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Gene expression profile changes in NB_4 cells induced by arsenic trioxide[1]

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KEY WORDS arsenicals; gene expression profiling; acute promyelocytic leukemia; apoptosis; cell differentiation

ABSTRACT

AIM: To investigate the gene expression profiles of acute promyelocytic leukemia (APL) cell line NB_4 treated with arsenic trioxide (As_2O_3) using cDNA microarray. **METHODS:** Two cDNA probes were prepared through reverse transcription from mRNA of NB_4 cells treated with or without arsenic trioxide. The probes were labeled with Cy3 and Cy5 fluorescence dyes individually, hybridized with cDNA microarray representing 1003 different human genes, and their fluorescent intensities were scanned. The genes were screened through the analysis of the difference in two gene expression profiles. **RESULTS:** The analysis of gene expression profiles indicated that after the treatment of arsenic trioxide (0.5 μmol/L) 3 genes were up-regulated, among which, PSMB6 gene was involved in proteasome degradation pathway, and 18 genes related to RNA processing, protein synthesis, and signal transduction were down-regulated. **CONCLUSION:** PSMB6 and ITGB1 genes may be related to the differentiation and/or apoptosis of NB_4 cells induced by As_2O_3.

INTRODUCTION

Arsenic trioxide (As_2O_3) is extremely effective in the treatment of acute promyelocytic leukemia[1]. As_2O_3 was shown to exert concentration-dependent dual effects in APL cells, and high-concentration (0.5-2.0 μmol/L) of As_2O_3 could induce *in vitro* growth inhibition and apoptosis of APL cells. However, low-concentration (0.1-0.5 μmol/L) As_2O_3-induced differentiation was also observed in APL cells. The apoptosis of APL cells induced by As_2O_3 was associated with down-regulation of *bcl*-2 gene expression[2]. The recent availability of microarray technology has made it possible to perform an extensive analysis of As_2O_3 modulating effects on genes in APL cells that play a role in the apoptosis and/or differentiation. To identify the genes modulated by arsenic trioxide, we monitor the gene expression profiles of the APL cells (NB_4 cells) upon treatment with As_2O_3.

MATERIALS AND METHODS

Reagents Arsenic trioxide was purchased from Sigma Chemical Company. As_2O_3 was prepared as a 0.1 mmol/L stock solution in RPMI-1640 medium, and used at a final concentration of 0.5 μmol/L.

Cell culture The NB_4 cells, obtained from Dr CHEN Zhu (Shanghai Institute of Hematology, Shanghai, China), were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, glutamine, and antibiotics (benzylpenicillin 100 kU/L and streptomycin 100 mg/L) in a humidified atmosphere of 95 % air/5 %

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CO_2 at 37 °C. Cells were maintained at less than 5×10^8 cells/L with daily adjusting cell density through adding fresh medium and corresponding concentrations of compounds. The cells treated with or without As_2O_3 (0.5 µmol/L) were collected at 48 h.

Construction of microarrays The BioDoor 1024D microarray consisted of 1003 novel or known genes (provided by United Gene Holdings, Ltd). These genes were amplified through PCR using universal primers. The length of PCR production was about 1000-3000 bp. These genes were purified and examined through agarose electrophoresis. They were dissolved in 3×SSC solution and then spotted on silylated slides (TeleChem, Inc) by Cartesian 7500 Spotting Robotics (Cartesian, Inc). After spotting, the slides were hydrated for 2 h, dried for 0.5 h, UV cross-linked, and then treated with 0.2 % SDS, H_2O, and 0.2 % $NaBH_4$ respectively for 10 min each. The slide was dried again for use.

