European Review for Medical and Pharmacological Sciences 2015; 19: 1055-1062

ATRA and As_2O_3 regulate differentiation of human hematopoietic stem cells into granulocyte progenitor via alteration of HoxB8 expression

W.-J. LIU, N.-J. JIANG, Q.-L. GUO, Q. XU

Department of Pediatrics, Affiliated Hospital of Luzhou Medical College, Luzhou, Sichuan, China

Abstract. – OBJECTIVE: This study aimed to investigate the effect of all-trans retinoic acid (ATRA) and/or arsenic trioxide (As_2O_3) on homeobox B8 (HOXB8) mRNA and protein expressions during the differentiation and proliferation of hematopoietic stem cells (HSCs) to colony forming unit-granulocyte (CFU-G) in order to explore the pathogenesis of leukemia mediated by HOXB8 at mRNA and protein level.

MATERIALS AND METHODS: Twelve cord blood samples were collected from the fetal placenta umbilical vein and cultured in vitro. The proliferation and differentiation of cord blood HSCs into CFU-G was continuously disrupted with 10 nmol/l of ATRA and/or 10 nmol/l of As_2O_3. The expression of HOXB8 mRNA and protein were detected by quantitative real-time polymerase chain reaction (qRT-PCR) and Western-blot, respectively.

RESULTS: HOXB8 mRNA/protein expression was detected in control, ATRA, As_2O_3 and ATRA +As_2O_3 groups on days 3, 7, and 12 of culture. HOXB8 mRNA/protein expression was detectable on day 3, reached its highest level on day 7 and decreased on day 12. HOXB8 mRNA/protein expression in ATRA, As_2O_3 and ATRA+As_2O_3 was upregulated compared with control group ($p < 0.05$).

CONCLUSIONS: There is a positive relationship between HOXB8 gene expression and granulocyte progenitor hematopoiesis. ATRA/As_2O_3 upregulate the expression of HOXB8 mRNA/protein, and treatment of leukemia with ATRA/As_2O_3 may regulate HOX gene expression.

Key Words:
Colony Forming Unit-Granulocyte, All-trans retinoic acid, Arsenic trioxide, Homeobox B8.

Introduction

Homeobox (HOX) gene is a class of regulatory gene regulating embryonic development and cell differentiation. Previous studies have shown that Hox genes not only play a role in embryonic development, but also involve in self-renewal and directed differentiation of hematopoietic stem cells (HSC). The clustered HOX proteins play crucial roles in development, hematopoiesis, and leukemia, yet the targets they regulate and their mechanisms of action are poorly understood[1]. Each transcription factor of the HOX genes has its unique function in the hematopoietic process including control of proliferation, differentiation, and self-renewal of HSC[2-5]. Several Hox genes, in particular HOXA9, and HOXA10, HOXB3, HOXB4, HOXB7, HOXB8 are expressed in the HSC compartment, but are subsequently downregulated upon differentiation[6-8].

In hematopoietic cells, abnormal expression of HOX genes, cell differentiation and maturation disorder can lead to the occurrence and development of leukemia[9]. HoxB8 was the first Hox protein found to be transcriptionally activated in acute myeloid leukemia (AML). Persistent expression of *HOXB8* prevents differentiation of factor-dependent myeloid progenitors[10], and cooperates with mutations that establish autocrine proliferation to generate overt AML[11].

All-trans retinoic acid (ATRA) can regulate the expression of Hox genes, and is widely used in clinical treatment in acute promyelocytic leukemia (APL), merely the longer-term use of the drug has been shown having certain toxic and side effects. Clinical experiments showed that a combination of arsenic trioxide (As_2O_3) and ATRA had a synergistic effect in term of their antitumor activities. So far, there is no study addressing the effect of As_2O_3 on the regulation of HOX gene, while it has been proven the exact effectiveness of a combination of arsenic trioxide (As_2O_3) and ATRA in the treatment of AML[12,13]. This study aims to determine whether ATRA and As_2O_3 can regulate the expression of HOXB8 mRNA/protein during the differentiation and proliferation of HSC.

Corresponding Author: Wen-jun Liu, MD; e-mail: lwjlyfy@qq.com

Materials and Methods

Samples

Placental cord blood samples from 12 full-term infants were collected at the Department of Obstetrics and Gynecology, Affiliated Hospital of Luzhou Medical College, Sichuan, China. All mothers were in good health and HBsAg negative. The indices of serum anti-HCMV-IgG and HCMV-IgM of samples detected by ELISA and HCMV-DNA assessed by PCR were negative. Written informed consent was provided prior to samples collection. Ethical approval was granted by the Ethics Committee of the Affiliated Hospital of Luzhou Medical College. ATRA and As_2O_3 were obtained from Chongqing Huapont Pharm Co. Ltd., China. Rabbit anti-human HOXB8 antibody was from Santa Cruz, Biotechnology Inc., Santa Cruz, USA).

