

Interferon- γ enhances promyelocytic leukemia protein expression in acute promyelocytic cells and cooperates with all-*trans*-retinoic acid to induce maturation of NB4 and NB4-R1 cells

PENGCHENG HE¹, YANFENG LIU¹, MEI ZHANG¹, XIAONING WANG¹,
JIEYING XI¹, DI WU¹, JING LI¹ and YUNXIN CAO²

¹Department of Hematology, First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi 710061;

²Department of Immunology, Fourth Military Medical University, Xi'an, Shaanxi 710032, P.R. China

Received December 8, 2011; Accepted February 3, 2012

DOI: 10.3892/etm.2012.488

Abstract. In order to investigate the effect and mechanisms of interferon (IFN)- γ in combination with all-*trans*-retinoic acid (ATRA) on NB4 cells [ATRA-sensitive acute promyelocytic leukemia (APL) cell line] and NB4-R1 cells (ATRA-resistant APL cell line) and to search for a novel approach to solve the problem of ATRA resistance in APL, we initially treated NB4 and NB4-R1 cells with IFN- γ , ATRA and IFN- γ in combination with ATRA, respectively. The cell proliferation was then tested by MTT assay, and the cell differentiation was tested through light microscopy, by NBT test and flow cytometry (FCM). The expression of promyelocytic leukemia (PML) protein was observed by indirect immune fluorescent test. Results showed that ATRA inhibited the growth of NB4 cells, however, it could not inhibit the growth of NB4-R1 cells. IFN- γ inhibited the growth of both NB4 and NB4-R1 cells. Meanwhile, the growth inhibition effect of IFN- γ in combination with ATRA on both NB4 and NB4-R1 cells was significantly stronger than that of any single drug treatment. The results of the NBT reduction test and CD11b antigen detection by FCM indicated that IFN- γ induces the differentiation of NB4 and NB4-R1 cells to some extent. Moreover, the maturation degree of both NB4 and NB4-R1 cells induced by IFN- γ in combination with ATRA was more significant than that of IFN- γ or ATRA alone. After treatment with IFN- γ , the number of fluorescent particles in NB4 and NB4-R1 cell nuclei was higher than those in the control group, which indicated that IFN- γ may induce the expression of PML protein. Together, IFN- γ augments the proliferation inhibition effect of ATRA on NB4 and NB4-R1

cells through enhancing the expression of PML protein. IFN- γ in combination with ATRA not only strengthens the induction differentiation effect of ATRA on NB4 cells, but also can partially induce the maturation of NB4-R1 cells with ATRA resistance.

Introduction

More than 90% of patients with acute promyelocytic leukemia (APL) achieve complete remission clinically using all-*trans*-retinoic acid (ATRA), a strong differentiation inducer (1). However, the rapid development of ATRA resistance brings a new problem to the treatment of APL. The relapse and refractoriness of APL remains one of the most difficult problems clinically (2). How to treat APL with ATRA resistance has become one of the hot spots of APL research.

Interferon (IFN), as an important cytokine, has broad biological activities. It not only inhibits the growth of tumor cells, but also reverses the drug resistance of chemotherapy. As proven by several studies, the mechanisms of ATRA resistance are probably related to the lack of certain important proteins which are synthesized by IFN (3-5). In order to solve the problem of ATRA resistance in APL, we studied the effect and mechanisms of IFN- γ in combination with ATRA on the proliferation/differentiation of NB4 cells (APL cell line with ATRA sensitivity) and NB4-R1 cells (APL cell line with ATRA resistance), respectively.

Materials and methods

Reagents. ATRA (Sigma Co.) was dissolved in absolute ethanol to a concentration of 10^{-3} mol/l and was stored at -20°C . IFN- γ (Shanghai Clone Biology Technical Co.) was dissolved in normal saline, diluted to 4×10^8 U/l and stored at -20°C . The rabbit anti-human polyclonal antibody was purchased from Chemicon Co. The goat anti-rabbit FITC-IgG antibody was purchased from KPL Co. The CD11b antibody for flow cytometry (FCM) was purchased from Immunotech Co.

