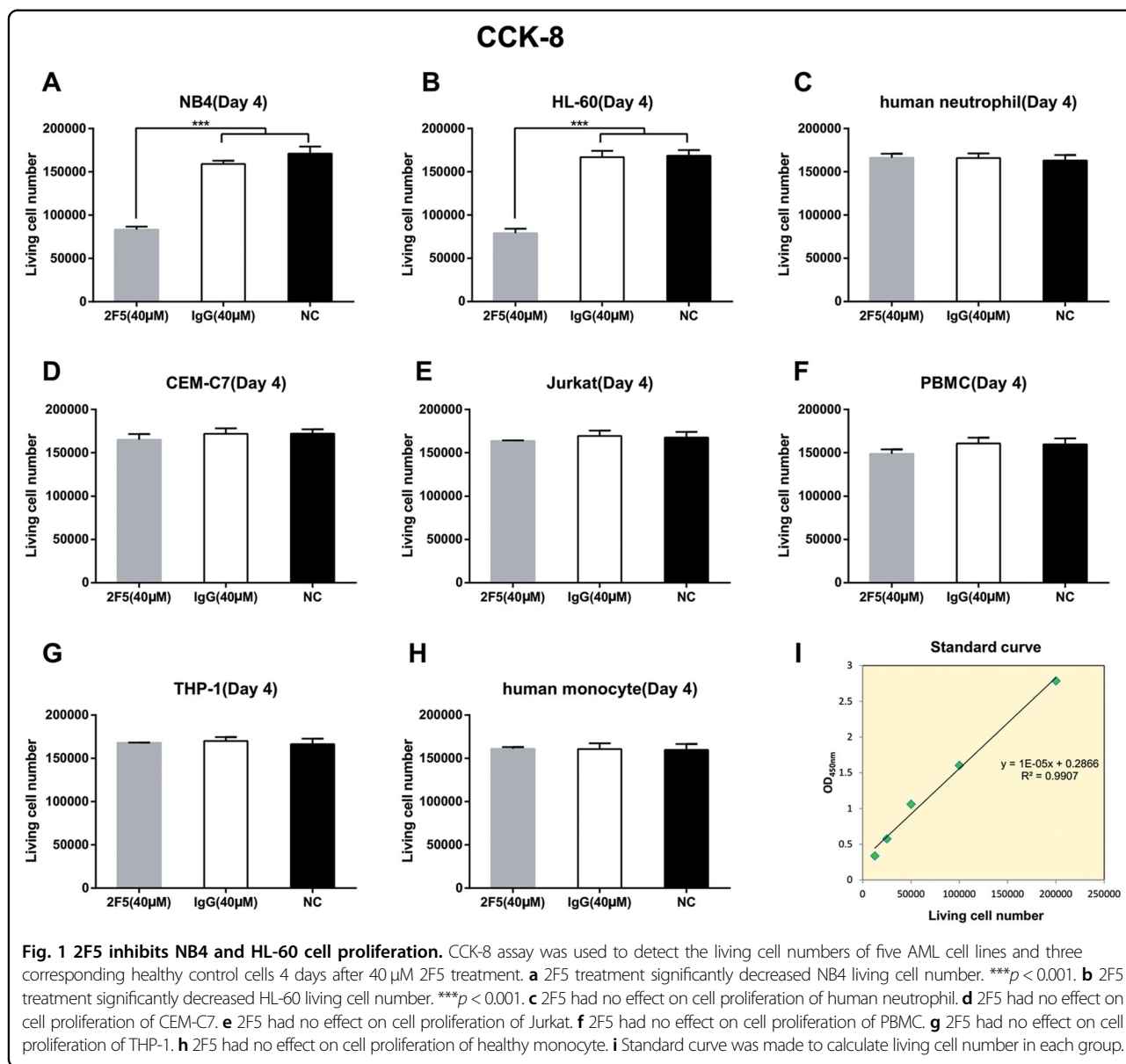


Fig. 1 2F5 inhibits NB4 and HL-60 cell proliferation. CCK-8 assay was used to detect the living cell numbers of five AML cell lines and three corresponding healthy control cells 4 days after 40 µM 2F5 treatment. **a** 2F5 treatment significantly decreased NB4 living cell number. ****p* < 0.001. **b** 2F5 treatment significantly decreased HL-60 living cell number. ****p* < 0.001. **c** 2F5 had no effect on cell proliferation of human neutrophil. **d** 2F5 had no effect on cell proliferation of CEM-C7. **e** 2F5 had no effect on cell proliferation of Jurkat. **f** 2F5 had no effect on cell proliferation of PBMC. **g** 2F5 had no effect on cell proliferation of THP-1. **h** 2F5 had no effect on cell proliferation of healthy monocyte. **i** Standard curve was made to calculate living cell number in each group.

Expression of DDX5 was elevated in APL cell lines

The expression of DDX5 was detected in five different leukemia cell lines, and corresponding healthy control cells. As shown in Fig. 2, DDX5 expression levels in NB4 and HL-60 cells were much higher than the other six cell lines (**p* < 0.05, Fig. 2a, b). Additionally, DDX5 levels in normal neutrophils, PBMCs and in monocytes were significantly lower than in APL (NB4 and HL-60), T-ALL (Jurkat and CEM-C7), and in monocyte leukemia (THP-1) cell lines (**p* < 0.05, ***p* < 0.01, Fig. 2b).

Downregulated DDX5 expression induced by 2F5 in APL cells

Five types of different leukemia cells were treated with 2F5. After 4 days, DDX5 expression was detected by

western blotting. Results indicated that DDX5 expression were significantly suppressed in NB4 cells by 2F5 (**p* < 0.05, Fig. 2d, e), as well as in HL-60 cells (**p* < 0.05, Fig. 2f–h). However, there was no significant difference of DDX5 expression in CEM-C7, Jurkat, THP-1, healthy neutrophil, PBMC, and monocyte treated with 2F5, compared with IgG or normal control (Fig. 2i–n).

G0/G1 phase arrest induced by 2F5 in APL cells

The cell cycle distribution was analyzed by flow cytometry and western blotting in NB4 and HL-60 cells at day 4 after 40 µM 2F5 treatment. A significant increase in the G0/G1 phase was detected both in NB4 (***p* < 0.01, Fig. 3a–d) and HL-60 cells (***p* < 0.001, Fig. 3h–k). Meanwhile, 2F5 significantly decreased the percentages of