Collection of CD34+ Progenitor Cells

The umbilical cord blood samples, collected within 2-4 hrs after birth, were procured from Affiliated Hospital of Luzhou Medical College. Mononuclear cells were isolated by density gradient centrifugation using lymphocytes separation medium. $CD34^+$ progenitor cells were enriched using $CD34^+$ cell isolation kit (Miltenyi Biotech Co., San Diego, CA, USA). Flow cytometry (Beckman-Coulter Co., Brea, CA, USA) was used to assess the purity of $CD34^+$. Fluorescein isothiocyanate (FITC)-conjugated anti-CD45+ and R-Phycoerythrin (PE)-conjugated anti-CD34+ were purchased from Immunotech Co. (San Mateo, CA, USA). The purity of CD34+ cells was 93.4 ± 2.3%.

Culture and Identification of CFU-G

The various study groups were designated as control (blank), ATRA, As_2O_3 and ATRA+ As_2O_3 groups. CD34+ cells were cultured in DMEM/F12 medium containing 30% fetal bovine serum (FBS), a method developed in our laboratory was used for CFU-G culture (Table I). For identification of granulocyte progenitor cells, cultured samples were collected at days 3, 7 and 12, stained by Wright-Giemsa staining and the morphology, size of cells were confirmed by microscopy.

Cell Proliferation Assay

Dosing intervention based on the above grouping, ATRA and As_2O_3 were dissolved in culture medium, the final concentration of ATRA and As_2O_3 were 100 nmol/l, 10 nmol/l, and 1 nmol/l. Cell morphology was observed for culture on days 1-14 under an inverted microscope and growth conditions were recorded. The number of CFU-G cells was measured on day 1, 3, 5, 7, 9, and 14 of culture. Cell proliferation was assessed using the MTT colorimetric assay. Cells were seeded into 96-well plates at a density of 2×10^4 per well (100 µl) and treated with ATRA or As_2O_3 at 1, 10 and 100 nmol/l. Wells with serum free medium were used as negative control. The cells were treated for 48 h, After 48 hours, the media was replaced with 3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT)-containing media (0.5 mg/ml), incubated at 37°C for 4 hours, The medium was removed and 100 µl of dimethyl sulfoxide (DMSO) was added into each well. The plate was gently rotated on an orbital shaker for 10 min to completely dissolve the precipitation. The absorbance was detected at 570 nm with a Microplate Reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA), and the results were expressed as a percentage of the absorbance of control (cell viability rate = $(OD_{EG}/OD_{Control}) \times 100\%$).

RNA Extraction and qRT-PCR

Total RNA was isolated from cells on days 3, 7 and 12 of culture using Trizol reagent, RNA integrity was checked by electrophoresis on 10% denaturing agarose and samples were stored at –80°C until use.

Table I. The composition of CFU-G culture media.

Culture composition	Control	ATRA	As_2O_3	As_2O_3 + ATRA
Fetal bovine serum (%)	30%	30%	30%	30%
2-mercaptoethanol (50 µmol/l)	+	+	+	+
CD34 + cells (1×10^5/ml)	3 ml	3 ml	3 ml	3 ml
ATRA (10 nmol/l)	–	+	–	+
As_2O_3 (10 nmol/l)	–	–	+	+
GM-SF (40 ng/ml)	+	+	+	+
DMEM/F12	+	+	+	+

One microgram RNA was subjected to reverse transcription using PrimeScript® 1st strand cDNA Synthesis Kit (Takara Bio Inc, Ahiga, Japan) according to manufacture instructions. The cDNA templates were serially diluted 10-fold and served as standards with presumed original copy number (10^4). A sample (5 μl) from each standard was added to a 30-μl reaction volume including 3 μl 10× buffer, 3 μl $MgCl_2$ (25 mmol/l), 9 μl dNTPs (10 mmol/l), 1 μl dNTPs (10 mmol/l), 1 μl each of upstream and downstream primers (10 μmol/l), 1 μl TaqMan probes (10 μmol/l; Table II), 0.3 μl Taq DNA polymerase, 14.8 μl diethylpirocarbonate (DEPC)-H_2O and 5 μl cDNA template. PCR reaction was performed using the following reaction conditions: initial denaturation at 95°C for 60 sec; followed by 45 cycles of denaturation at 95°C for 10 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min.