Cell culture. NB4 is an APL cell line established from a patient with APL by Lanotte. NB4-R1 is an ATRA-resistant APL

Correspondence to: Dr Mei Zhang or Dr Pengcheng He, Department of Hematology, The First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, No. 277 Yanta West Road, Xi'an, Shaanxi 710061, P.R. China
E-mail: zhangmei@medmail.com.cn
E-mail: hepc@163.com

Key words: acute promyelocytic leukemia, interferon- γ , all-*trans*-retinoic acid, all-*trans*-retinoic acid-resistant

cell line. They were all donated by the Hematology Research Institute of Ruijin Hospital, Shanghai. NB4 and NB4-R1 cells were inoculated at a density of $1 \times 10^6/l$ in RPMI-1640 medium, respectively, and cultured at 37°C in a humidified 5% CO_2 incubator.

Detection of proliferation by MTT assay (6,7). NB4 or NB4-R1 cells were inoculated to each well of 96-well culture plates with a concentration of $5 \times 10^4/l$ and $100 \mu\text{l}$ for each well, respectively. ATRA, IFN- γ and IFN- γ + ATRA were added respectively to each experimental group. The final concentration of ATRA was $1 \times 10^{-6} \text{ mol/l}$ and the final concentration of IFN- γ was $1 \times 10^6 \text{ U/l}$. RPMI-1640 medium ($100 \mu\text{l}$) was added to the control group. Each well volume was kept at $200 \mu\text{l}$ and made up the residual volume with RPMI-1640 medium. Cell proliferation was detected by MTT assay on days 1, 3, 5 and 8, respectively. The results were expressed as the mean absorbance (A_{490 nm}) value of triplicates.

Observation of cell morphology. NB4 or NB4-R1 cells were cultured in 25-ml culture flasks at a concentration of $5 \times 10^4/l$ and 5 ml for each flask, respectively. The subgroups and treatment methods were in accordance with the MTT assay. The cells were harvested on days 1, 3, 5 and 8, respectively. The cell sediments were made into smears and stained with Wright's solution, and then observed by light microscopy.

NBT reduction assay (8,9). Cell culture and treatment were the same as for observation of cell morphology. Cells were harvested by centrifugation and removal of supernatant, and the samples were blended with 0.5 ml NBT reaction solution (1 mg/ml NBT and 100 ng/ml TPA), incubated at 37°C for 1 h and centrifuged for 5 min. The cell sediments were made into smears and stained with Wright's solution, and then detected under immersion objective. NBT-positive cell rates were calculated in every 200 cells. The test was performed three times and the results were expressed as a mean of triplicates: NBT-positive cell rates = NBT-positive cell counts/total cell counts $\times 100\%$.

Detection of CD11b antigen. Cell culture and treatment were the same as for observation of cell morphology. Mouse anti-human antibody FITC-CD11b ($10 \mu\text{l}$) was added to a $100\text{-}\mu\text{l}$ cell suspension (total cell counts were $\sim 5 \times 10^5$) and blended completely. The samples were stained for 15-30 min at 25°C and protected from light. After washing with PBS twice, centrifugation and removal of the supernatant, the samples were fixed with $500 \mu\text{l}$ 2% paraform solution. The expression of the CD11b antigen was detected by FCM.

Detection of the promyelocytic leukemia (PML) protein. Cell culture and treatment were the same as for observation of cell morphology. The cells were harvested by centrifugation and removal of supernatant. The cell suspension was diluted to a concentration of $1 \times 10^6/l$ with PBS. The cell suspension (0.1 ml) was extracted to collect specimens and the indirect immune fluorescence test was carried out. After being fixed with 4% paraform solution for 20 min, the slides were washed with PBS and BSA-PBS twice. Then, the slides were treated with 0.1% Triton X-100 for 10 min and incubated with anti-PML anti-

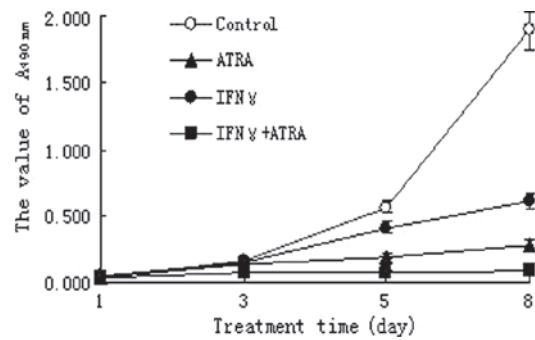


Figure 1. Effect of IFN- γ and/or ATRA on the proliferation of NB4 cells. Cellular growth curves showed that the growth of NB4 cells in the IFN- γ , ATRA and IFN- γ + ATRA treatment groups was significantly inhibited, as compared to the control group ($P < 0.05$). Meanwhile, the growth inhibition effect in the IFN- γ + ATRA treatment group was the strongest among the three experimental groups ($P < 0.05$).