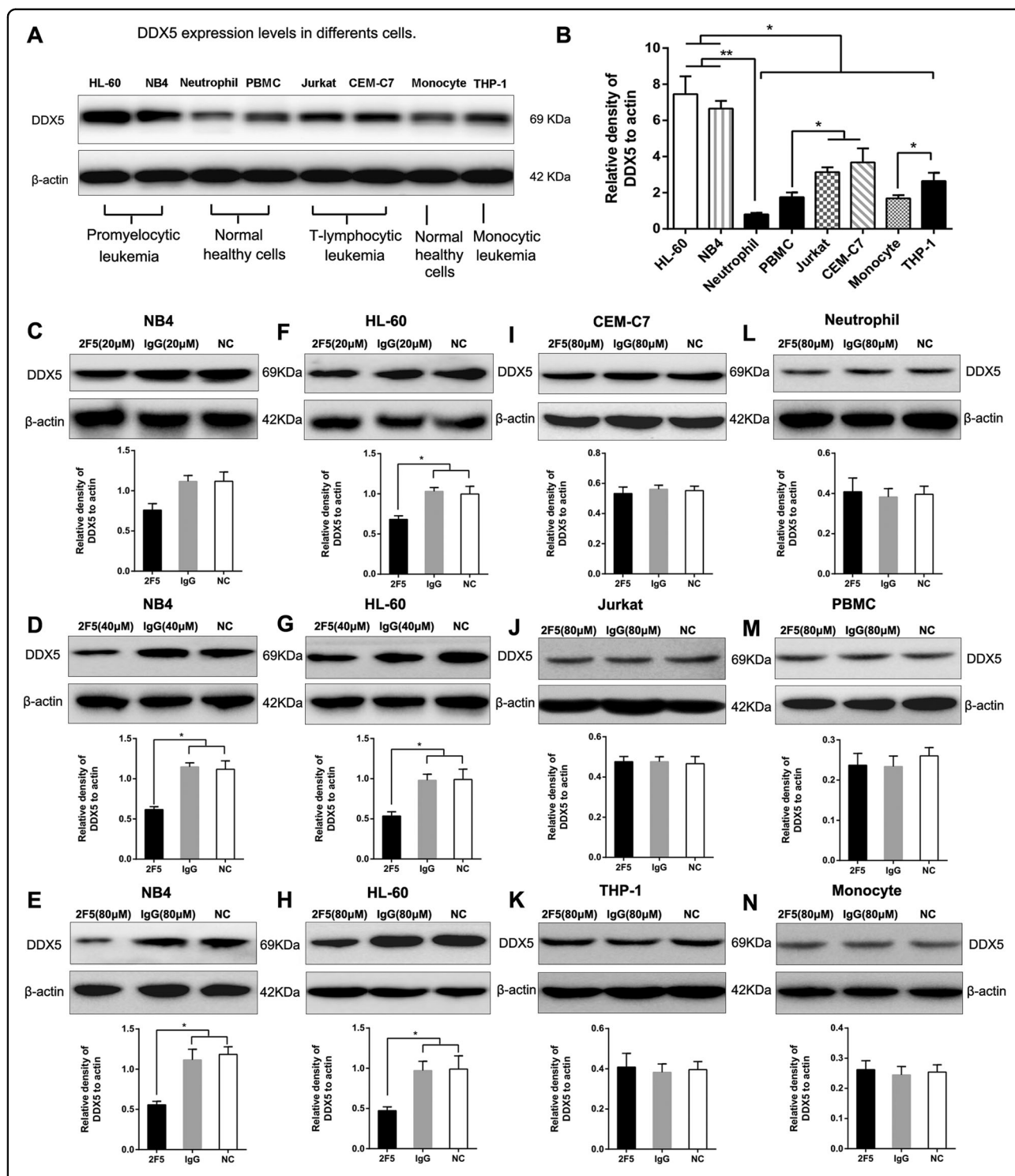
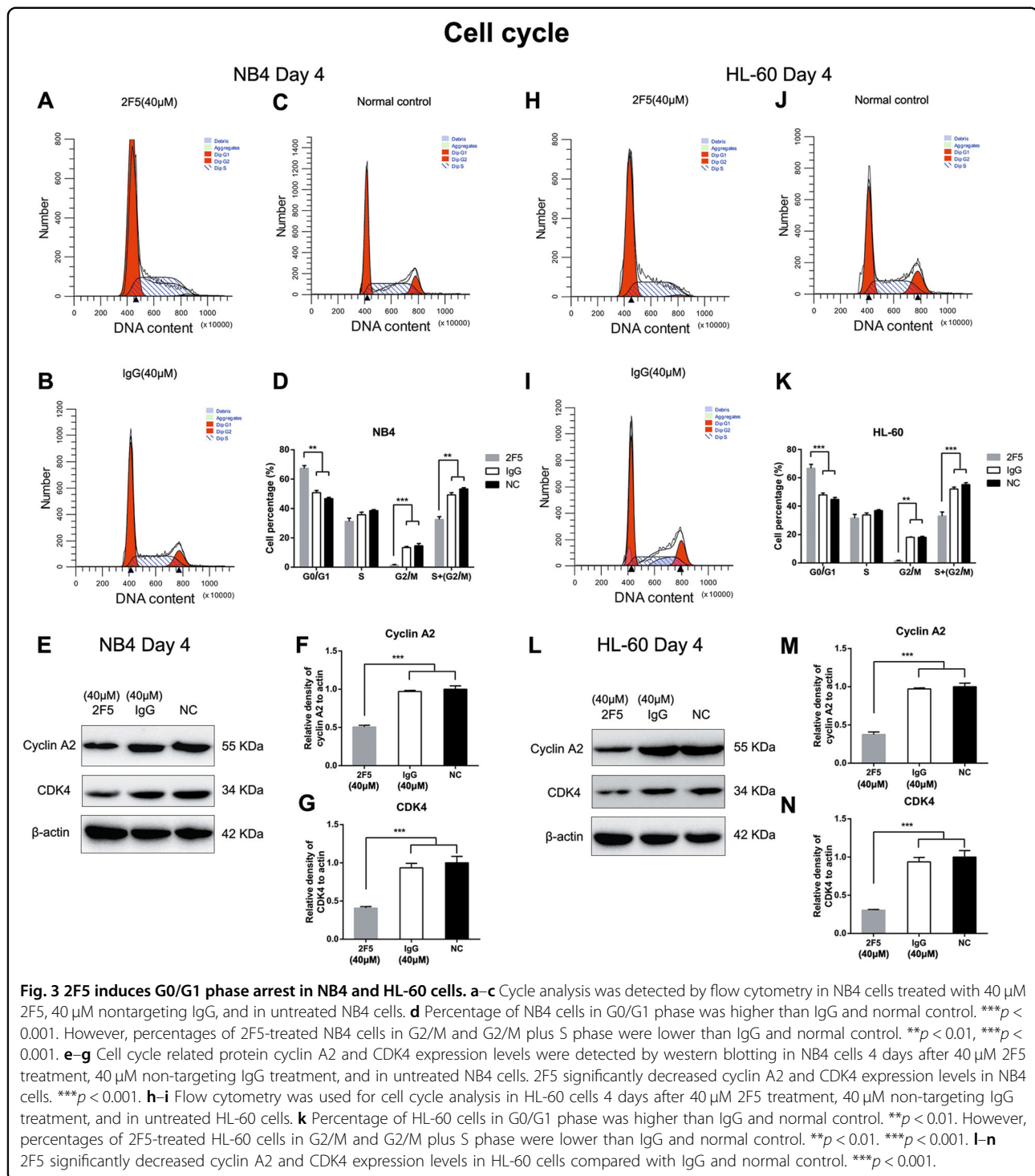