The specificity of PCR amplification was checked by melting curve analysis. The copy numbers of target genes were calculated from Ct values by using standard curves. The fold change in expression for each sample was calculated using $2^{-\Delta\Delta Ct}$ and the values for HOXB8 expression levels were represented as means ± SD.

Western Blot

In each group, $1{\times}10^6$ cells were collected at 3, 7, and 12 days. Cells were washed with phosphate buffered saline (PBS), and total proteins were extracted using RIPA buffer with protease inhibitors. The samples (20 μl aliquots) were stored at –80°C until use. The extracted proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), first at 80V for 20 min then at 120V for 50 min. After electrophoresis, the proteins were electroblotted to nitrocellulose membrane (at 200 mA for 80 min). The membranes were blocked in tris buffered saline (TBS) with 10% skim milk solution at room temperature for 2 hrs and then treated with primary antibody (4°C, overnight). The membranes were washed thoroughly with TBS and then treated with secondary antibodies at room temperature for 1 hr. Nitrocellulose membranes were exposed to X-ray films in dark and developed. Results were analyzed using image analysis software and HOXB8/actin ratio was determined to measure relative amount of protein expression in the samples. Results for HOXB8 expression levels were represented as means ± SD of at least three independent experiments.

Table II. Target gene sequences.

HOXB8-fw	5'-ACACAGCTCTTCCCCTGGAT-3'
HOXB8-rv	5'-CTTCTCCAGCTCCAGGGTCT-3'
β-actin-fw	5'-GAAGATCAAGATCATTGCTCCT-3'
β-actin-rv	5'-TACTCCTGCTTGCTGATCCA-3'

Statistical Analysis

The data were calculated by a comparative threshold method using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). A pair wise comparison of mean values between groups (LSD method) was performed after detecting a significant difference by Homogeneity test for variance and two-way analysis of variance. Data were analyzed using Mann-Whitney rank sum test (except for "competitive (lymphomyeloid) repopulating units/CRU" assay). All p-values < 0.05 were considered statistically significant.

Results

Effect of ATRA and As_2O_3 on Cell Proliferation of CFU-G

Test results are shown in Figure 1: in the control as well as ATRA and As_2O_3 treated groups, the number of CFU-G cells were gradually increased; reached its maximum level on day 7 then gradually decreased (Figure 1 A, C). Our results also show that 100 nmol/l ATRA significantly inhibited cell proliferation of CFU-G at all studied time points starting from day 3 ($p < 0.05$) while 1 and 10 nmol/l had minimal effects (Figure 1 B). Similarly, 100 nmol/l As_2O_3 significantly inhibited cell proliferation of CFU-G at all studied time points while 1 and 10 nmol/l had minimal effects (Figure 1 D). According to the above results, the final concentration for drug intervention selected was 10 nmol/l in order to guarantee the stability of cell growth and achieve good experimental effect.

HOXB8 mRNA Expression

As shown in Figure 2, the expression of HOXB8 mRNA in CFU-G progenitor was time-dependent and showed significant increase after 7 days of treatment compared with the 3 days ($p < 0.05$), while its expression significantly decreased after 12 days ($p < 0.05$). HOXB8 mRNA expression in ATRA, As_2O_3 and ATRA+As2O3 was upregulated compared with control group ($p < 0.05$) at all studied time points.