body (1:200) for 1 h at 37°C in a waterbath. After washing with BSA-PBS and PBS twice, 1:10 diluted fluorescence-labeled goat anti-rabbit IgG was added to the slides for 1 h at 37°C in a waterbath. After being washed with BSA-PBS and PBS, the slides were investigated using a fluorescent microscope (wavelength 488 nm) and images were captured.

Statistical analysis. All data are expressed as the means \pm standard deviation (SD). Statistical analysis of data was carried out using the Student's t-test. $P < 0.05$ was considered to denote statistical significance.

Results

Effect of IFN- γ and/or ATRA on the proliferation of NB4 and NB4-R1 cells. The growth of NB4 cells in the IFN- γ , ATRA and IFN- γ + ATRA treatment groups was significantly inhibited after day 5 compared to that in the control group ($P < 0.05$). Meanwhile, the growth inhibition effect of IFN- γ + ATRA treatment on the NB4 cells was the strongest among the three experimental groups. The growth inhibition effect of ATRA was next and last was the IFN- γ treatment ($P < 0.05$) (Fig. 1).

The growth of NB4-R1 cells in the IFN- γ and IFN- γ + ATRA treatment groups was significantly inhibited after day 5 compared to that in the control group ($P < 0.05$). However, there were no significant differences in growth inhibition rates between the ATRA treatment group and the control group ($P > 0.05$). Meanwhile, the growth inhibition effect of the IFN- γ + ATRA treatment on NB4-R1 cells was stronger than that of the IFN- γ treatment ($P < 0.05$) (Fig. 2).

Effect of IFN- γ and/or ATRA on the differentiation of NB4 and NB4-R1 cells

Morphological observation. Morphological differentiation of NB4 cells was not observed in the IFN- γ treatment group on day 3. However, it was observed in both the ATRA and IFN- γ + ATRA treatment groups. Moreover, the differentiation degree of NB4 cells in the IFN- γ + ATRA treatment group was higher than that in the ATRA treatment group. Morphological differentiation of NB4-R1 cells was not observed in both the IFN- γ and ATRA treatment groups on day 3, but it was observed in the IFN- γ + ATRA treatment group. Morphological

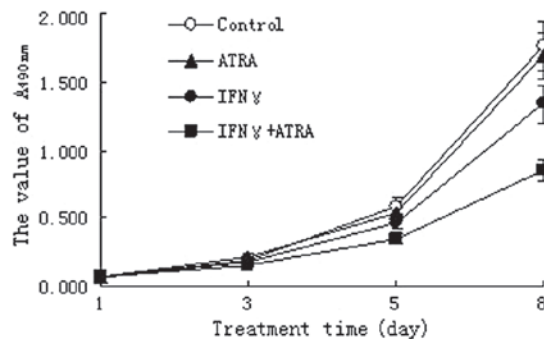


Figure 2. Effect of IFN- γ and/or ATRA on the proliferation of NB4-R1 cells. Cellular growth curves showed that the growth of NB4-R1 cells in the IFN- γ and IFN- γ + ATRA treatment groups was significantly inhibited, as compared to the control group ($P < 0.05$). However, there were no significant differences in the growth rates between the ATRA and control groups ($P > 0.05$). Meanwhile, the growth inhibition effect in the IFN- γ + ATRA treatment group was stronger compared to the IFN- γ group ($P < 0.05$).

differentiation of NB4 and NB4-R1 cells was manifested as the reduction in nuclear size, the increase in cytoplasm and the disappearance of nucleoli under Wright's staining (data not shown, refer to Fig. 3).