Fig. 2 The effect of 2F5 on DDX5 expression levels in different leukemia cell lines. **a** Western blotting was performed to analyze the basal expression level in five leukemia cell lines and three corresponding healthy control cells. β-actin was used as an internal control. **b** DDX5 levels in NB4 and HL-60 cells are much higher than other six types of cells. **p* < 0.05. DDX5 levels in three healthy control cells (neutrophil, PBMC and monocyte) are lower than in promyelocytic lymphoblastic and monocytic leukemia cells respectively. **p* < 0.05. ***p* < 0.01. **c–e** DDX5 expression levels were detected by western blotting in NB4 cells 4 days after 20, 40, and 80 μM 2F5 treatment. **p* < 0.05. **f–h** DDX5 expression levels were detected by western blotting in HL-60 cells 4 days after 20, 40, and 80 μM 2F5 treatment. **p* < 0.05. **i** 80 μM 2F5 had no effect on DDX5 expression levels in CEM-C7 cells. **j** 80 μM 2F5 had no effect on DDX5 expression levels in Jurkat cells. **k** 80 μM 2F5 had no effect on DDX5 expression levels in THP-1 cells. **l** 80 μM 2F5 had no effect on DDX5 expression levels in neutrophils. **m** 80 μM 2F5 had no effect on DDX5 expression levels in PBMCs. **n** 80 μM 2F5 had no effect on DDX5 expression levels in monocytes.



G2/M and G2/M plus S phase in NB4 and HL-60 cells (** $p < 0.01$, *** $p < 0.001$, Fig. 3d, k). Consistently, cell cycle related protein Cyclin A2 and CDK4 expression levels were also found dramatically decreased in NB4 (*** $p < 0.001$, Fig. 3e–g) and HL-60 cells (*** $p < 0.001$, Fig. 3l–n) after 2F5 treatment.

APL cells differentiation induced by 2F5 had no harmful effects on normal neutrophils and tissues

To examine whether 2F5 contributed to APL cell differentiation, Wright–Giemsa staining and NBT reduction assay were performed. Morphological features of cell differentiation were observed in 2F5-treated HL-60 cells

and ATRA positive control group, such as a lower nucleocytoplasmic ratio and chromatin condensation (Fig. S3). Specially, 2F5-treated HL-60 cells showed granulocytic differentiation morphological features, including shrinkage nucleus, lobulated nucleus, and grayish cytoplasm (Fig. S3e, f).

Furthermore, NBT reduction assay was performed to detect cell differentiation. The result of NBT reduction assay showed that in NB4 cells, 40 μ M 2F5 induce a significant increase of NBT reduction activity at day 4, 8, and 12 after 2F5 treatment ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, Fig. S4a–c). Two curves showed that 2F5 played a time-dependent increasing effect on NBT reduction in NB4 and HL-60 cells (Fig. S4d, h).

Cells were found dead 8 days after ATRA treatment (Fig. S4 and Fig. 4), suggesting critical toxic effect of ATRA on cells. The population of CD11b-positive and CD14-positive HL-60 cells significantly increased following 2F5 or ATRA treatment compared with control ($*p < 0.05$, Fig. 4b, e). Interestingly, even if 2F5 exposure was stopped at day 12, the increasing trend of CD11b-positive and CD14-positive cells were kept to day 16. At day 16, CD14-positive HL-60 cells of 2F5 treatment group was higher than that in ATRA positive control group at day 8 (Fig. 4e). The percentage of CD11b-positive and CD14-positive cells persistently increased from day 4 to day 16 (Fig. 4c, f). Flow cytometry result showed that 2F5 promoted the cell differentiation with a time-dependent instead of dose-independent manner. In addition, similar results were acquired in NB4 cells that both CD11b-positive and CD14-positive cells were significantly increased by 2F5 treatment ($***p < 0.001$, Fig. 5).

In order to confirm the biological safety of 2F5, neutrophils were extracted from the blood of healthy volunteer. Then cell proliferation was detected by CCK-8 assay. The results indicated that there was no significant difference of cell proliferation between 2F5-treatment, normal control, and IgG control (Fig. S3m). Morphological staining was also performed on mice tissues. In animal model, no significant pathological changes were found in brain, kidney, or liver of mice with 2F5 intravenous injection (Fig. S3i–k).

ROS production induced by 2F5 in NB4 and HL-60 cells

It has been reported that increased ROS levels can induce AML cell differentiation¹⁴, and ROS production is also critical for macrophage differentiation¹⁵. Hence, we sought to determine ROS level in APL cells treated with 2F5 by CM-H₂DCFDA ROS detection assay. Compared with IgG control, 20 μ M and 40 μ M 2F5 induced a significant increase of ROS production at day 4, 8, and 12 ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, Fig. 6a–h). The 80 μ M 2F5 significantly increased ROS production at day 8 and