Probe preparation Total RNA were extracted at 48 h using the Trizol reagent (Gibco BRL Life Technologies, Grand Island, NY), and then mRNA were purified using Oligotex mRNA Midi Kit (Qiagen,Inc). The fluorescent cDNA probes were prepared through reverse transcription and then they were purified referring to the protocol of Schena[3]. The probes from NB_4 cells without As_2O_3 treatment were labeled with Cy3-dUTP, and those from NB_4 cells treated with As_2O_3 for 48 h were labeled with Cy5-dUTP. The probes were mixed and precipitated by ethanol, and resolved in 20 µL hybridization solution (5×SSC, 0.2 % SDS).

Hybridization and washing Chip was prehybridized with hybridization solution at 42 °C for 5 h. After denaturation at 95 °C for 2 min, the probe mixture was added on the prehybridized chip and covered with glass. The chip was incubated at 42 °C for 15-17 h. The slide was washed with solutions of 2×SSC, 0.2 % SDS and 0.1×SSC, 0.2 % SDS, and then 0.1×SSC respectively, 10 min each, then dried at room temperature.

Detection and analysis The chip was scanned by ScanArray3000 laser scanner (General Scanning, Inc) at different wavelengths. The acquired image was analyzed by ImaGene 3.0 software (BioDiscovery, Inc). The intensity of each spot at the different wavelengths represents the quantity of Cy3-dUTP and Cy5-dUTP, respectively. Each ratio of Cy3 to Cy5 was computed.

RESULTS

In order to monitor the preparation and hybridization of the microarray, we arranged 92 housekeeping genes as positive controls, and 8 plant genes, spotting solution (without DNA, 49 spots) as negative control spots in the array. We noticed that these positive control spots showed high intensity of signal and negative control spots showed low intensity after hybridization, which proved the reliability of the experiment. RT-PCR was performed to further prove the reliability of results. The results of RT-PCR of ITGB1 gene were consistent with those of cDNA microarray (data not shown).

The genes with intensity value above 200 and a 2-fold difference of ratio were regarded as differentially expressed genes. The analysis of gene expression profiles indicated that 3 genes were up-regulated and 18 genes were down-regulated. The scanning results of hybridizing signals on cDNA microarray were shown with scatter plot (Fig 1, 2). The data of scatter plot revealed that the spots which were far from 45° angle line were differentially expressed genes.

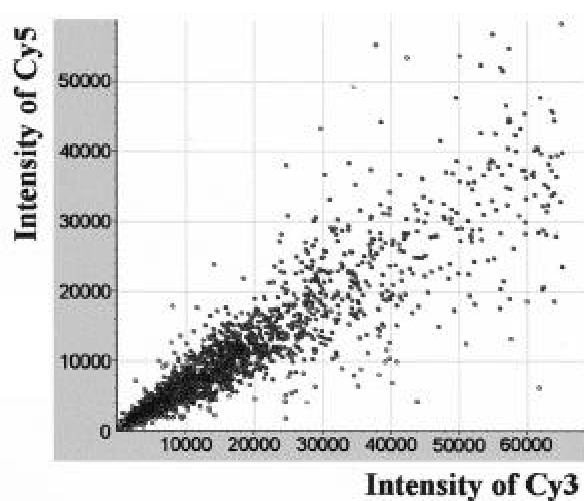

Fig 1. The scatter plots of hybridizing signals on gene chip.

According to the above standard, the up-regulated genes (GeneBank Accession) included: degradation of proteins: D29012 (PSMB6); cytoskeletal proteins: J05243 (SPTAN1) and U03271 (CAPZB). The down-regulated genes included: 1) synthesis of proteins: L06845 (CARS), NM_000985 (RPL17), NM_002139 (RBMX), U13045 (GABPB1), and X57958 (RPL7); 2) cell signaling and communication proteins: AF012872 (PIK4CA), D50683 (TGFBR2), L13463 (RGS2), and