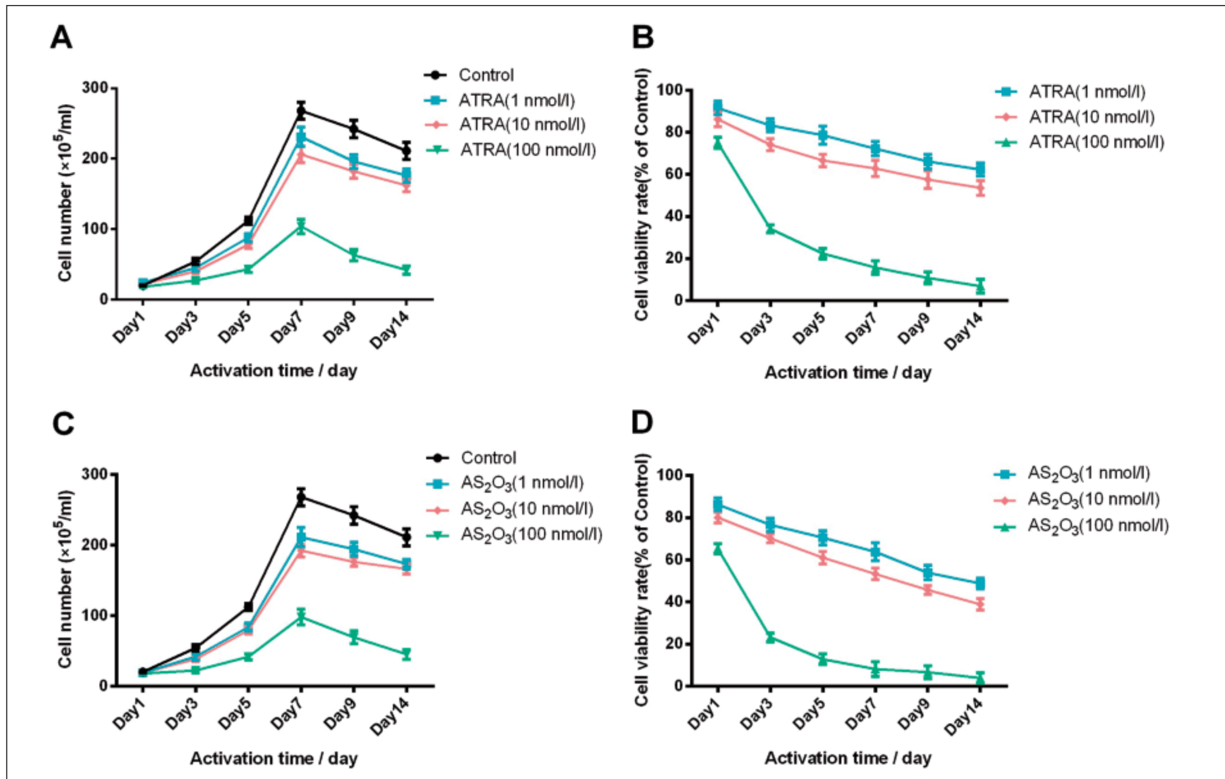

Figure 1. Effect of ATRA and AS_2O_3 on CFU-G progenitor cell growth and viability rate. Results are expressed as mean ± SD.

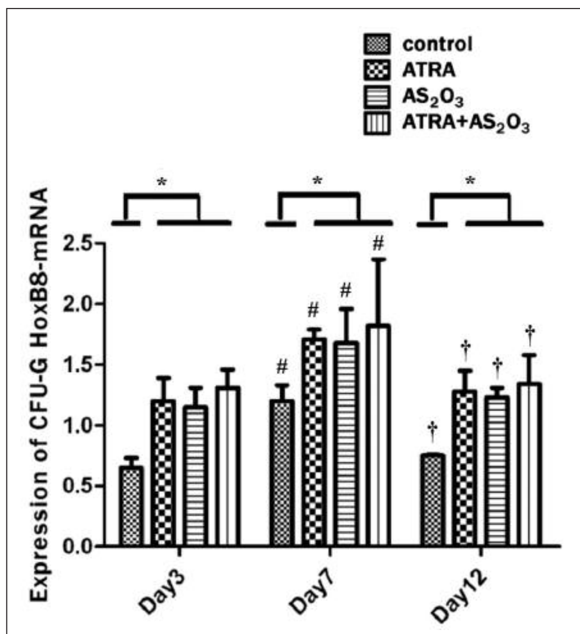

Figure 2. Time dependent mRNA expression of HOXB8 in CFU-G progenitors. Results are expressed as mean ± SD. *Statistical significance compared to control group at the same time point ($p < 0.05$); #Statistical significance compared to 3rd day ($p < 0.05$); †Statistical significance compared to 7th day ($p < 0.05$).

HOXB8 Protein Expression

HOXB8/Actin protein expression in CFU-G progenitor is shown in Figure 3 and Figure 4, As shown in Figure 4, HOXB8 protein expression in ATRA, As_2O_3 and ATRA+As_2O_3 was upregulated compared with control group ($p < 0.05$). The expression of HOXB8 protein in CFU-G progenitor was time-dependent and showed the 7 days than the 3 days and 12 days the expression was significantly raised, the difference was statistically significant ($p < 0.05$), and compared with 12 days, three days there was no statistically significant difference ($p > 0.05$).