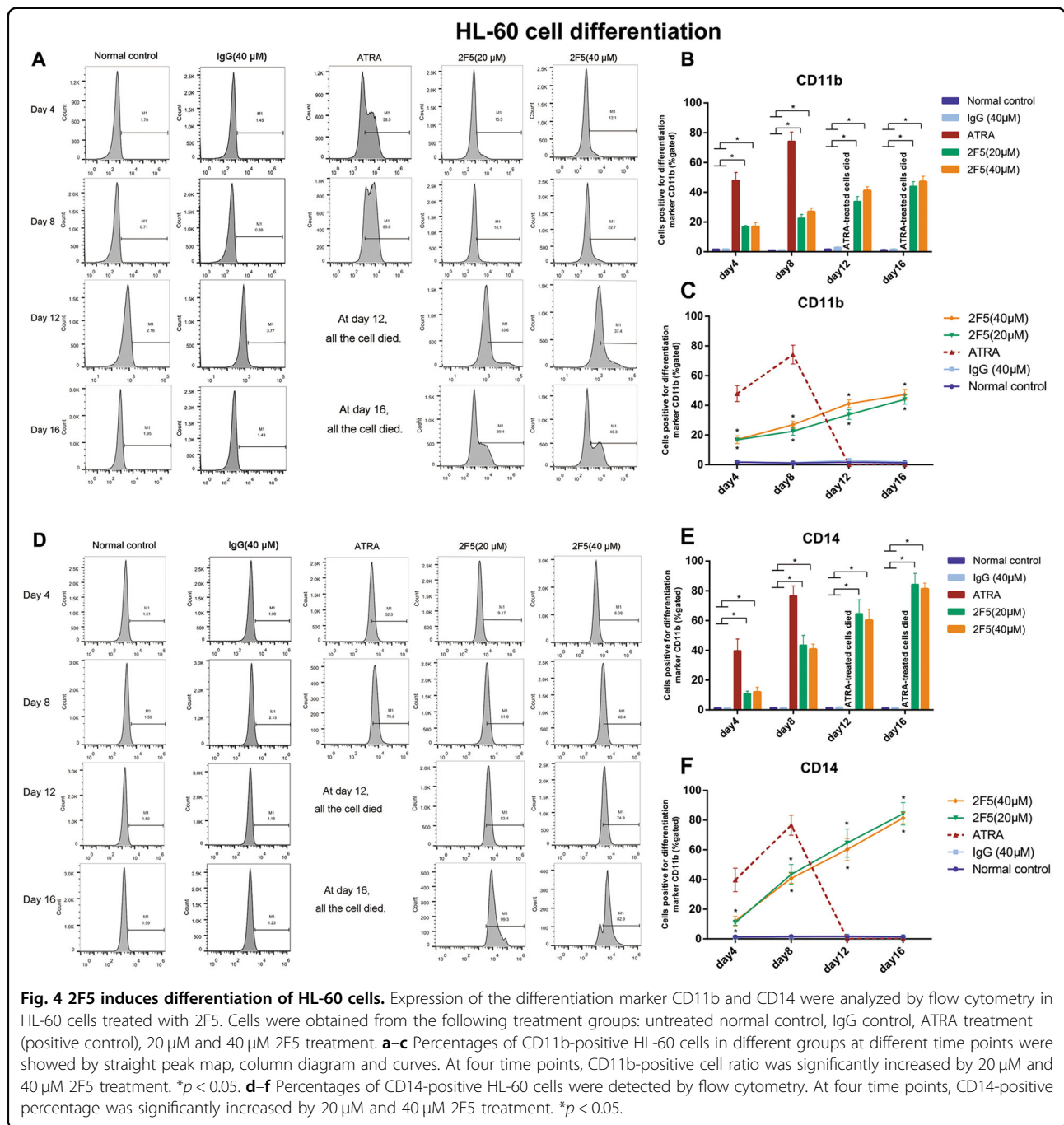
day 12 ($**p < 0.01$, $***p < 0.001$, Fig. 6j–l) except the day 4 compared with IgG control (Fig. 6i). The curve diagram showed that the effect of 2F5 on ROS production in HL-60 cells was enhanced with the prolongation of treatment time, but not dose-dependent (Fig. 6d, h, l). These findings were consistent with the results of NBT assay (Fig. S4) and flow cytometry (Fig. 4). Furthermore, increased ROS production was also detected in NB4 cells treated with 2F5, but not in CEM-C7, Jurkat and THP-1 cells ($**p < 0.01$, Fig. S5). These results indicated that 2F5 may exert its role to promote leukemia cell differentiation by ROS production.

ROS inhibitor blocked 2F5-induced cell differentiation

To determine whether ROS are required to maintain the promoting differentiation effect of 2F5, ROS inhibitor NAC was employed in NB4 and HL-60 cells, and then the ROS production, DDX5 expression and cell differentiation were analyzed. Flow cytometric detection of ROS showed that NAC could block the ROS promotion by 2F5 in NB4 and HL-60 cells (Fig. 7e, m). Compared with cells treated with 2F5 only, ROS production was significantly decreased in APL cells treated with NAC combined 2F5 ($***p < 0.001$, Fig. 7d, l). Moreover, DDX5 expression was significantly decreased in 2F5-treated NB4 and HL-60 cells, while increased combined with NAC treated group ($***p < 0.001$, Fig. 7h, p). These results suggested that the inhibition effect of 2F5 on DDX5 expression could be reversed by ROS inhibitor. In addition, flow cytometry detection of CD14 showed that compared with 2F5-treatment group, CD14-positive APL cells treated with 2F5 combined NAC were decreased significantly (Fig. S6). Collectively, these data indicated that the effect of 2F5 on promoting APL cell differentiation and inhibition of DDX5 expression is dependent on ROS production.

The role of DDX5 silence on cell proliferation, ROS production, and cell differentiation

To determine whether DDX5 plays an essential role in regulating APL cell differentiation, siRNAs targeting DDX5 were transfected in NB4 and HL-60 cells. Western blotting analysis revealed significant decrease of DDX5 expression in siDDX5 transfected NB4 and HL-60 cells compared to control scramble siRNA respectively ($*p < 0.05$, $**p < 0.01$, Fig. 8a–d). Compared with siDDX5, 2F5 had similar ability on specific down-regulating DDX5 expression level, which revealed that 2F5 treatment is sufficient to inhibit DDX5 activity. siDDX5 significantly reduced the living cell number of NB4 and HL-60 cells ($***p < 0.001$, Fig. 8e, f). Moreover, similar to 2F5, siDDX5 also increased ROS production and CD14-positive cell percentages in NB4 and HL-60 cells ($***p < 0.001$, Fig. 8m, s, γ).