NBT reduction assay. NBT-positive rates of NB4 cells in the ATRA, IFN- γ and IFN- γ + ATRA treatment groups were significantly higher than those in the control group ($P < 0.05$). NBT-positive rates of NB4-R1 cells in the three experimental groups were significantly higher than those in the control group ($P < 0.05$). Meanwhile, in both the NB4 and NB4-R1 cells, the NBT-positive rates in the IFN- γ + ATRA treatment group were the highest among the three experimental groups, respectively ($P < 0.05$) (Table I, Fig. 3).

Detection of the CD11b antigen. Expression of the CD11b antigen on the NB4 cell surface in the ATRA, IFN- γ and IFN- γ + ATRA treatment groups on day 3 was markedly higher than that in the control group ($P < 0.05$). Moreover, the expression of the CD11b antigen on the NB4-R1 cell surface in the IFN- γ and IFN- γ + ATRA treatment groups was significantly higher than that in the control group ($P < 0.05$). However, there was no significant difference in the expression of CD11b antigen on the NB4-R1 cell surface between the ATRA treatment group and the control group ($P < 0.05$). Meanwhile, both on the cell surfaces of the NB4 and NB4-R1 cells, the expression of the CD11b antigen in the IFN- γ + ATRA treatment group was the highest among the four groups, respectively ($P < 0.05$; Table II).

Effect of IFN- γ and/or ATRA on the expression of PML protein in NB4 and NB4-R1 cells. After the NB4 and NB4-R1 cell control groups were stained with the rabbit anti-human PML multiple antibody and goat anti-rabbit FITC-IgG, many diffuse, fine fluorescent particles were noted in the nuclei of both the NB4 and NB4-R1 cells. These were called 'swollen furuncle' structures and implied that the nuclear body (NB) structures were destroyed (Fig. 4A and E). After treatment with ATRA for 3 days, the swollen furuncle structures in the NB4 cell nuclei disappeared and bulky fused fluorescent particles (NB structures) appeared, which implied that the NB structures in the NB4 cells were restored (Fig. 4B). However, the swollen furuncle structures in the NB4-R1 cell nuclei remained, which implied that the NB structures in the

Table I. Results of NBT reduction assay of NB4 and NB4-R1 cells in the four treatment groups.

Treatment group	NB4	NB4-R1
Control	1.1 \pm 0.1	1.0 \pm 0.1
ATRA	74.7 \pm 1.5 ^a	5.2 \pm 0.1 ^a
IFN- γ	19.3 \pm 0.5 ^{a,b}	16.8 \pm 0.3 ^{a,b}
IFN- γ + ATRA	93.3 \pm 2.1 ^{a-c}	30.4 \pm 0.8 ^{a-c}

Results are expressed as the means \pm SD ($n=3$). ^a $P < 0.05$ vs. the control group; ^b $P < 0.05$ vs. the ATRA treatment group; ^c $P < 0.05$ vs. the IFN- γ treatment group.

NB4-R1 cells were not restored (Fig. 4F). After treatment with IFN- γ , the size and number of the fluorescent particles in the NB4 and NB4-R1 cell nuclei were significantly increased compared to the control group, which implied that the expression of PML protein in both the NB4 and NB4-R1 cells was increased. Nevertheless, no bulky fused fluorescent particles (NB structures) appeared in both the NB4 and NB4-R1 cell nuclei, which implied that NB structures in both NB4 and NB4-R1 cells were not restored (Fig. 4C and G). After treatment with IFN- γ + ATRA, the size and number of the fluorescent particles were increased and bulky fused fluorescent particles (NB structures) appeared in the NB4 cell nuclei, implying that the expression of PML protein was increased and NB structures were restored (Fig. 4D). Although the size and number of the fluorescent particles were increased, still no bulky fused fluorescent particles were noted in the NB4-R1 cell nuclei, which was similar to the IFN- γ group (Fig. 4H).

Discussion

As a negative regulating factor of cell growth, IFN inhibits the growth of many tumor cells (10). IFN has traditionally been applied to the treatment of many malignant hematologic diseases, such as chronic myelocytic leukemia (CML), multiple myeloma (MM) and hairy cell leukemia (HCL) (11-13). ATRA, as a strong differentiation inducer, also inhibits the proliferation of APL cells (14). In our study, MTT assay showed that IFN- γ in combination with ATRA significantly enhanced the growth inhibition effect of ATRA on both NB4 and NB4-R1 cells.

