Analysis of triptolide-regulated gene expression in Jurkat cells by complementary DNA microarray[1]

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ABSTRACT

AIM: To investigate the global gene expression profile changes in Jurkat cells after triptolide treatment in order to find the possible triptolide targets. **METHODS:** Jurkat cells were treated with or without triptolide 10 μg/L for 2 h. Total RNA were isolated and used as templates for reverse transcriptional labeling of fluorescent cDNA probes. High density DNA microarray chips with a set of 13 872 human genes/Ests were used to generate the expression profile of triptolide-treated or untreated control Jurkat cells by hybridizing with fluorescent labeled probes. Array image was acquired and analyzed with array analyzing software GeneSpring. **RESULTS:** Triptolide significantly suppressed expression of 117 genes in Jurkat cells. Among these 117 genes, 30 % were Ests or genes without known functions, 13 % were transcription factors, 9 % were signal transduction pathway regulators, and 9 % were DNA binding proteins. Notably, the expression of mitogen-activated protein kinase kinase kinase kinase 5 (MAP kinase 5) and phosphoinositide-3-kinase (PI-3 kinase) was inhibited more than 100-fold. Moreover, the expression of genes involved in lipid transportation and metabolism was down-regulated by triptolide. **CONCLUSION:** High-density microarray provided an effective approach to identify drug targeting molecules. It is suggested that the widely known immune suppressive and antitumor effects of triptolide were mediated at least in part by suppression of MAP kinase and PI-3 kinase gene expression.

INTRODUCTION

Triptolide is an effective ingredient of the Chinese herb *Tripterygium wilfordii* Hook F (TWHF), which has been historically used in traditional Chinese medicine for the treatment of rheumatoid arthritis, chronic nephritis, and other pulmonary diseases[1,2]. TWHF is a multi-functional drug and has been reported to have immunosuppressive and anti-inflammatory[1-3], antitumor[4-7], and antifertility activities[8]. Recently, the molecular mechanism of the immunosuppressive and anti-inflammatory effects of TWHF has been investigated. It had been found that TWHF inhibited IL-2 production by activated T-cells via a pathway that is different from cyclosporin A[9]. TWHF also suppressed IL-2 receptor α expression induced by phorbol 12-myristate 13-acetate and CD40 ligand expression induced by ionomycin[10]. Triptolide, a chloroform/methanol extract of TWHF, inhibits mitogen or antigen-induced proliferation of human peripheral blood T-cells and B-cells, IL-2 production by T-cells and immunoglobulin production by B cells[11]. Triptolide has a potent inhibitory

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effect on the clonogenic response of human bone marrow cells to exogenous hematopoietic growth factors by suppression of the activation of NF-κB[12]. Furthermore, triptolide induces apoptosis of T-cell hybridomas and peripheral T-cells by increasing caspase activity[3]. Several groups had reported that TWHF inhibited tumor cell growth *in vitro* and *in vivo*[5,7]. Triptolide reduced the number of soft-agar clone formation by more than 70 % in breast cancer MCF-7 and BT-20 cells, stomach cancer MKN-45 and MKN-7 cells[7]. Triptolide inhibits cell growth, induces apoptosis, and suppresses NF-κB and AP-1 transactivation in gastric cancer through the p53 pathway[5]. Though the above-mentioned studies shed some lights on triptolide actions, there are still many gaps left and hard to explain why triptolide has such a broad biological functions.

Recently, complementary DNA (cDNA) microarray has been used to identify gene expression patterns in a variety of organisms. cDNA microarray provides an efficient method to analyze gene expression profile with a high throughput of thousands of genes in parallel. In contrast to the traditional molecular techniques that focus on one to a few genes at a time, cDNA microarray allows gene expression patterns to be analyzed on a genomic scale[13]. In this study, the global gene expression changes in Jurkat T-cells treated with triptolide were investigated by this modern high density microarray technique.

MATERIALS AND METHODS

Drug treatment Triptolide was first dissolved in Me_2SO (0.1 g/L) and then diluted with culture medium to 1 mg/L. The acute T-cell leukemia cell line Jurkat cells were obtained from the American Type Culture Collection (ATCC) and were maintained in RPMI-1640 supplemented with 10 % fetal bovine serum. Cells were grown at 37 ºC in a humidified 5 % CO_2 atmosphere. Triptolide was added to the medium in a final concentration of 10 μg/L for 2 h at the cell concentration of 2×10^9 /L. Cells treated with the same concentration Me_2SO were used as controls. Both cells were centrifuged at 300×*g* for 5 min. The pellets were used for total RNA isolation.