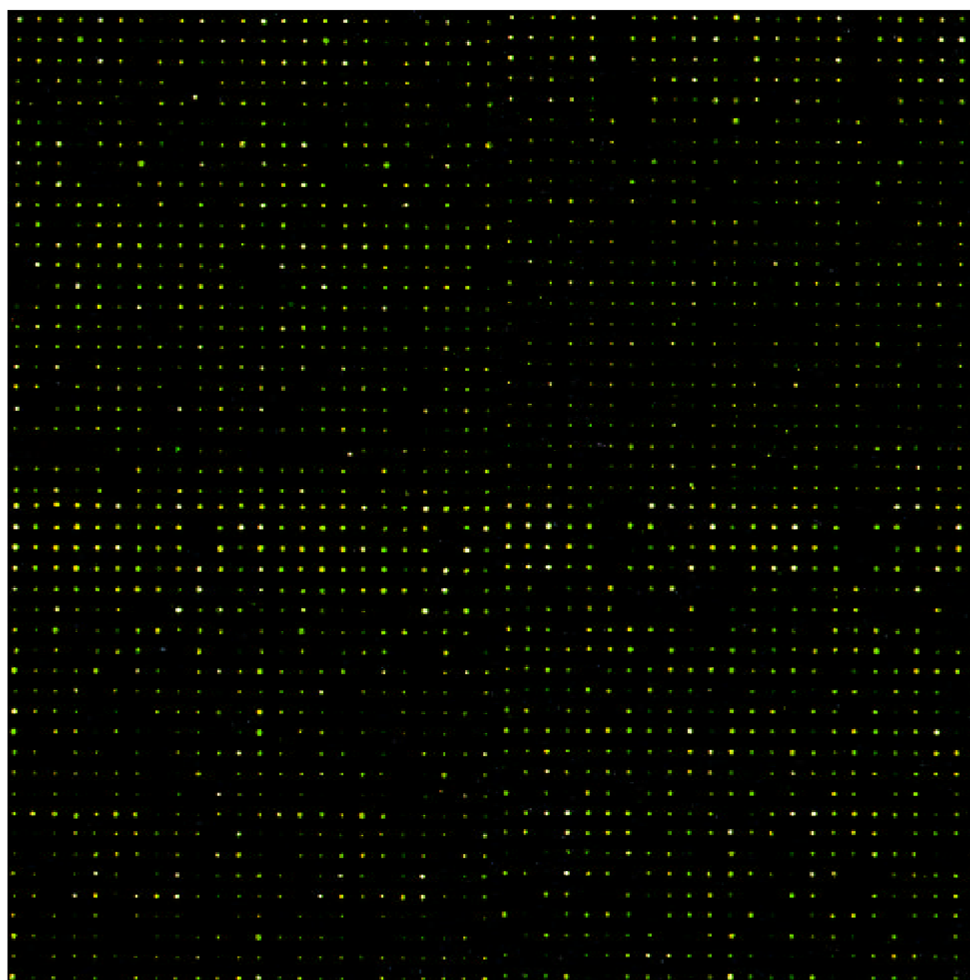

Fig 2. Scanning results of hybridizing signals on gene chip.

NM_002211(ITGB1); 3) cytoskeletal proteins: NM_006825 (CKAP4) and J04621 (SDC2); 4) other proteins: AB002360 (MCF2L), AF052108, AF052119, AL157424 (SYNJ2), L14561(ATP2B1), M55210 (LAMC1), and X80910 (PPP1CB).

DISCUSSION

During the last few years, several groups have investigated As_2O_3-regulated genes during As_2O_3-triggered apoptosis of APL cells. However, there was no systematic survey of gene expression regulation upon the effect of As_2O_3, and few genes were known to be associated with the differentiation of APL cells induced by As_2O_3. Only recently have innovative tools, such as cDNA microarray, allowed a more global approach of the analysis of transcriptional regulation in APL cells. As_2O_3 induce both apoptosis and partial differentiation of NB_4 cells at 0.5 μmol/L[1], so the genes related to apoptosis and partial differentiation could be modulated. In the present work, 3 genes were found to be up-regulated by As_2O_3 at 0.5 μmol/L, while 18 genes were down-regulated.