Buonamici *et al* (15) found that both type I and II IFN induced the expression of the PML protein. The PML gene promoter has an IFN-stimulated response element (ISRE) -GAGAATCGAAACT- and an IFN- γ -activated site (GAS) -TTTACCGTAAG-. IFN combines with ISRE or GAS of the PML gene to induce transcription and expression of the PML gene directly (16-19). In our study, the results of the indirect immune fluorescent test showed that the size and the number of the fluorescent particles in the NB4 and NB4-R1 cells were significantly increased compared to the control group after they were treated with IFN- γ , which implied that IFN- γ induces the expression of the PML protein. Our findings are consistent with the reports of Buonamici *et al*.

As a type of tumor growth inhibitor, the PML protein inhibits the growth of many types of tumor cells (20,21). In

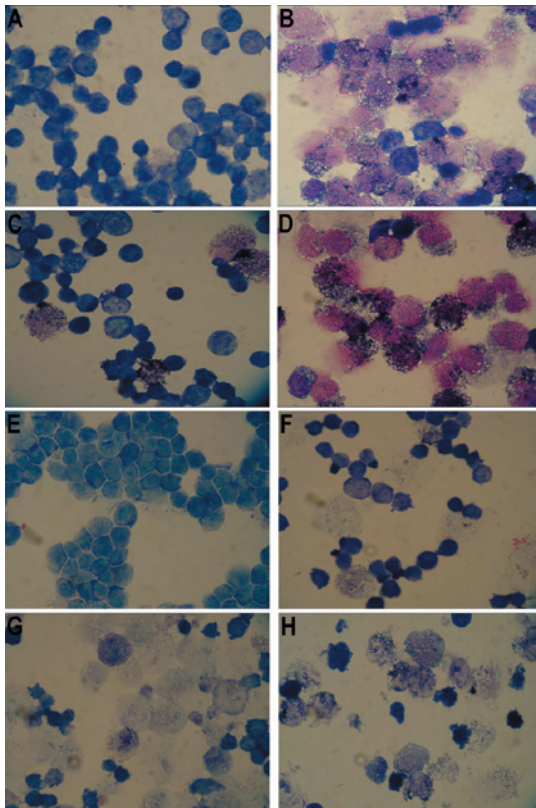


Figure 3. Photomicrographs of the NBT reduction assay of NB4 cells in (A) the control group, (B) the ATRA treatment group, (C) the IFN- γ treatment group, (D) the IFN- γ + ATRA treatment group, and of NB4-R1 cells in (E) the control group, (F) the ATRA treatment group, (G) the IFN- γ treatment group and (H) the IFN- γ + ATRA treatment group (Wright's stain; magnification, $\times 1,000$). (A and E) There were no black particles (formazan) in the control group of NB4 and NB4-R1 cells. (B) There were many black particles (formazan) in the ATRA group of NB4 cells, so-called NBT-positive cells, which indicated that the NB4 cells had differentiated. (F) However, almost no black particles (formazan) appeared in the ATRA group of the NB4-R1 cells. (C and G) There were a few black particles (formazan) in the IFN- γ group of the NB4 and NB4-R1 cells. (D and H) Notably, in both the NB4 and NB4-R1 cells, the NBT-positive rates in the IFN- γ + ATRA treatment group were the highest among the four groups, respectively.

APL cells, chromosome translocation of t(15;17) leads to the formation of the PML-RAR α fusion gene and the expression of PML-RAR α protein. The latter sequesters the PML protein by forming a heterodimer with the PML protein, which results in the inactivity of PML protein and the occurrence of APL (22-24). Accordingly, we hypothesized that there are close relationships between the up-regulation of expression of the PML protein induced by IFN- γ and the growth inhibitory effect of IFN- γ on NB4 and NB4-R1 cells.

The NBT reduction assay is an index which is often used to reflect the differentiation of APL cells functionally. The CD11b antigen is usually expressed on the surface of mature myeloid cells, which is also used to represent the maturation degree of APL cells. In our study, the results of the NBT test and CD11b antigen detection by FCM suggested that IFN- γ enhances the induction differentiation effects of ATRA on NB4 cells. Most importantly, it induces the differentiation of NB4-R1 cells with ATRA resistance when it cooperates with ATRA. The same results were drawn from the cell morphological observation (Fig. 3).