Discussion

Transcription factors encoded by Hox genes are associated with the development of the hematopoietic system, and involve in the proliferation, differentiation and maturation of hematopoietic stem/progenitor cells (HSPC). In hematopoietic cells, the abnormal expression of HOX genes may cause the disorder of cell differentiation and maturation, reduced hematopoietic abilities, or even result in occurrence and devel-

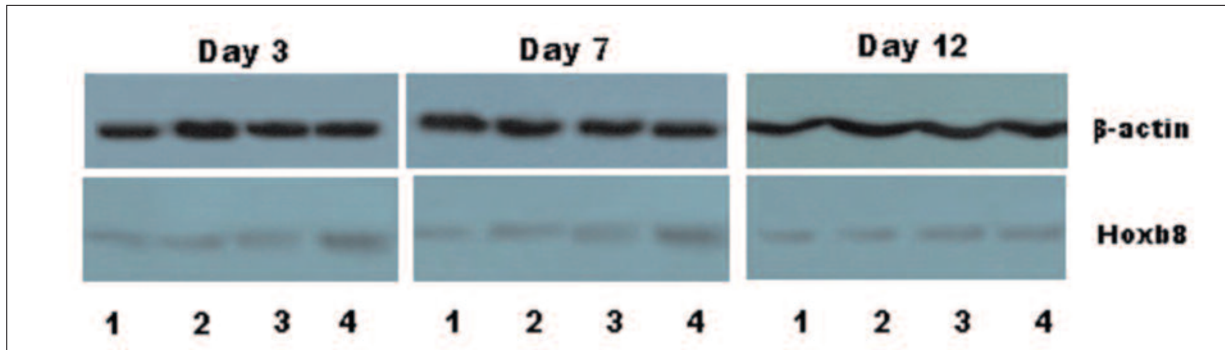

Figure 3. HOXB8 protein expression in CFU-G at Day 3; Day 7 and Day 12. (1) Control group; (2) AS_2O_3; (3) ATRA; and (4) ATRA+ AS_2O_3.

opment of blood system diseases[14]. Previous studies showed that HOXB8 gene can prevent differentiation by directly influencing cellular gene expression, and by its interaction with Pbx proteins[15]. Several studies have been conducted on HOX genes in CD34+ cell subpopulations isolated from normal human bone marrow (BM); HOXB2, HOXB4, HOXB6 are mainly related to the generation of erythroid progenitor cells, while HOXB3 are involved in the generation of myeloid progenitor cells, on the other hand,

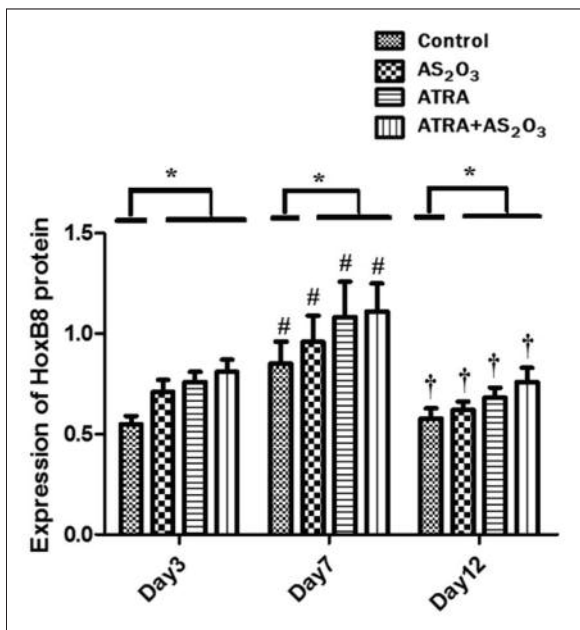

Figure 4. Time dependent expression of HOXB8 protein in CFU-G progenitors. Results are expressed as mean ± SD. * Statistical significance compared to control group at the same time point ($p < 0.05$); #Statistical significance compared to 3rd day ($p < 0.05$); †Statistical significance compared to 7th day ($p < 0.05$).

HOXB7, HOXB8 regulate the development of granulopoiesis[8]. HOXB8 sustained expression in macrophage cell line has been shown to maintain macrophages vitality[16] and it was also found enforcing the expression of HOXB6, HOXB7 in hematopoietic cell lines will lead to granulopoiesis abnormal differentiation[17]. MicroRNA-196 (miR-196) inhibits HOXB8 expression in regulating myeloid differentiation of acute myeloid leukemia cells (HL-60), inferred that HOXB8 gene may be one of the regulatory genes of proliferation and differentiation of granulopoiesis[18]. In the current study, HOXB8 mRNA/protein were positively expressed in the control group and the drug intervention, hence HOXB8 gene may be associated with the normal proliferation and differentiation process from hematopoietic stem and progenitor cells to myeloid.