RNA isolation Total RNA was extracted using standard Trizol RNA isolation protocol (Life Technologies, Inc, Grand Island, NY). Briefly, 1 mL of Trizol reagent was mixed with Jurkat cell pellet per 10^7 cells by repeated pipetting. DNA and protein were excluded by chloroform phase separation. RNA in the aqueous phase was precipitated with isopropanol and then resuspended in DEPC water.

Reverse-transcriptional labeling The first strand cDNA synthesis was performed with CyScribe first strand cDNA labeling kit (Amersham Pharmacia Biotech). Briefly, 25 μg of total RNA was denatured in the presence of 1 μL of anchored oligo dT(18)VN primer in 10 μL at 70 ºC for 3 min followed by quenching on ice. The RNA from both control and triptolide samples were reciprocally labeled with Cy3-dCTP and Cy5-dCTP separately in a volume of 20 μL in the presence of 1× CyScript buffer, DTT 10 mmol/L, 1 μL dCTP nucleotide mix, 1 μL Cy3/Cy5-dCTP and 1 μL CyScript reverse transcriptase. The reaction was carried on at 42 ºC for 2 h in water bath in the dark. To degrade RNA, 2 μL of NaOH 2.5 mol/L was added into labeling reactions and incubated at 37 ºC for 15 min. NaOH in the solution was neutralized with 10 μL of HEPES 2 mol/L free acid. The labeling products were purified with PCR purification columns (Qiagen, Hilden, Germany).

Microarray hybridization Cy3- and Cy5-labelled cDNA were dried in a speedvac concentrator in amber microcentrifuge tubes and resuspended in 11 μL of nuclease free water. Having been denatured by heating at 95 ºC for 2 min, the labeled cDNA was incubated with 1 μL of polyA 5 g/L, 3 μL of human Cot-1 DNA 10 g/L at 75 ºC for 45 min. Following incubation, 15 μL of 4×microarray hybridization buffer and 30 μL of 100 % (v/v) formamide were mixed to the above solution and then gently applied onto the microarray slides on which 14 000 oligoes corresponding to known genes and EST were printed in duplicates (Operon, Alameda, California, USA) and covered with a cover slip. Hybridization was performed in a humid hybridization chamber at 42 ºC for 14-18 h. After hybridization, the slides were first washed with 1×SSC with 0.2 % SDS at RT for 10 min using a rotatory shaker, followed by washing with 0.1×SSC with 0.2 % (w/v) SDS twice at RT for 10 min. After final wash in distilled water for 10 s, the slides were dried with gentle air stream and scanned with a microarray scanner.

Array quantification and data process Cy3 and Cy5 images were acquired separately. Each spot was defined by manually positioning a grid of circles over the array image. For each fluorescent image, the average pixel intensity within each circle was determined. The local background was computed for each spot equal to the median pixel intensity in a circle of 5 pixels in

width around the signal area. Net signal was determined by subtraction of this local background from the average intensity for each spot. Spots deemed that they were unsuitable for accurate quantification because array artifacts were manually flagged and excluded from further analysis. Signal intensities were first normalized by dividing all intensities measured with the median fluorescence intensity (MFI) in the same channel, and then the computed intensity of each spot was further divided by mean of the same spot from two different channels. This effectively defined the signal intensity-weighted "average" spot on each array to have a ratio of 1.0. To minimize the possibility of fault positive, only those genes with a signal intensity ≥300 and the standard division between duplicated spots <1 were used for further consideration. A gene was considered to be differentially expressed when the difference of normalized MFI between the two fluorochromes was more than 5-fold in two separated experiments with reciprocally labeled cDNA. Except the expressed sequence tags, the differentially expressed genes were further divided into groups based on their molecular functions and biological processes reported in the literature.

Statistical analysis The calculation of fluorescence intensity, standard division of duplicated spots and normalization between different arrays were performed by specialized array analyzing software GeneSpring from Silicon Genetics.