Table II. Expression of the CD11b antigen on the NB4 and NB4-R1 cell surface in the four treatment groups.

Subgroup	NB4	NB4-R1
Control	2.91 \pm 0.06	1.58 \pm 0.04
ATRA	61.67 \pm 1.12 ^a	2.19 \pm 0.07
IFN- γ	22.58 \pm 0.37 ^{a,b}	9.30 \pm 0.16 ^{a,b}
IFN- γ + ATRA	77.14 \pm 1.61 ^{a-c}	18.45 \pm 0.31 ^{a-c}

Results are expressed as the means \pm SD (n=3). ^aP<0.05 vs. the control group; ^bP<0.05 vs. the ATRA treatment group; ^cP<0.05 vs. the IFN- γ treatment group.

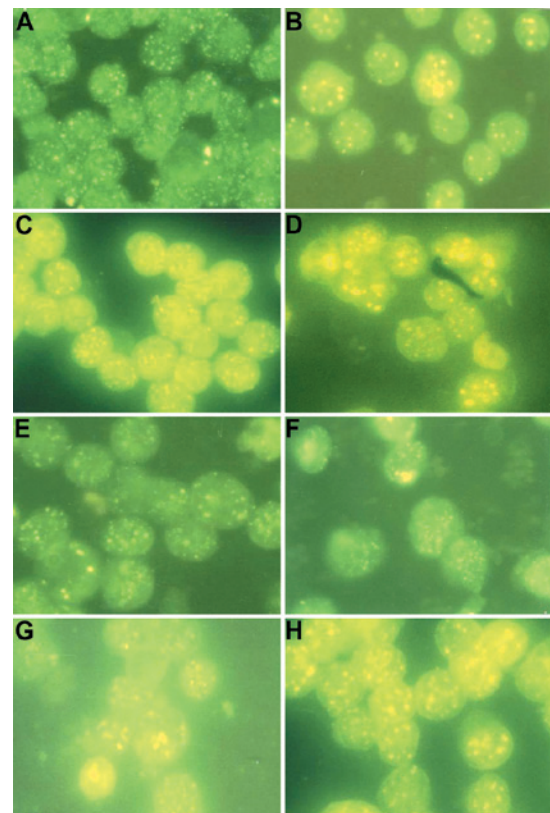


Figure 4. Photomicrographs of indirect immune fluorescent test of NB4 cells in (A) the control group, (B) the ATRA treatment group, (C) the IFN- γ treatment group, (D) the IFN- γ + ATRA treatment group, and of NB4-R1 cells in (E) the control group, (F) the ATRA treatment group, (G) the IFN- γ treatment group and (H) the IFN- γ + ATRA treatment group (magnification, $\times 1,000$). (A and E) Many diffused, fine fluorescent particles are noted in the nuclei of the NB4 and NB4-R1 cells, which implies that nuclear body (NB) structures were destroyed. (B) Some bulky fused fluorescent particles (NB structures) appear in the NB4 cell nuclei, which implies that NB structures were restored. (F) However, the fluorescent particles in the NB4-R1 cell nuclei remained diffused and fine, which implies that NB structures were not restored. (C and G) The size and number of the fluorescent particles in the NB4 and NB4-R1 cell nuclei were increased compared to those in the control group, but there were no bulky fused fluorescent particles, which implies that the expression of PML protein was increased, but NB structures were not restored. (D) The size and number of the fluorescent particles were increased and bulky fused fluorescent particles (NB structures) are noted in the NB4 cell nuclei, which implies that the expression of PML protein was increased and NB structures were restored. (H) Although the size and number of the fluorescent particles were increased, no bulky fused fluorescent particles (NB structures) are noted in the NB4-R1 cell nuclei, which is similar to the IFN- γ treatment group.

Wang *et al* (25) found that the differentiation of hematopoietic progenitor cells which was induced by retinoic acid (RA) required the participation of the PML protein. In PML^{+/+} bone marrow cells, RA induced the terminal differentiation of hematopoietic progenitor cells. On the contrary, in PML^{-/-} bone marrow cells, RA did not induce the terminal differentiation of hematopoietic progenitor cells, even at an extremely high concentration.