ATRA can regulate the expression of HOX genes. Retinoic acid (RA) can induce the expression of HOX genes mainly depends on its retinoic acid receptor (RAR) and retinoid (RXR) receptors. RA and receptor assembled into dimer then directly bind to specific retinoic acid response element (retinoic acid response element, RARE), will cause activation or inhibition in gene transcriptional process[19]. It has been reported that RARE exists in the promoter region of the HOX genes, such as HOXA1, HOXA4, HOXA7, HOXA9, HOXB1, and HOXD4[20]. ATRA could stimulate the terminal differentiation of granulocytic leukemia cells, and could also interfere with the differentiation of hematopoietic stem and progenitor cells in mammalian embryonic period, RAR and RXR as ATRA receptors are major nuclear receptors of the monophyletic hematopoietic differentiation[21,22]. It has been observed that ATRA can stimulate the de-

velopment of late committed colony-forming unit-granulocyte, macrophage and inhibit the early differentiation of normal human myeloid progenitor cells[23,24]. ATRA up-regulate the erythroid progenitor cells (colony-forming-units erythroid, CFU-E) and lymphoid progenitor cells (Colony-forming-units T-lymphocyte,CFU-TL)[25]. The homeohox gene HOX-B8 was shown to cooperate with hematokines to induce leukemia, and to enhance self-renewal of immature myeloid progenitors when expressed alone[15,26]. In this work, HOXB8 has been chosen as target gene, using ATRA to interfere with the directed proliferation and differentiation process from human cord blood hematopoietic stem and progenitor cell to myeloid, we aimed to observe whether ATRA could affect the expression of the HOXB8 gene and its protein. Many genes can be upregulated by ATRA involved in differentiation, the oxidase activation pathway and adhesion molecules. ATRA may play an important role in HL-60 cells differentiation pathway by inducing differential expression of a variety of genes from several pathways[27-29]. Retinoic acid induces transcription of genes encoding transcription factors and signaling proteins that further modify gene expression such as HoxA1, FOX03A, Sox9, TRAIL by binding to RARs, to regulate stem cell differentiation[30]. It demonstrates that ATRA-induced apoptosis of NB4 cells by suppressing HOXA cluster antisense RNA 2 (HOXA-AS2), which is a long non-coding RNA located between the HOXA3 and HOXA4 genes in the HOXA cluster[31]. Our results showed that CFU-G colony number was gradually increased in presence of ATRA (10 nmol/l) and reached its maximum level on day 7 then gradually decreased; treatment with ATRA significantly induced HOXB8 mRNA and protein expressions compared with control group, suggesting that ATRA can increase the expression of HOXB8 gene and protein.

Recently, it was discovered that As_2O_3 is one of the effective drugs for treating leukemia, and it can also inhibit the growth of various tumor cells *in vitro*, induce the apoptosis of tumor cells, moreover, have less toxic and side effect than conventional chemotherapy drugs and no cross-resistance. Some studies had confirmed[32-34] that As_2O_3 could be sensitive to the tumor cells of conventional chemotherapy drug resistance, and promote the apoptosis of drug-resistant tumor cells, these advantages make arsenical getting much attention as a unique anticancer drug. As_2O_3 has apoptosis-inducing effect on HL-60 cells, and inhibits the expression of their telomerase subunit hTERT mRNA, both of which shows a concentration-dependent effect[35]. As_2O_3 in high doses triggers apoptosis, while in lower concentrations it induces partial differentiation[36]. The As_2O_3 apoptosis-inducing effect is associated with the collapse of mitochondrial transmembrane potentials in a thiol-dependent manner, whereas the mechanisms underlying APL cell differentiation induced by low dose arsenic remain to be explored[37]. It is known to cause degradation of PML-RARalpha with subsequent induced myeloid differentiation. But it found that ATO by itself does not cause differentiation of the PML-RARalpha negative HL-60 cells, but enhances ATRA's capability to cause differentiation[38]. Our previous[39] study has been found that the regulation of the HOXA9 mRNA and protein expression is associated with the treatment of leukemia by ATRA and As_2O_3.

Conclusions

In the current study, CFU-G colony number was gradually increased in presence of As_2O_3 (10 nmol/l) and reached its maximum level on day 7 then gradually decreased; As_2O_3 significantly induced HOXB8 mRNA and protein expressions compared with control group, suggesting that As_2O_3 plays a regulatory role on the expression of the HOXB8 protein and mRNA.

Acknowledgements

We thank the Health Bureau of Sichuan Province for financial support (Grant No. 20130040).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Huang Y, Sitwala K, Bronstein J, Sanders D, Dandekar M, Collins C, Robertson G, MacDonald J, Cezard T, Bilenky M, Thiessen N, Zhao Y, Zeng T, Hirst M, Hero A, Jones S, Hess JL. Identification and characterization of Hoxa9 binding sites in hematopoietic cells. Blood 2012; 119: 388-398.
2) Abramovich C, Humphries RK. Hox regulation of normal and leukemic hematopoietic stem cells. Curr Opin Hematol 2005; 12: 210-216.