Among the 3 up-regulated genes, PSMB6 gene is involved in ubiquitin-proteasome degradation pathway. The proteasome is essential for the rapid elimination of highly abnormal proteins, arising via mutation or by post-translational damage[5]. The 26S proteasome is the key enzyme complex in the ubiquitin/ATP-dependent pathway of protein degradation. The catalytic core of this large complex is formed by the 20S proteasome. PSMB6 gene encodes a subunit of 20S complex. It is well known that the differentiation of NB_4 cells is associated with the degradation of PML/RARα and the reconstitution of the nuclear bodys (NBs). The proteasome degradation pathway is involved in the reconstitution of NB and the degradation of PML/RARα fusion protein induced by As_2O_3 or retinoic acid[6-8]. The findings in-

dicate that up-regulation of PSMB6 may be associated with the degradation of fusion protein and the NB reorganization and play a role in the differentiation of NB_4 cells. The proteasome can also modulate apoptosis by affecting the half-life of bcl-2 family members[9]. Whether PSMB6 gene plays a role in the apoptosis of NB_4 cells is still unclear.

According to the result from present work, a few of genes related to signal transduction were down-regulated, such as the ITGB1 gene encoding the integrin β1. The integrins comprise a large family of cell surface receptors involved in diverse adhesion and signaling events in animals. At least 20 different αβ heterodimeric integrins are important for processes such as embryonic morphogenesis, leukocyte migration, platelet aggregation, and regulation of cell proliferation and differentiation. Integrin β1 is a negative stimulus for differentiation[10]. The expression of β1-integrins decreased after ATRA-induced APL cell differentiation[11]. Activation of β1 integrins mediates proliferation and inhibits apoptosis of lymphocytes[12]. The antibody of integrin β1 can inhibit proliferation of lymphocyte[13]. Whether the down-regulation of integrin β1 leads to the differentiation of NB_4 cells is still unclear. RGS2 gene regulates G-protein signaling 2, and may play a role in leukemogenesis[14]. Wu *et al* studied differential expression of the RGS2 gene. They examined RGS2 expression in normal and malignant hematopoietic cells and found that it was expressed in 28 of 30 samples of acute myelogenous leukemia and in 9 of 11 samples of adult acute lymphoblastic leukemia, regardless of clinical classification. Furthermore, RGS2 mRNA was detected in all cases tested of chronic myelogenous leukemia (CML) in blast crisis; however, RGS2 transcript was not detected in CML patients in chronic phase or in normal bone marrow or other hematopoietic cells[14]. The down-regulation of RGS2 may be associated with the remission of leukemia.

Some genes related to RNA processing and protein synthesis were down-regulated. Ribosomes, the organelles that catalyze protein synthesis, consist of a small 40 S subunit and a large 60 S subunit. RPL7 (ribosomal protein L7) encodes a ribosomal protein that is a component of the 60 S subunit[15]. RPL17 (ribosomal protein L17) belongs to the l22 p family of ribosomal proteins[16]. The down-regulation of the genes encoding ribosomal proteins indicates that the synthesis of proteins may decrease. It is in accordance with the phenomenon that the growth of NB_4 cells was inhibited by As_2O_3. CARS (cysteinyl-tRNA synthetase) is involved in protein synthesis and RNA processing/modification[17]. The down-regulation of genes related to RNA processing and protein synthesis may be because of the down-regulation of many other genes after induction of the As_2O_3.

In summary, the genes found through the cDNA microarray provide some new clues for further studying of molecular mechanism of differentiation and apoptosis induced by As_2O_3. The ubiquitin-proteasome degradation pathway may play an important role in the differentiation of NB_4 cells. PSMB6 and ITGB1 genes may be involved in the differentiation and/or apoptosis of NB_4 cells induced by As_2O_3.

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