Nuclear body (NB) structures are nuclear protein compounds, which exist in the nucleus of normal cells (26). The breakage of NB structures in APL cells suggested that the APL cells had lost the abilities of differentiation or maturation (27). We found that although IFN- γ could not restore the NB structures in NB4 and NB4-R1 cells, it did augment the expression of the PML protein and enhance the induction differentiation effect of ATRA in both NB4 and NB4-R1 cells (Fig. 4). Therefore, we considered that IFN- γ in combination with ATRA enhanced the differentiation effects of ATRA on NB4 cells and induced the maturation of NB4-R1 cells with ATRA-resistant, and this may be related to the up-regulation of PML protein expression induced by IFN- γ . Many studies have shown that IFN and ATRA have a synergistic effect on modulating proliferation and differentiation in many cell lines, which may be related to the fact that IFN and ATRA can induce expression of certain genes, such as RIG-G, ISGs and P21WAF1/CIP1, synergistically (28-31).

Taken together, IFN- γ enhances the growth inhibition effect of ATRA in both NB4 and NB4-R1 cells. Most importantly, IFN- γ cooperates with ATRA to induce the maturation of NB4 and NB4-R1 cells with ATRA resistance, which may relate to the up-regulation of PML protein expression.

Acknowledgements

The authors would like to thank Ms. Fang Wang, Dr Qunling Zhang, Ms. Yiping Geng and Dr Ya Zhao for the technical assistance in this study. The project was supported by the National Natural Science Foundation of China (No. 30701133), and the Shaanxi Province Science and Technology Development Fund, China (2006K09-G5).