3) Lawrence HJ, Sauvageau G, Humphries RK, Largman C. The role of HOX homeobox genes in normal and leukemic hematopoiesis. Stem Cells 1996; 14: 281-291.

4) Owens BM, Hawley RG. HOX and non-HOX homeobox genes in leukemic hematopoiesis. Stem Cells 2002; 20: 364-379.

5) Thorsteinsdottir U, Sauvageau G, Humphries RK. Hox homeobox genes as regulators of normal and leukemic hematopoiesis. Hematol Oncol Clin N Amer 1997; 11: 1221-1237.

6) Giampaolo A, Sterpetti P, Bulgarini D, Samoggia P, Pelosi E, Valtieri M, Peschle C. Key functional role and lineage-specific expression of selected HOXB genes in purified hematopoietic progenitor differentiation. Blood 1994; 84: 3637-3647.

7) Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. Science 2002; 298: 601-604.

8) Sauvageau G, Lansdorp PM, Eaves CJ, Hogge DE, Dragowska WH, Reid DS, Largman C, Lawrence HJ, Humphries RK. Differential expression of homeobox genes in functionally distinct CD34+ subpopulations of human bone marrow cells. Proc Natl Acad Sci USA 1994; 91: 12223-12227.

9) He M, Chen P, Arnovitz S, Li Y, Huang H, Neilly MB, Wei M, Rowley JD, Chen J, Li Z. Two isoforms of HOXA9 function differently but work synergistically in human MLL-rearranged leukemia. Blood Cells Mol Dis 2012; 49: 102-106.

10) Blatt C, Aberdam D, Schwartz R, Sachs L. DNA rearrangement of a homeobox gene in myeloid leukaemic cells. EMBO J 1988; 7: 4283-4290.

11) Perkins A, Kongsuwan K, Visvader J, Adams JM, Cory S. Homeobox gene expression plus autocrine growth factor production elicits myeloid leukemia. Proc Natl Acad Sci USA 1990; 87: 8398-8402.

12) Mathieu J, Besancon F. Arsenic trioxide represses NF-kappaB activation and increases apoptosis in ATRA-treated APL cells. Ann NY Acad Sci 2006; 1090: 203-208.

13) Xu JL, Jiang M, Halida Y, Duan XL, Guo XH, Hao JP, Fu ML, Qu JH, Wang L. Stratification therapy in patients with acute promyelocytic leukemia after a complete remission by all-trans retinoic acid]. Zhonghua yi xue za zhi 2011; 91: 2254-2257.

14) Wen-jun L, qu-lian G, Hong-ying C, Yan Z, Mei-Xian H. Studies on HOXB4 expression during differentiation of human cytomegalovirus-infected hematopoietic stem cells into lymphocyte and erythrocyte progenitor cells. Cell Biochem Biophys 2012; 63: 133-141.

15) Knoepfler PS, Sykes DB, Pasillas M, Kamps MP. HoxB8 requires its Pbx-interaction motif to block differentiation of primary myeloid progenitors and of most cell line models of myeloid differentiation. Oncogene 2001; 20: 5440-5448.

16) Rosas M, Osorio F, Robinson MJ, Davies LC, Dierkes N, Jones SA, Reis e Sousa C, Taylor PR. Hoxb8 conditionally immortalised macrophage lines model inflammatory monocytic cells with important similarity to dendritic cells. Eur J Immunol 2011; 41: 356-365.

17) Nemeth MJ, Curtis DJ, Kirby MR, Garrett-Beal LJ, Seidel NE, Cline AP, Bodine DM. Hmgb3: an HMG-box family member expressed in primitive hematopoietic cells that inhibits myeloid and B-cell differentiation. Blood 2003; 102: 1298-1306.

18) Kawasaki H, Taira K. MicroRNA-196 inhibits HOXB8 expression in myeloid differentiation of HL60 cells. Nucleic Acids Symp Ser 2004; 211-212.

19) Thomas M, Sukhai MA, Kamel-Reid S. An emerging role for retinoid X receptor alpha in malignant hematopoiesis. Leukemia Res 2012; 36: 1075-1081.

20) Wada H, Escriva H, Zhang S, Laudet V. Conserved RARE localization in amphioxus Hox clusters and implications for Hox code evolution in the vertebrate neural crest. Dev Dyn 2006; 235: 1522-1531.

21) Evans T. Regulation of hematopoiesis by retinoid signaling. Exp Hematol 2005; 33: 1055-1061.

22) Urahama N, Ito M, Sada A, Yakushijin K, Yamamoto K, Okamura A, Minagawa K, Hato A, Chihara K, Roeder RG, Matsui T. The role of transcriptional coactivator TRAP220 in myelomonocytic differentiation. Genes to Cells 2005; 10: 1127-1137.