RESULTS

Global expression analysis of genes regulated by triptolide The overall expression of the 13 872 genes in Jurkat cells was shown (Fig 1). A representative partial array image was shown (Fig 2). The fluroscence intensity was correlated with the brightness of spots. Each gene/Est was deposited as duplicate for statistic analysis and quality control. The arrowhead indicated differentially expressed gene in untreated (Fig 2A) and triptolide-treated Jurkat cells (Fig 2B). There 547 (4 %) genes were expressed in a high abundance (the signal fluorescence intensity ranges from 1000 to 64 000), 7072 (51 %) genes were expressed from low to intermediate high level (fluorescence intensity ranges from 100 to 1000), 6253 genes (45 %) were either not expressed or in the margin level of expression (fluorescence intensity less than 100).

Genes suppressed by triptolide Among the 13872 genes/Ests in the array set, 117 were identified as triptolide-suppressed genes/Ests which showed decreased expression of at least 5-fold in triptolide-treated Jurkat cells than control. Among those, the expressions of 20 genes/Ests were inhibited more than 100-fold (Tab 1). Based on their molecular functions, these 117 genes were classified into 21 different groups (Fig 3A). The group with most genes (34 genes, 29 % of total suppressed genes) did not have reported functional data and classified as molecular function unknown. Other groups were transcription factors (15 genes, 13 %), nucleic acid binding proteins (11 genes, 9 %), and regulatory molecules (11 genes, 6 %). Some examples of the genes in the above mentioned last three groups include U33761 S-phase kinase-associated protein 2 (p45), U77129 mitogen-activated protein kinase kinase kinase kinase 5, X77794 cyclin G1, and S67334 phosphoino-sitide-3-kinase. In accordance with classification by molecular function, the 171 triptolide-suppressed genes/Ests had similar distribution as classified by biological process (Fig 3B). Thirty-three genes/Ests belonged to a group of protein without reported biological functions. The other 83 genes were grouped into 21 different subgroups according to the biological processes. In these subgroups, most of the genes (28 genes, 24 % of total) belong to molecules that were involved in nucleoside, nucleotide and nucleic acid metabolism including DNA replication, mRNA transcription, and pre-mRNA processing. Other subgroup genes were protein metabolism and modification (9 genes, 8 %),

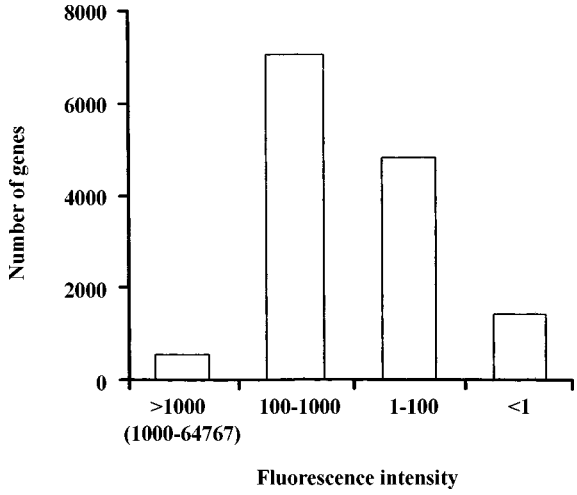

Fig 1. Global expression of genes in Jurkat cells. The expression level of 13 872 genes in Jurkat cells as indicated by fluorescence intensity by array analysis. About half were expressed at an intermediate level (100-1000 pixels), half were expressed at low level or not expressed at all. Less than 4 % genes were high abundant in transcript.

Tab 1. Triptolide-suppressed genes.