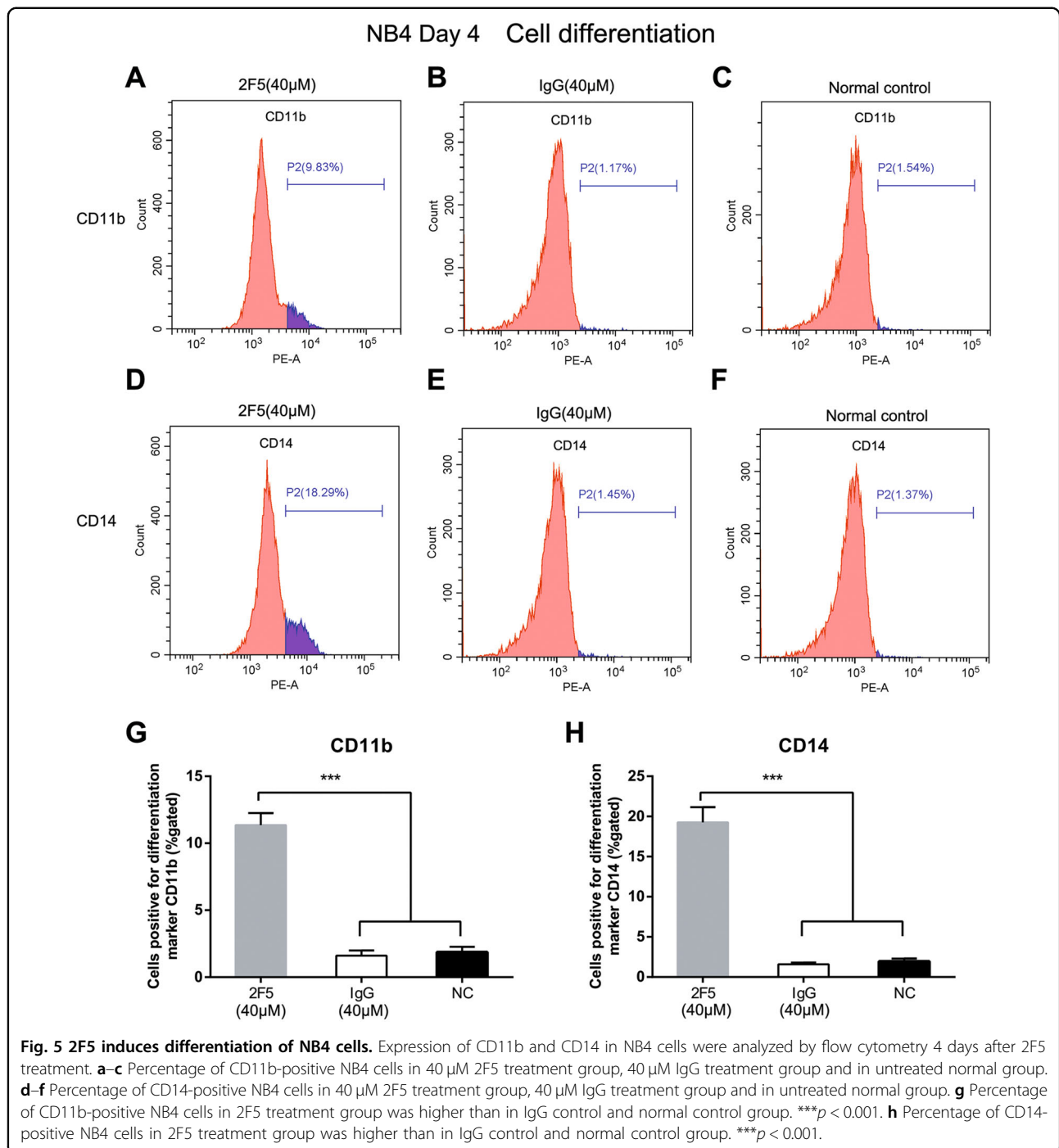


Discussion

The therapeutic management of APL has considerably evolved during the past two decades. ATRA in combination with chemotherapy has provided high cure rates. But this approach shows significant toxicity including severe myelosuppression and occurrence of relapse/refractory leukemia¹⁶. In recent years, the advent of ATO and its use in combination with ATRA with or without chemotherapy has further improved patient outcome by maintaining high antileukemic efficacy. However, common

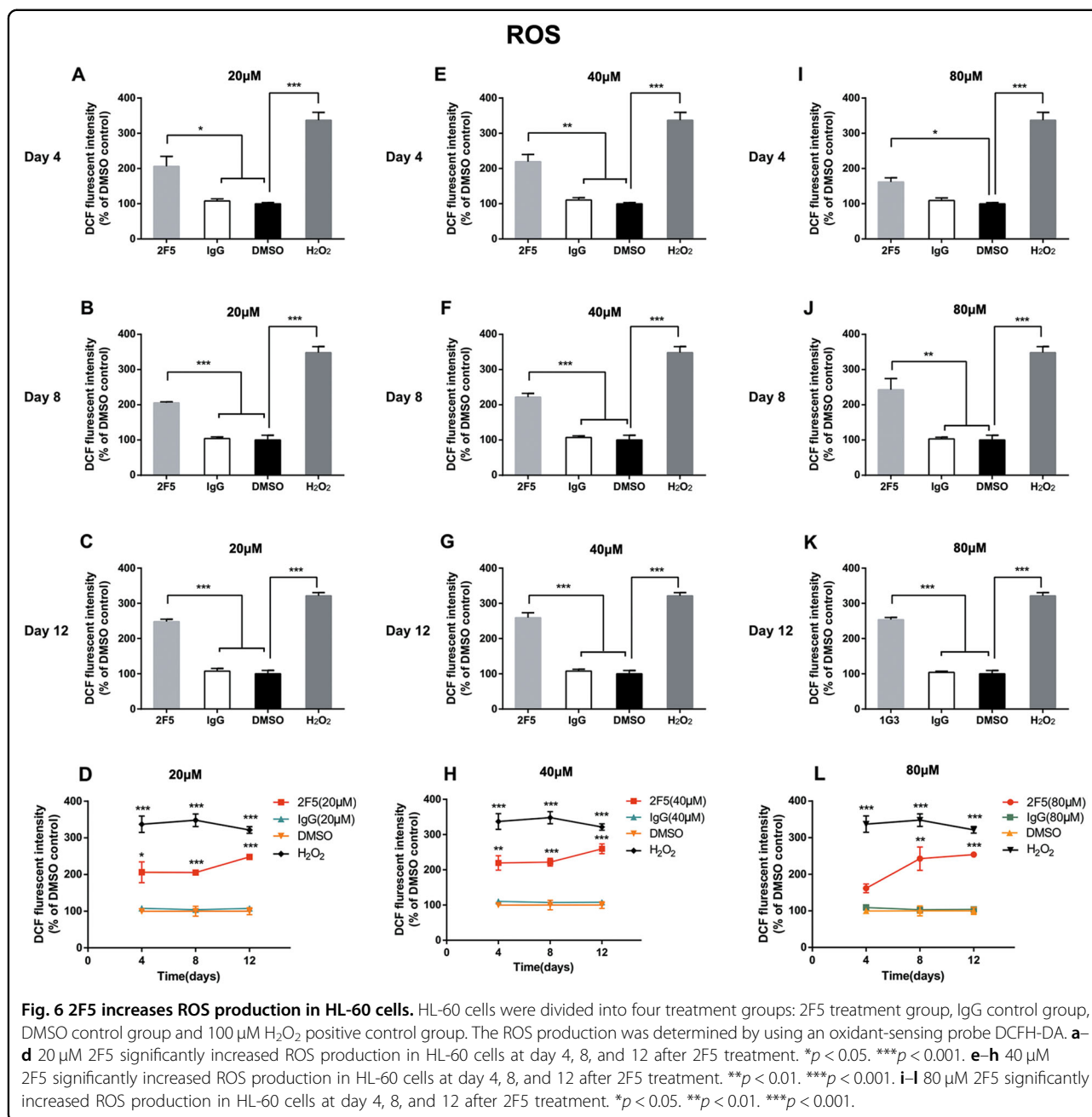
complications such as differentiation syndrome and hepatic toxicity induced by ATO causes serious harm to patients¹⁷. Monitoring liver function and adjusting treatment regimens at any time during ATO therapy also bring problem and complexity to APL treatment¹⁸. Therefore, finding new management strategy without toxicity is dramatically valuable for targeting treatment of APL.

Autoantibody is immunoglobulin directed against an individual's self-antigens, the function of which has been abundantly explored in SLE patients. In recent years,



researchers have become more interested in the role and application of autoantibodies in treatment of human malignant tumors¹⁹. Numerous epidemiological studies showed that risk of some cancers in SLE were decreased²⁰. For example, the risk of endometrial cancer in SLE patients is decreased more than 50%^{21,22}, which implies that certain autoantibodies in people with SLE may prevent carcinogenesis.