23) Sammons J, Ahmed N, Khokher MA, Hassan HT. Mechanisms mediating the inhibitory effect of all-trans retinoic acid on primitive hematopoietic stem cells in human long-term bone marrow culture. Stem Cells 2000; 18: 214-219.

24) Szatmari I, Iacovino M, Kyba M. The retinoid signaling pathway inhibits hematopoiesis and uncouples from the Hox genes during hematopoietic development. Stem Cells 2010; 28: 1518-1529.

25) Liu WJ, Huang MX, Guo QL, Chen JH, Shi H. Effect of human cytomegalovirus infection on the expression of Hoxb2 and Hoxb4 genes in the developmental process of cord blood erythroid progenitors. Mol Med Rep 2011; 4: 1307-1311.

26) Krishnaraju K, Hoffman B, Liebermann DA. Lineage-specific regulation of hematopoiesis by HOX-B8 (HOX-2.4): inhibition of granulocytic differentiation and potentiation of monocytic differentiation. Blood 1997; 90: 1840-1849.

27) Kim SH, Yoo JC, Kim TS. Nargenicin enhances 1,25-dihydroxyvitamin D(3)- and all-trans retinoic acid-induced leukemia cell differentiation via PKCbetaI/MAPK pathways. Biochem Pharmacol 2009; 77: 1694-1701.

28) Wang J, Fong CC, Tzang CH, Xiao P, Han R, Yang M. Gene expression analysis of human promyelocytic leukemia HL-60 cell differentiation and cytotoxicity induced by natural and synthetic retinoids. Life Sci 2009; 84: 576-583.

29) Yedjou CG, Tchounwou PB. Modulation of p53, c-fos, RARE, cyclin A, and cyclin D1 expression in human leukemia (HL-60) cells exposed to arsenic trioxide. Mol Cell Biochem 2009; 331: 207-214.

30) Gudas LJ, Wagner JA. Retinoids regulate stem cell differentiation. J Cell Physiol 2011; 226: 322-330.
31) Zhao H, Zhang X, Frazao JB, Condino-Neto A, Newburger PE. HOX antisense lincRNA HOXA-AS2 is an apoptosis repressor in all Trans retinoic acid treated NB4 promyelocytic leukemia cells. J Cell Biochem 2013; 114: 2375-2383.
32) Kinjo K, Kizaki M, Muto A, Fukuchi Y, Umezawa A, Yamato K, Nishihara T, Hata J, Ito M, Ueyama Y, Ikeda Y. Arsenic trioxide (As_2O_3)-induced apoptosis and differentiation in retinoic acid-resistant acute promyelocytic leukemia model in hGM-CSF-producing transgenic SCID mice. Leukemia 2000; 14: 431-438.
33) Wang DH, Wei HL, Zhao HS, Hao CY, Min ZH, Liu JM. Arsenic trioxide overcomes apoptosis inhibition in K562/ADM cells by regulating vital components in apoptotic pathway. Pharmacol Res 2005; 52: 376-385.
34) Ya-li Z, Hu-lai W, Lu G. Changes of mdr1 and Survivin genes expressions during apoptosis induced by Arsenic trioxide in multidrug-resistant human leukemia K562/ADM cells. Chin J Cancer Prev Treat 2006; 13: 577-581.
35) Yao Z, Yan Z, C. C-J. Experimental study on the effect of arsenic trioxide on HL-60 cell apoptosis and telomerase hTERT-mRNA expression. Chin Pharmacol Bull 2003; 19: 206-208.
36) Hoffman E, Mielicki WP. Arsenic trioxide: impact on the growth and differentiation of cancer cells and possible use in cancer therapy. Postepy Hig Med Dosw (Online) 2013; 67: 817-827.
37) Zhang TD, Chen GQ, Wang ZG, Wang ZY, Chen SJ, Chen Z. Arsenic trioxide, a therapeutic agent for APL. Oncogene 2001; 20: 7146-7153.
38) Nayak S, Shen M, Bunaciu RP, Bloom SE, Varner JD, Yen A. Arsenic trioxide cooperates with all trans retinoic acid to enhance mitogen-activated protein kinase activation and differentiation in PML-RARalpha negative human myeloblastic leukemia cells. Leuk Lymphoma 2010; 51: 1734-1747.
39) Guo WW, Liu WJ. [Expression of homeobox A9 in myeloid leukemia cell line HL-60 and effect of drugs on its expression]. Zhongguo shi yan xue ye xue za zhi / Zhongguo bing li sheng li xue hui = J Exp Hematol 2012; 20: 300-304.