Accession # and name	Molecular function	Biological process	Fold
AB033073 similar to glucosamine-6-sulfatases	Hydrolase	Carbohydrate metabolism	>100
Z82022 dolichyl-phosphate	Transferase	Protein metabolism and modification	>100
U33761 S-phase kinase-associated protein 2 (p45)	Select regulatory molecule	Cell proliferation and differentiation	>100
AK000459 hypothetical protein FLJ20452	Molecular function unknown	Biological process unknown	>100
U46691 suppressor of Ty (S cerevisiae) 6 homolog	Transcription factor	Nucleoside and nucleic acid metabolism	>100
U77129 mitogen-activated protein kinase kinase kinase kinase 5	Kinase	Signal transduction	>100
U76638 BRCA1 associated RING domain 1	Select regulatory molecule	Apoptosis	>100
D85429 heat shock 40 kDa protein 1	Chaperone	Protein metabolism and modification	>100
AL080110 Homo sapiens mRNA	Molecular function unknown	Biological process unknown	>100
X76534 glycoprotein (transmembrane) nmb	Molecular function unknown	Biological process unknown	>100
AF151030 hypothetical protein	Molecular function unknown	Biological process unknown	>100
S67334 phosphoinositide-3-kinase_ catalytic_ beta	Kinase	Signal transduction	>100
D87438 KIAA0251 protein	Molecular function unknown	Biological process unknown	>100
AK000808 Homo sapiens cDNA FLJ20801	Molecular function unknown	Biological process unknown	>100
AB006630 transcription factor 20 (AR1)	Transcription factor	Nucleoside and nucleic acid metabolism	>100
U21092 TNF receptor-associated factor 3	Select regulatory molecule	Apoptosis	>100
U90919 Human clones 23667 and 23775	Molecular function unknown	Biological process unknown	>100
X77794 cyclin G1	Select regulatory molecule	Cell cycle	>100
NM_000754 catechol-*O*-methyltransferase	Transferase	Neuronal activities	>100
AL137330 Homo sapiens mRNA	Molecular function unknown	Biological process unknown	>100
U74612 forkhead box M1	Transcription factor	Nucleoside and nucleic acid metabolism	59.5
L41162 collagen_ type IX_ alpha 3	Extracellular matrix	Cell adhesion	44.2
U20180 iron-responsive element binding protein 2	Nucleic acid binding	Nucleoside and nucleic acid metabolism	43.6
X87237 glucosidase I	Hydrolase	Carbohydrate metabolism	42.5
AK002011 hypothetical protein FLJ11149	Molecular function unknown	Biological process unknown	42.3
L04490 NADH dehydrogenase (ubiquinone) 1 alpha	Oxidoreductase	Electron transport	41.0
L07633 proteasome activator subunit 1 (PA28 alpha)	Protease	Protein metabolism and modification	35.2
L10910 splicing factor (CC1.3)	Nucleic acid binding	Nucleoside and nucleic acid metabolism	23.5
NM_002101 glycophorin C (Gerbich blood group)	Miscellaneous function	Blood circulation and gas exchange	23.0
AF053070 NADH dehydrogenase flavoprotein 1 (51 kDa)	Oxidoreductase	Electron transport	22.1
M31642 hypoxanthine phosphoribosyltransferase 1	Transferase	Coenzyme and prosthetic group metabolism	20.6
D89859 zinc finger protein homologous to Zfp161	Select regulatory molecule	Biological process unknown	19.9
AF034544 7-dehydrocholesterol reductase	Oxidoreductase	Lipid, fatty acid and steroid metabolism	19.1
AF043644 protein tyrosine phosphatase	Phosphatase	Signal transduction	17.8
M16591 hemopoietic cell kinase	Kinase	Signal transduction	17.4
AK000087 hypothetical protein FLJ20080	Molecular function unknown	Biological process unknown	16.7
AL031778 nuclear transcription factor Y_ alpha	Transcription factor	Nucleoside and nucleic acid metabolism	16.2
D90359 TATA box binding protein associated factor	Transcription factor	Nucleoside and nucleic acid metabolism	15.9