References

- Wang Z and Chen Z: Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood* 111: 2505-2515, 2008.
- Zhou GB, Zhang J, Wang ZY, Chen SJ and Chen Z: Treatment of acute promyelocytic leukaemia with all-trans retinoic acid and arsenic trioxide: a paradigm of synergistic molecular targeting therapy. *Philos Trans R Soc Lond B Biol Sci* 362: 959-971, 2007.
- Wang X, Yin Y, Ma P, *et al*: Apoptosis of human pancreatic carcinoma cells induced by all-trans retinoic acid and interferon. *Chin J Cancer Res* 21: 224-228, 2009.
- Park HH, Kim M, Lee BH, *et al*: Intracellular IL-4, IL-10, and IFN-gamma levels of leukemic cells and bone marrow T cells in acute leukemia. *Ann Clin Lab Sci* 36: 7-15, 2006.
- Hoyer KK, Herling M, Bagrintseva K, *et al*: T cell leukemia-1 modulates TCR signal strength and IFN-gamma levels through phosphatidylinositol 3-kinase and protein kinase C pathway activation. *J Immunol* 175: 864-873, 2005.
- Montoro E, Lemus D, Echemendia M, *et al*: Comparative evaluation of the nitrate reduction assay, the MTT test, and the resazurin microtitre assay for drug susceptibility testing of clinical isolates of *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 55: 500-505, 2005.
- Huang Y, Liu J, Wang LZ, Zhang WY and Zhu XZ: Neuroprotective effects of cyclooxygenase-2 inhibitor celecoxib against toxicity of LPS-stimulated macrophages toward motor neurons. *Acta Pharmacol Sin* 26: 952-958, 2005.
- Owen J, Bates J and Lewis S: Differential effects of nitroblue tetrazolium on the hemodynamic responses elicited by activation of alpha(1)-adrenoceptors and 5-HT(2) receptors in conscious rats. *Eur J Pharmacol* 535: 248-252, 2006.
- Choi HS, Kim JW, Cha YN and Kim C: A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. *J Immunoassay Immunochem* 27: 31-44, 2006.
- Shi J, Zhang Y, Su JY, *et al*: Cytotoxicity of IFN-gamma-activated dendritic cells to freshly isolated acute myeloid leukemia cells. *J Exp Hematol* 13: 1071-1075, 2005.
- Burchert A, Muller MC, Kostrewa P, *et al*: Sustained molecular response with interferon alfa maintenance after induction therapy with imatinib plus interferon alfa in patients with chronic myeloid leukemia. *J Clin Oncol* 28: 1429-1435, 2010.
- Khoo TL, Vangsted AJ, Joshua D and Gibson J: Interferon-alpha in the treatment of multiple myeloma. *Curr Drug Targets* 12: 437-446, 2011.
- Benz R, Stussi G and Fehr J: Interferon as an alternative to purine analogues in the treatment of hairy cell leukaemia. *Brit J Haematol* 148: 664-666, 2010.
- Gupta V, Yi QL, Brandwein J, *et al*: Role of all-trans-retinoic acid (ATRA) in the consolidation therapy of acute promyelocytic leukaemia (APL). *Leukemia Res* 29: 113-114, 2005.
- Buonamici S, Li D, Mikhail F, *et al*: EVI1 abrogates interferon-alpha response by selectively blocking PML induction. *J Biol Chem* 280: 428-436, 2005.
- Reineke EL and Kao HY: Targeting promyelocytic leukemia protein: a means to regulating PML nuclear bodies. *Int Biol Sci* 5: 366-376, 2009.
- Chelbi-Alix MK, Vidy A, El Bougrini J and Blondel D: Rabies viral mechanisms to escape the IFN system: the viral protein P interferes with IRF-3, Stat1, and PML nuclear bodies. *J Interferon Cytokine Res* 26: 271-280, 2006.
- Crowder C, Dahle O, Davis RE, Gabrielsen OS and Rudikoff S: PML mediates IFN-alpha-induced apoptosis in myeloma by regulating TRAIL induction. *Blood* 105: 1280-1287, 2005.
- Dror N, Rave-Harel N, Burchert A, *et al*: Interferon regulatory factor-8 is indispensable for the expression of promyelocytic leukemia and the formation of nuclear bodies in myeloid cells. *J Biol Chem* 282: 5633-5640, 2007.
- Bernardi R, Papa A and Pandolfi PP: Regulation of apoptosis by PML and the PML-NBs. *Oncogene* 27: 6299-6312, 2008.
- Li L and He DL: Transfection of promyelocytic leukemia in retrovirus vector inhibits growth of human bladder cancer cells. *Acta Pharmacol Sin* 26: 610-615, 2005.
- Ravindranath Y, Gregory J and Feusner J: Treatment of acute promyelocytic leukemia by using all-trans retinoic acid (ATRA). *Leukemia* 18: 1576-1577, 2004.
- Gupta V, Yib QL, Brandwein J, *et al*: Clinico-biological features and prognostic significance of PML/RARalpha isoforms in adult patients with acute promyelocytic leukemia treated with all trans retinoic acid (ATRA) and chemotherapy. *Leuk Lymphoma* 45: 469-480, 2004.
- Zhang X, Yan X, Zhou Z, *et al*: Arsenic trioxide controls the fate of the PML-RAR α oncoprotein by directly binding PML. *Science* 328: 240-243, 2010.
- Wang ZG, Delva L, Gaboli M, *et al*: Role of PML in cell growth and the retinoic acid pathway. *Science* 279: 1547-1551, 1998.
- Abreu ELR, Baruffi MR, de Lima AS, *et al*: The co-expression of PML/RAR alpha and AML1/ETO fusion genes is associated with ATRA resistance. *Brit J Haematol* 128: 407-409, 2005.
- Mueller BU, Pabst T, Fos J, *et al*: ATRA resolves the differentiation block in t(15;17) acute myeloid leukemia by restoring PU.1 expression. *Blood* 107: 3330-3338, 2006.
- Andrianifahanana M, Agrawal A, Singh AP, *et al*: Synergistic induction of the MUC4 mucin gene by interferon-gamma and retinoic acid in human pancreatic tumour cells involves a reprogramming of signalling pathways. *Oncogene* 24: 6143-6154, 2005.
- Liu CH: Study on the synergic effect of all-trans retinoic acid combined with IFN-alpha on the proliferation and differentiation of HL-60 cells. *Chin J Cell Mol Immunol* 21: 637-639, 642, 2005.
- Gu ZM, Wu YL, Zhou MY, *et al*: Pharinin B stabilizes retinoic acid receptor-alpha and presents synergistic differentiation induction with ATRA in myeloid leukemic cells. *Blood* 116: 5289-5297, 2010.
- Wang J, Peng Y, Sun YW, *et al*: All-trans retinoic acid induces XAF1 expression through an interferon regulatory factor-1 element in colon cancer. *Gastroenterology* 130: 747-758, 2006.