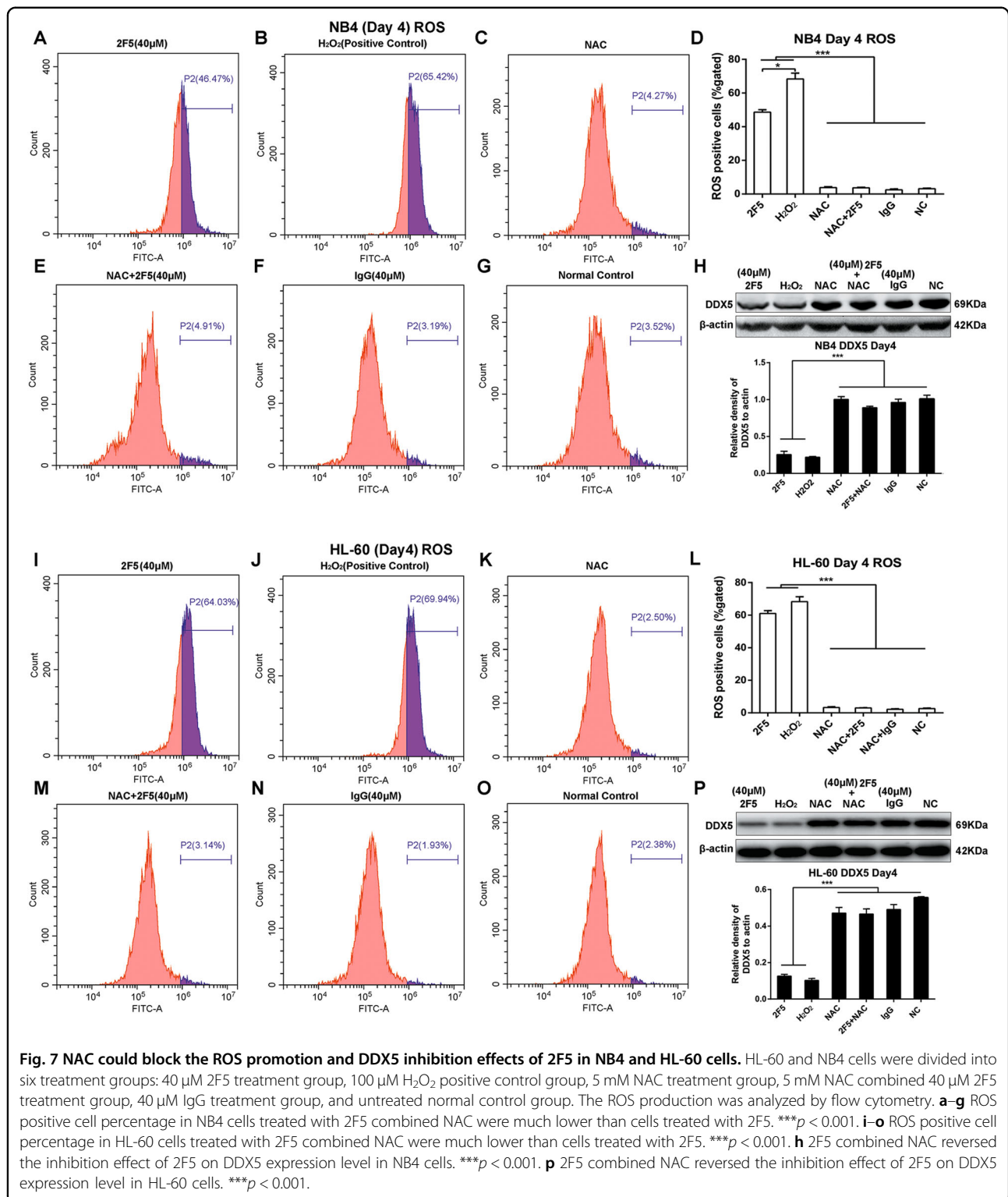
Currently, existing monoclonal antibody drugs used for treating leukemia mostly targeted lymphocyte surface molecules, such as CD20, CD3, or CD19 and so on²³. Beside those cell surface biomarkers, some transcriptional regulators have been reported to be aberrantly expressed in many cancers, and are linked to the regulation of many cancer-related pathways. Among those transcriptional regulators, the DDX5 plays a key role in the



tumorigenesis, invasiveness, and metastasis²⁴. It has been reported that AML is dependent on DDX5, and inhibition of DDX5 slows AML cell proliferation in vitro and AML progression in vivo, but is not toxic to cells from normal bone marrow⁹. These investigation conclusions provide an insight that DDX5-targeting monoclonal auto-antibodies might be potential new agents for APL therapy.

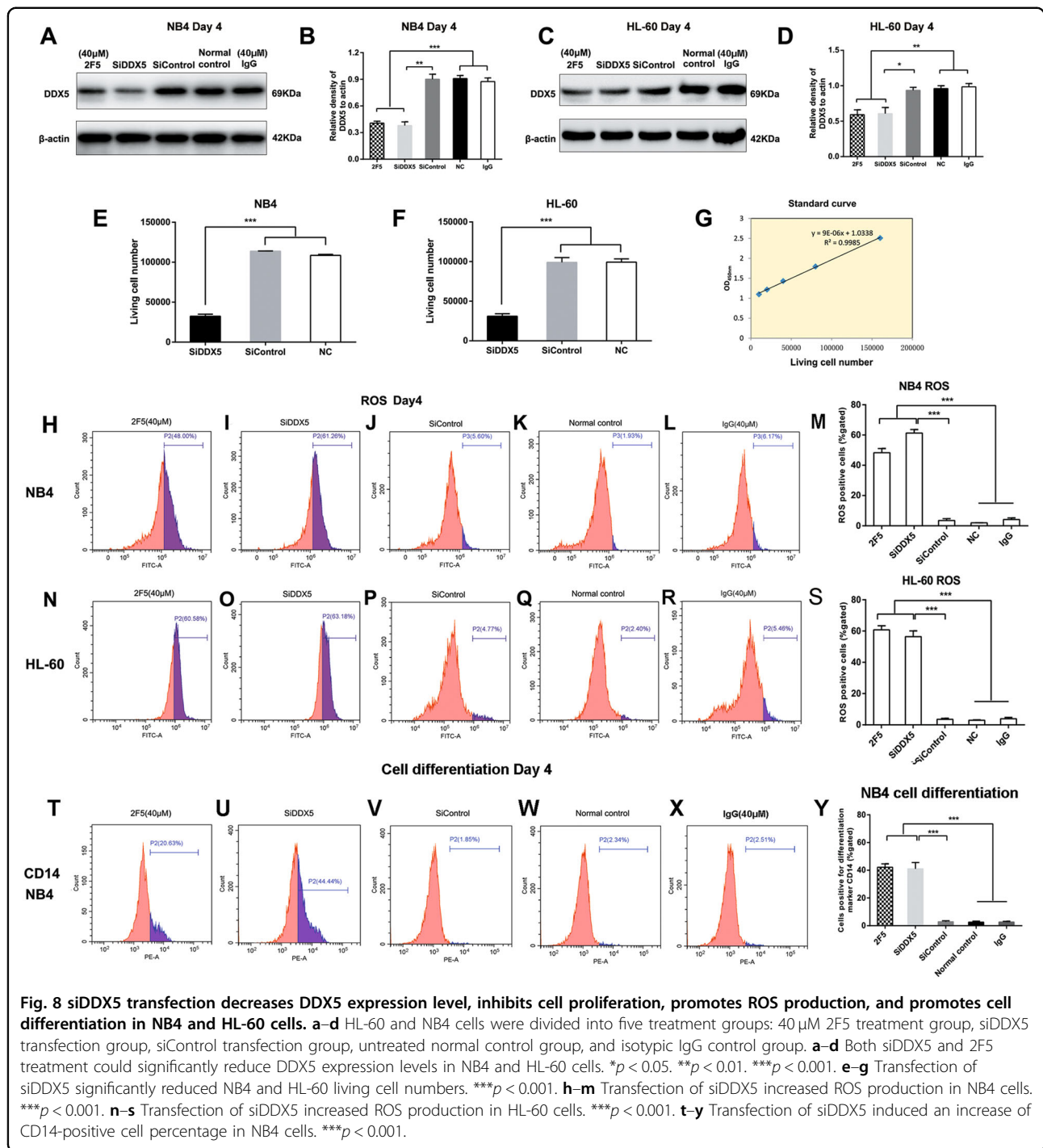
DDX5 expression is closely correlated with the onset of organ differentiation²⁵. The function of DDX5 is responsible for the expression of proteins that contribute to cell differentiation. Otherwise, studies showed that