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Accession # and name	Molecular function	Biological process	Fold
NM_014420 Dickkopf gene 4	Select regulatory molecule	Developmental processes	15.0
M13755 interferon-stimulated protein_ 15 kDa	Chaperone	Protein metabolism and modification	14.9
D49396 antioxidant protein 1	Oxidoreductase	Electron transport	14.3
X13293 v-myb avian myeloblastosis viral oncogene	Viral protein	Oncogenesis	13.3
AL137406 translocase of inner mitochondrial membrane	Molecular function unknown	Biological process unknown	13.2
AK002051 Homo sapiens cDNA FLJ11189	Molecular function unknown	Biological process unknown	13.1
AF026291 chaperonin containing TCP1 subunit 4	Chaperone	Intracellular protein traffic	12.7
AL021395 Human DNA sequence	Molecular function unknown	Biological process unknown	12.7
NM_005258 GTP cyclohydrolase I feedback regulatory protein	Select regulatory molecule	Lipid, fatty acid and steroid metabolism	12.3
D85758 enhancer of rudimentary (Drosophila) homolog	Molecular function unknown	Nucleoside and nucleic acid metabolism	12.3
X69550 Rho GDP dissociation inhibitor (GDI) alpha	Signaling molecule	Signal transduction	11.9
AK001749 hypothetical protein FLJ10450	Molecular function unknown	Biological process unknown	11.8
NM_000055 butyrylcholinesterase	Synthase and synthetase	Neuronal activities	11.7
M64098 high density lipoprotein binding protein (vigilin)	Transfer/carrier protein	Transport	10.7
AF038406 NADH dehydrogenase	Oxidoreductase	Electron transport	10.7
J03626 uridine monophosphate synthetase	Synthase and synthetase	Nucleoside and nucleic acid metabolism	10.6
D50918 KIAA0128 protein; septin 2	Cytoskeletal protein	Cell structure and motility	9.5
U65590 interleukin 1 receptor antagonist	Defense/immunity protein	Immunity and defense	9.5
U32944 dynein_ cytoplasmic_ light polypeptide	Cytoskeletal protein	Cell structure and motility	9.4
AL049650 small nuclear ribonucleoprotein polypeptides	Nucleic acid binding	Nucleoside and nucleic acid metabolism	9.3
AF039029 snuportin-1	Transfer/carrier protein	Transport	9.1
U94831 transmembrane 9 superfamily member 1	Molecular function unknown	Biological process unknown	8.9
D21260 clathrin_ heavy polypeptide (Hc)	Cytoskeletal protein	Cell structure and motility	8.7
NM_013349 secreted protein of unknown function	Molecular function unknown	Biological process unknown	8.7
AL021878 cytochrome P450_ subfamily IID	Molecular function unknown	Biological process unknown	8.6
AJ007509 E1B-55 kDa-associated protein 5	Transcription factor	Nucleoside and nucleic acid metabolism	8.4
AL050341 zinc metalloproteinase_ STE24	Protease	Protein metabolism and modification	8.3
AB014560 Ras-GTPase activating protein	Select regulatory molecule	Signal transduction	8.3
AB007935 immunoglobulin superfamily_ member 3	Defense/immunity protein	Immunity and defense	8.1
D14710 ATP synthase_ H+ transporting_ mitochondria	Oxidoreductase	Electron transport	8.0
NM_006755 transaldolase 1	Transferase	Carbohydrate metabolism	8.0
U44378 MAD (mothers against decapentaplegic)	Transcription factor	Nucleoside and nucleic acid metabolism	7.9
AK000451 Homo sapiens cDNA FLJ20444	Molecular function unknown	Biological process unknown	7.5
AF218818 nudix (nucleoside diphosphate linked moiety X)	Hydrolase	Nucleoside and nucleic acid metabolism	7.5
AJ245416 Homo sapiens mRNA for G7b protein	Molecular function unknown	Biological process unknown	7.4
AK002079 Homo sapiens cDNA FLJ11217	Molecular function unknown	Biological process unknown	7.3
AF055006 similar to *S. cerevisiae* Sec6p	Molecular function unknown	Biological process unknown	7.2
AK000672 CGI-90 protein	Molecular function unknown	Biological process unknown	7.1
M14170 alkaline phosphatase_ placental	Phosphatase	Lipid, fatty acid and steroid metabolism	7.0
AK001052 Homo sapiens cDNA FLJ10190	Molecular function unknown	Biological process unknown	6.6

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Accession # and name	Molecular function	Biological process	Fold
AC005263 splicing factor 3a_ subunit 2_ 66 kDa	Nucleic acid binding	Nucleoside and nucleic acid metabolism	6.6
D26599 proteasome subunit_ beta type_ 2	Protease	Protein metabolism and modification	6.5
NM_013242 similar to mouse Glt3	Transcription factor	Nucleoside and nucleic acid metabolism	6.4
NM_006600 nuclear distribution gene C homolog	Membrane traffic protein	Protein targeting and localization	6.4
AK001809 HSPC182 protein	Molecular function unknown	Biological process unknown	6.2
AJ132592 zinc finger protein 281	Transcription factor	Nucleoside and nucleic acid metabolism	6.2
AJ276003 GAR1 protein	Nucleic acid binding	Nucleoside and nucleic acid metabolism	6.2
AJ000098 eyes absent (Drosophila) homolog 1	Molecular function unknown	Developmental processes	6.1
NM_012087 general transcription factor IIIC	Transcription factor	Nucleoside and nucleic acid metabolism	6.1
AJ001810 pre-mRNA cleavage factor Im (25 kDa)	Nucleic acid binding	Nucleoside and nucleic acid metabolism	6.1
U09953 ribosomal protein L9	Nucleic acid binding	Nucleoside and nucleic acid metabolism	6.1
AF001891 zinc finger protein-like 1	Transcription factor	Nucleoside and nucleic acid metabolism	6.0
X71661 lectin_ mannose-binding_ 1	Membrane traffic protein	Protein targeting and localization	5.9
NM_005090 phospholipase A2_ group IVB (cytosolic)	Signaling molecule	Lipid, fatty acid and steroid metabolism	5.9
AF000984 DEAD/H (Asp-Glu-Ala-Asp/His) box	Nucleic acid binding	Nucleoside and nucleic acid metabolism	5.9
NM_006811 tumor differentially expressed 1	Molecular function unknown	Biological process unknown	5.8
U73824 eukaryotic translation initiation factor 4 gamma_ 2	Nucleic acid binding	Protein metabolism and modification	5.8
D42041 KIAA0088 protein	Molecular function unknown	Biological process unknown	5.8
U82130 tumor susceptibility gene 101	Chaperone	Protein metabolism and modification	5.7
D55654 malate dehydrogenase 1_ NAD (soluble)	Oxidoreductase	Electron transport	5.7
L41067 nuclear factor of activated T-cells_ cytoplasmic 3	Transcription factor	Nucleoside and nucleic acid metabolism	5.7
AF042081 SH3-binding domain glutamic acid-rich protein	Oxidoreductase	Electron transport	5.6
AK001901 uncharacterized hypothalamus protein HT010	Molecular function unknown	Biological process unknown	5.5
Y15724 ATPase_ Ca^{++} transporting_ ubiquitous	Ion channel	Transport	5.5
M63959 low density lipoprotein-related protein	Select regulatory molecule	Lipid, fatty acid and steroid metabolism	5.5
AB014563 KIAA0663 gene product	Molecular function unknown	Biological process unknown	5.5
AL110164 Homo sapiens mRNA	Molecular function unknown	Biological process unknown	5.4
U10061 POU domain_ class 4_ transcription factor 3	Transcription factor	Nucleoside and nucleic acid metabolism	5.3
L03411 RD RNA-binding protein	Nucleic acid binding	Nucleoside and nucleic acid metabolism	5.3
X05276 tropomyosin 4	Cytoskeletal protein	Muscle contraction	5.3
D83781 KIAA0197 protein	Molecular function unknown	Biological process unknown	5.3
NM_006717 spindlin	Select regulatory molecule	Cell cycle	5.2
AC005154 Homo sapiens PAC clone DJ0777O23	Molecular function unknown	Biological process unknown	5.2
AC002544 eukaryotic translation initiation factor 3	Nucleic acid binding	Protein metabolism and modification	5.2
U29175 SWI/SNF regulator of chromatin_ subfamily 4	Transcription factor	Nucleoside and nucleic acid metabolism	5.2
U66617 SWI/SNF regulator of chromatin_ subfamily d	Transcription factor	Nucleoside and nucleic acid metabolism	5.2
X54199 phosphoribosylglycinamide formyltransferase	Synthase and synthetase	Nucleoside and nucleic acid metabolism	5.1
AK001548 GTP-binding protein	Signaling molecule	Signal transduction	5.1
AL033379 Human DNA sequence from clone 417O22	Molecular function unknown	Biological process unknown	5.1