downregulation of DDX5 in gastric and lung cell lines abrogates proliferation^{26,27}, which suggested that DDX5 could affect cancer cell proliferation. DDX5 is considered to be required for T-ALL pathogenesis since it regulates the notch signaling pathway which is required for the growth and survival of T-ALL cells. This is evidenced by Lin et al. that DDX5 depletion decreased survival rate and inhibited proliferation of KOPT-K1 and HPB-ALL cells¹⁰. Similar conclusion was also acquired in present study that both DDX5-targeting 2F5 and siRNA could inhibit APL cell proliferation by downregulation of DDX5. Moreover,



2F5 induced G0/G1 phase arrest in APL cells (Fig. 3). Interestingly, unlike the finding related to DDX5 by Lin et al., we found that DDX5-targeted 2F5 has no effect on the proliferation of T-ALL cell line (Jurkat and CEM-C7).

Our results showed that the basal expression levels of DDX5 in different leukemia cell lines were also different, DDX5 basal level in APL cell lines were much higher than that in T-ALL cell line (Fig. 2). These findings indicated



that the different basal level of DDX5 might determine the susceptibility of different leukemia subtypes to 2F5. Moreover, 2F5 makes no harmful effect on healthy neutrophils and tissues (Fig. S3). This provides evidence to safety of 2F5 in potential further application.

The differentiation promotion effect of 2F5 was evaluated by NBT reduction assay, morphological staining and differentiation biomarker analysis (Figs. 4, 5 and

Figs. S3 and 4). CD11b and CD14 have been known as characteristic surface markers on mature granulocyte and mature monocyte, respectively²⁸. Those two surface markers decide the differentiation trends of APL cells. In APL cells treated with 2F5, the percentage of CD14-positive cells was obviously higher than CD11b-positive cells, thus it is speculated that 2F5 could induce APL cell differentiation along the monocytic lineage more than

myeloid lineage. Moreover, APL cells were found dead at day 8 after ATRA treatment, while APL cells were found still alive at day 16 after 2F5 treatment. It is suggested that 2F5 exerts its pro-differentiation effect on APL cell via other pathway that different from the pro-apoptosis mechanism of ATRA²⁹. Of note, 2F5 exerted promotion effect on APL cell differentiation up to day 16, even though the treatment of 2F5 was stopped at day 12 (Fig. 4). It means that 2F5 exerts its pro-differentiation effect on APL cells for a long time.

Up to now, there is no monoclonal antibody medicine used clinically for APL therapy with differentiation inducing mechanism. A monoclonal antibody against CD44 used alongside ATRA and FICZ (6-Formylindolo (3, 2-b) carbazole) has been shown to increase cell apoptosis³⁰. Another monoclonal antibody called HuM195 is used for APL targeted radiotherapy as a molecular chaperone agent by targeting CD33³¹. HuM195/rGel is a compound prepared by HuM195 coupling to plant toxin gelonin, which has been proven to be cytotoxic to HL-60 cells³². As a direct pro-differentiation monoclonal autoantibody, 2F5 has its own specific advantages and mechanism of action. In APL cells, 2F5 was found to inhibit DDX5 expression and promote ROS production significantly. Moreover, siRNA-targeted DDX5 played a similar role to 2F5 on APL cell proliferation, differentiation, and ROS production. However, the ROS inhibitor reversed the effect of 2F5 on DDX5 and ROS in APL cells. These findings indicated that 2F5 could promote APL cell differentiation by inhibition of DDX5 and activation of ROS.

It has been demonstrated that the decrease of DDX5 is the cause of ROS induction⁹. In addition, it has also been reported that NADPH oxidase-derived ROS plays a critical role in HL-60 cell monocytic differentiation¹³. ROS is involved in the HL-60 cell monocytic differentiation induced by isoliquiritigenin¹³. Combined with results in present study that ROS plays a dual role in promotion effect of APL cell differentiation. On one hand, DDX5 depletion-induced ROS production promotes APL cell differentiation. On the other hand, NAC-mediated ROS inhibition also blocks the decreasing effect of 2F5 on DDX5 and subsequent promotion effect of 2F5 on ROS and cell differentiation (Fig. S7). All these findings suggested that 2F5 promoted APL cell differentiation via targeted reducing DDX5-mediated ROS production. For the mechanisms by which autoantibodies penetrate living cells, researchers have suggested that Fc-receptor-mediated or some special cell membrane proteins mediate endocytosis to facilitate the cell penetration of autoantibodies³³. However, endosomes prevent 2F5 to recognize and bind to its targeting protein DDX5¹⁹. For this reason, it is suggested that 2F5 decreased DDX5 level via endosome-independent pathway. It is speculated that

2F5 binds to extracellular nucleotide and transports to the cell plasma via equilibrative nucleoside transporter³⁴, and then 2F5 neutralizes free DDX5 consequently (Fig. S7).

This study concluded that DDX5-targeting autoantibody 2F5 inhibited proliferation, induced a G0/G1 phase arrest, and promoted differentiation of APL cells by decreasing DDX5 and increasing ROS levels without cytotoxicity to healthy tissues and cells. This finding provides a novel and valid approach to solve the problem in therapy of relapse/refractory APL.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (NSFC) (81601428; 81670530; 81700380), and the Zhejiang Provincial Natural Science Foundation (ZJNSF) (LY20H250001; LQ19C080001), and the Research Start-Up Funds of Hangzhou Normal University (2019QDL017; 2018QDL035; 2019QDL008; 2019QDL007).

Author details

¹Department of Basic Medicine of Medical School, Department of Infectious Diseases of Affiliated Hospital, Institute of Liver and Metabolic Diseases, Key Laboratory of Aging and Cancer Biology of Zhejiang Province, and Key Laboratory of Inflammation and Immunoregulation of Hangzhou, Hangzhou Normal University, 310000 Hangzhou, Zhejiang, P.R. China. ²Department of Laboratory Medicine, The Second Affiliated Hospital of Harbin Medical University, 150001 Harbin, Heilongjiang, P.R. China. ³Peking University People's Hospital, Peking University Hepatology Institute, Beijing, P.R. China

Conflict of interest

The authors declare that they have no conflict of interest.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information accompanies this paper at (<https://doi.org/10.1038/s41419-020-02759-5>).