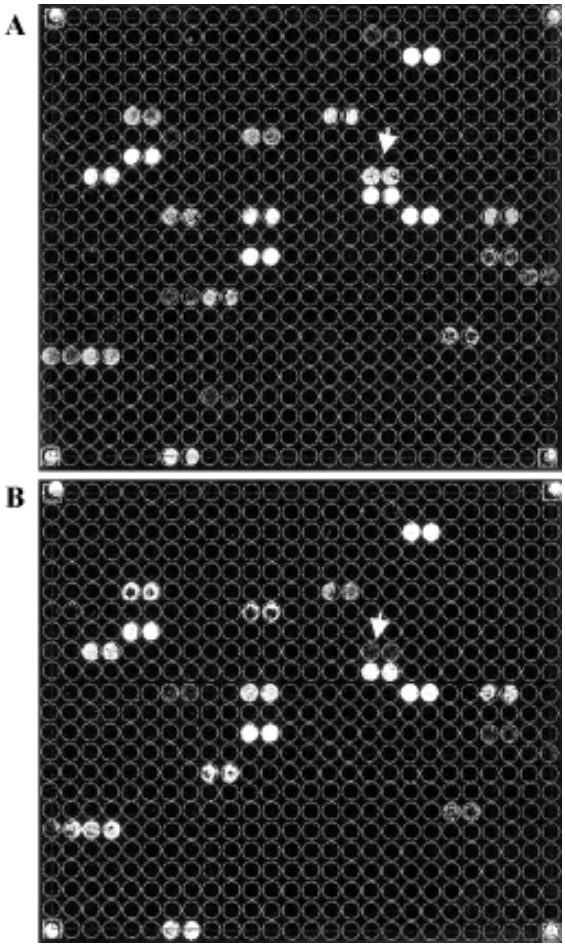

Fig 2. A representative partial microarray image from control cDNA (A) and triptolide-treated cDNA (B). Brightness of spots corresponding to fluorescence intensity and abundance of gene expression. ↓ indicate the genes that were differentially expressed by two samples.

electron transport (7 genes, 6 %), and signal transduction (7 genes, 6 %).

DISCUSSION

Triptolide had been reported to be a multifunctional compound that could induce apoptosis of T-lymphocytes[3], prostate epithelial cells[4], breast and gastric cancer cells[5,7]. It also had profound effects on male fertility, immune and inflammation[1,14,15]. Here, we reported the molecular profile changes of triptolide treatment on acute human T-cell leukemia cell line Jurkat cells by high density DNA array.

Our present results show that there are at least half of transcripts among the 13 872 genes/Ests represented in the array set expressed in Jurkat cells (Fig 1). Among those expressed transcripts, 171 genes/Ests were shut off or down-regulated at least 5-folds by triptolide in Jurkat cells. Some of the most significantly regulated genes (more than 100 times lower in triptolide-treated cells, Tab 1), U33761 S-phase kinase-associated protein 2 (p45), U77129 mitogen-activated protein kinase kinase kinase kinase 5, S67334 phosphoinositide-3-kinase, and X77794 cyclin G1 were suppressed more than 100-fold by triptolide. All these genes are well-known signal transduction pathway components and involved critically in a broad spectrum of biological process such as cell cycle, gene transcription, cell survival and cell death. Down-regulation of these molecules provided a new insight for the molecular mechanisms of triptolide-induced cell death, antifertility and immunsuppression. Triptolide treatment may also influence chromatin structure by inhibiting genes that either directly interact with or regulate chromatin remodulation complex such as U76638 BRCA1-associated RING domain 1 and U29175 SWI/SNF regulator of chromatin. Another class of genes suppressed by triptolide were oxidoreductase and electron transport protein, namely AF053070 NADH dehydrogenase flavoprotein, AF038406 NADH dehydrogenase, and AL021878 cytochrome P450. It remains to be illustrated whether this is the direct effects of triptolide or resulted from reduced metabolic activity of triptolide-treated cells. In addition to the suppressive effects, triptolide also activated or up-regulated gene transcription. The 43 genes/Ests were up-regulated, however most of them are either molecular function unknown or biological procession unknown. The few known genes, U27266 myosin-binding protein H, AJ238098 Sm protein F, Z80782 H2B histone family member K may represent non-specific cell response to triptolide treatment (data not shown).

In accordance with previous reports, our data indicated that the inhibitory effects of triptolide on immune responses were at least partially mediated by suppressing T-cell activation (L41067 nuclear factor of activated T-cells), reducing production of immunoglobulin (AB007935 immunoglobulin superfamily), and inhibiting interferon pathway (M13755 interferon-stimulated protein)[9]. In spite of the immune suppressive effect, triptolide may also affect lipid metabolism. At least 3-lipid and cholesterol metabolism related genes (AF034544 7-dehydrocholesterol reductase, M64098 high density lipoprotein binding protein, M63959 low density lipoprotein-related protein) were shown to be down-regulated in our experiment. The significance of this discovery deserves further investigation.

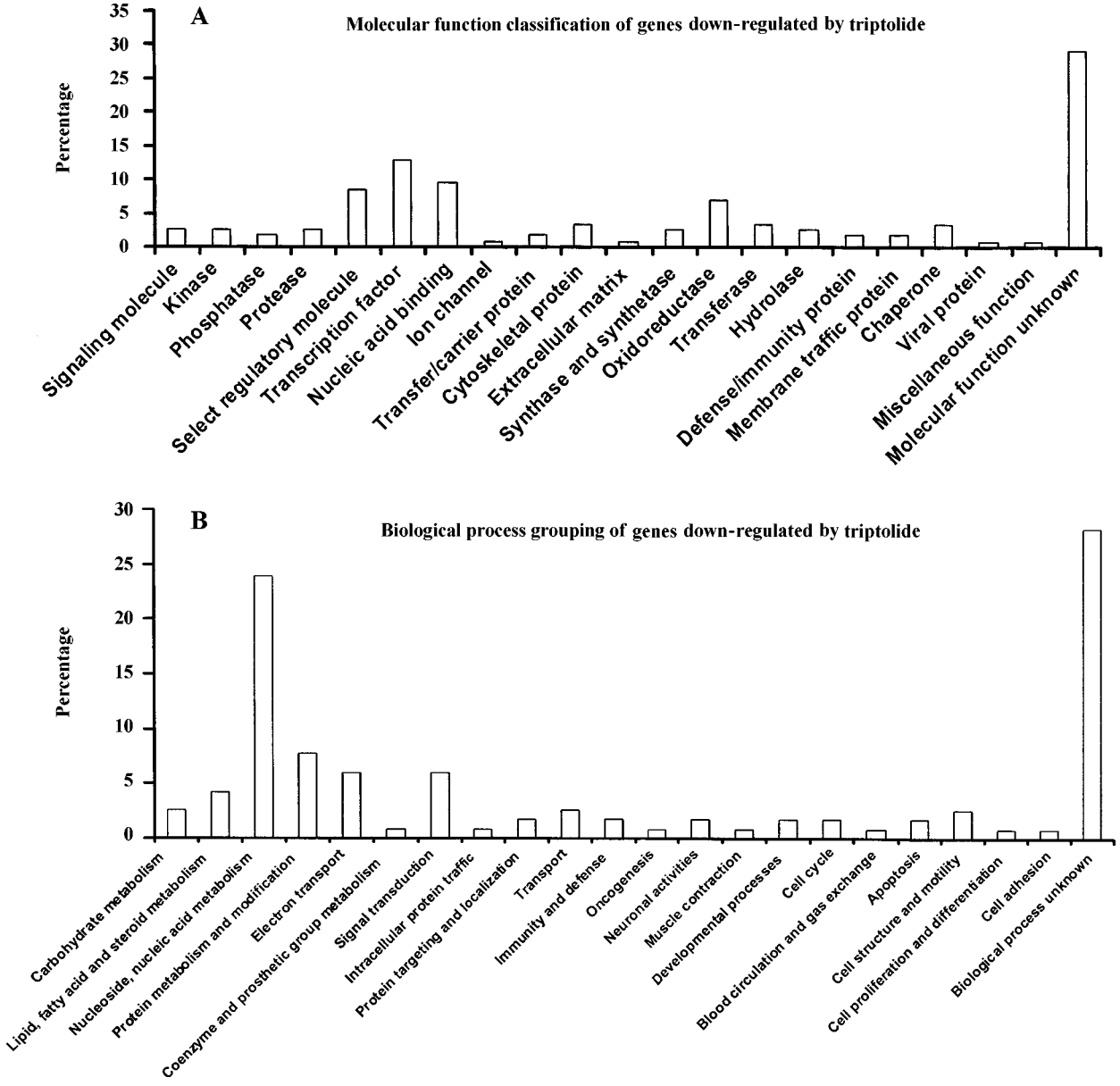

Fig 3. Distribution of genes down-regulated by triptolide according to their molecular function or biological process. A) The 117 genes down-regulated by triptolide were subgrouped into 21 different classes according to their molecular functions reported. B) The same genes were subgrouped into 22 different classes according to the biological process.

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