Received: 13 November 2019 Revised: 7 July 2020 Accepted: 9 July 2020
Published online: 20 July 2020

References

1. Ablain, V. et al. Activation of a promyelocytic leukemia-tumor protein 53 axis underlies acute promyelocytic leukemia cure. *Nat. Med.* **20**, 167–174 (2014).
2. Wang, L. et al. Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* **105**, 4826–4831 (2008).
3. Lehmann-Che, J., Bally, C. & de Thé, H. Resistance to therapy in acute promyelocytic leukemia. *N. Engl. J. Med.* **371**, 1170–1172 (2014).
4. Breccia, M. & Lo-Coco, F. Arsenic trioxide for management of acute promyelocytic leukemia: current evidence on its role in front-line therapy and recurrent disease. *Expert. Opin. Pharm.* **13**, 1031–1043 (2012).
5. Lo-Coco, F. et al. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N. Engl. J. Med.* **369**, 111–121 (2013).
6. Norsworthy, K. J. & Altman, J. K. Optimal treatment strategies for high-risk acute promyelocytic leukemia. *Curr. Opin. Hematol.* **23**, 127–136 (2016).
7. Kojima, M. et al. Refractory acute promyelocytic leukemia successfully treated with combination therapy of arsenic trioxide and tamibarotene: a case report. *Leuk. Res. Rep.* **5**, 11–13 (2016).
8. Cai, W. et al. Wanted DEAD/H or alive: helicases winding up in cancers. *J. Natl. Cancer Inst.* **109**, 1–15 (2017).
9. Mazurek, A. et al. Acquired dependence of acute myeloid leukemia on the DEAD-box RNA helicase DDX5. *Cell Rep.* **7**, 1887–1899 (2014).
10. Lin, S. et al. DDX5 is a positive regulator of oncogenic NOTCH1 signaling in T cell acute lymphoblastic leukemia. *Oncogene* **32**, 4845–4853 (2013).

11. Misaki, R. et al. Recombinant production and characterization of human anti-influenza virus monoclonal antibodies identified from hybridomas fused with human lymphocytes. *Biologicals* **44**, 394–402 (2016).
12. Soni, P. et al. Evaluation of the fusion partner cell line SPYMEG for obtaining human monoclonal antibodies against influenza B virus. *J. Vet. Med. Sci.* **80**, 1020–1024 (2018).
13. Chen, H. et al. NADPH oxidase-derived reactive oxygen species are involved in the HL-60 cell monocytic differentiation induced by isoliquiritigenin. *Molecules* **17**, 13424–13438 (2012).
14. Callens, C. et al. Targeting iron homeostasis induces cellular differentiation and synergizes with differentiating agents in acute myeloid leukemia. *J. Exp. Med.* **207**, 731–750 (2010).
15. Zhang, Y., Choksi, S., Chen, K., Pobezińska, Y. & Linnoila, I. ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages. *Cell Res.* **23**, 898–914 (2013).
16. Cicconi, L. & Lo-Coco, F. Current management of newly diagnosed acute promyelocytic leukemia. *Ann. Oncol.* **27**, 1474–1481 (2016).
17. Ling, S. et al. Metformin ameliorates arsenic trioxide hepatotoxicity via inhibiting mitochondrial complex I. *Cell Death Dis.* **8**, e3159 (2017).
18. Stahl, M. & Tallman, M. S. Differentiation syndrome in acute promyelocytic leukaemia. *Br. J. Haematol.* **187**, 157–162 (2019).
19. Wu, J. et al. The roles and applications of autoantibodies in progression, diagnosis, treatment and prognosis of human malignant tumours. *Autoimmun. Rev.* **16**, 1270–1281 (2017).
20. Igusa, T. et al. Autoantibodies and scleroderma phenotype define subgroups at high-risk and low-risk for cancer. *Ann. Rheum. Dis.* **77**, 1179–1186 (2018).
21. Mao, S., Shen, H. & Zhang, J. Systemic lupus erythematosus and malignancies risk. *J. Cancer Res. Clin.* **142**, 253–262 (2016).
22. Kantarjian, H. et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N. Engl. J. Med.* **376**, 836–847 (2017).
23. Marcus, R. et al. Obinutuzumab for the first-line treatment of follicular lymphoma. *N. Engl. J. Med.* **377**, 1331–1344 (2017).
24. Nyamao, R. M., Wu, J., Yu, L., Xiao, X. & Zhang, F. M. Roles of DDX5 in the tumorigenesis, proliferation, differentiation, metastasis and pathway regulation of human malignancies. *BBA* **1871**, 85–98 (2019).
25. Ponomartsev, N. V. & Enukashvily, N. E. The DDX5 protein is involved in cell proliferation and differentiation. *Tsitologiya* **57**, 111–118 (2015).
26. Sha, M. et al. Long non-coding RNA MIAT promotes gastric cancer growth and metastasis through regulation of miR-141/DDX5 pathway. *J. Exp. Clin. Cancer Res.* **37**, 58–70 (2018).
27. Wang, Z. D. et al. DDX5 promotes proliferation and tumorigenesis of non-small-cell lung cancer cells by activating β -catenin signaling pathway. *Cancer Sci.* **106**, 1303–1312 (2015).
28. Moeenrezakhanlou, A., Shephard, L., Lam, L. & Reiner, N. E. Myeloid cell differentiation in response to calcitriol for expression CD11b and CD14 is regulated by myeloid zinc finger-1 protein downstream of phosphatidylinositol 3-kinase. *J. Leukoc. Biol.* **84**, 519–528 (2008).
29. Wang, R., Xia, L., Gabrilove, J., Waxman, S. & Jing, Y. Sorafenib inhibition of Mcl-1 accelerates ATRA-induced apoptosis in differentiation-responsive AML cells. *Clin. Cancer Res.* **22**, 1211–1221 (2016).
30. Amanzadeh, A. et al. New synergistic combinations of differentiation-inducing agents in the treatment of acute promyelocytic leukemia cells. *Leuk. Res.* **68**, 98–104 (2018).
31. Chen, P. et al. Nuclear localizing sequences promote nuclear translocation and enhance the radiotoxicity of the anti-CD33 monoclonal antibody HuM195 labeled with ¹¹¹In in human myeloid leukemia cells. *J. Nucl. Med.* **47**, 827–836 (2006).
32. Duzkale, H. et al. Bone marrow purging studies in acute myelogenous leukemia using the recombinant anti-CD33 immunotoxin HuM195/rGel. *Biol. Blood Marrow Transplant.* **9**, 364–372 (2003).
33. Karsten, C. M. & Kohl, J. The immunoglobulin, IgG Fc receptor and complement triangle in autoimmune diseases. *Immunobiology* **217**, 1067–1079 (2012).
34. Weisbart, R. H. et al. DNA-dependent targeting of cell nuclei by a lupus autoantibody. *Sci. Rep.* **5**, 1–6 (2015).