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IL-18 gene expression pattern in exogenously treated AML cells

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IL-18 production may enhance immune system defense against KG-1 cells ; NB4 cells, which are associated with good prognosis, do not produce IL-18. In this study, we treated KG-1 cells with IL-18 and used microarray technology to assess subsequent effects on gene expression. In UniGene-array of 7488 human genes, expression of 57 genes, including stress related genes, increased at least 2-fold, whereas expression of 48 genes decreased at least 2-fold. Following exogenous exposure of KG-1 cells to IL-18, expression of CRYGC, NFκBIA and NACA gene were monitored. The latter is a transcriptional coactivator potentiating c-Jun-mediated transcription. NFκBIA is an inhibitor of NFκB, and affects growth regulation, apoptosis and hypoxic stress. Studies, such as this one, are beginning to clarify the differences between cells associated with good and bad cancer prognoses, which may ultimately assist in medical treatment for acute myeloid leukemia. [BMB reports 2008; 41(6): 461-465]

INTRODUCTION

Cytokine/cytokine receptors (C/CR) affect leukemic cell growth and survival (1). The goal of this study was to investigate possible correlations between gene expression patterns of C/CR, clinical features, and outcome in leukemic cells.

In hematopoiesis, physiological cell death (apoptosis) can be induced by immunoregulatory cytokines. Interleukin (IL)-18, originally called IFN-r inducing factor (IGIF), is a novel proinflammatory cytokine that is produced by activated macrophages and Kupper cells. IL-18 has been linked to the ability of cancers cells to evade immune responses of the host (2). Consistent with this, a marked difference in IL-18 gene expression has been obtained in high risk and intermediate risk groups (2). For example, IL-18 is produced in KG-1 cells, an acute myeloid leukemia (AML) cell line which is associated with poor prognosis. In contrast, IL-18 is not produced in AML cell line (NB4) which is associated with a more optimistic prognosis (3). Similar to matrix metalloproteinases (MMP)-9 and -2, IL-18 is over-expressed in some hematologic malignancies such as AML, precluding a poor clinical outcome (4). Furthermore, IL-18 induces the maturation of these cells towards a dendritic phenotype (5) and is able to mediate interferon (IFN) gamma production (5).

IL-18 function(s) can be suppressed by the inhibition of p38 mitogen-activated protein kinase (MAPK) activity and nuclear factor-kappaB (NF-κB) function (5). Specific suppression of T-bet induction impairs the secretion of IFN gamma by KG-1 cells under the influence of IL-18, and therapeutic application of IL-18 has the potential to profoundly affect the biology of AML predendritic cells, such as KG-1 (5). IL-18 is an important regulator of both innate and acquired immune responses. The aberrant expression of IL-18 is associated with severe inflammatory conditions such as autoimmune diseases and allergies (6). Based on their potent effects in up-regulating inflammatory responses such as the induction of IFN-γ and the augmentation of cytotoxic activity in natural killer (NK) and CD8 T cells, IL-18 and -12 have been tested for use in tumor immunotherapy. IL-18 activity against various tumor cell lines in vivo has been reported (7).

To examine the effect of IL-18 in KG-1 cells, cultured cells were treated with human IL-18, while the expressions of three genes (CRYGC, NFκBIA and NACA) were monitored. CRYGC-gamma-crystallins are involved in cataract formation (8). NFκBIA is an inhibitor of NF-κB as a consequence of binding to the Rel domain of NF-κB. This inhibition affects growth regulation, apoptosis and hypoxic stress (9). NACA is a human brain **n**ascent polypeptide **a**ssociated **c**omplex **a**lpha subunit that functions as a transcriptional coactivator potentiating c-Jun-mediated transcription (10).

In this study, we assessed the influence of IL-18 in KG-1 AML cell line. KG-1 cells were exogenously treated with IL-18 and the resultant gene expression pattern changes were assessed using microarray analysis, reverse transcription-polymerase chain reaction (RT-PCR) and Western blot.

RESULTS

Gene profiling in IL-18 treated and untreated KG-1 cells

Expression levels of 57 genes, including stress related genes, increased at least 2-fold, whereas expression of 48 genes decreased at least 2-fold in IL-18-treated KG-1 cells (Table 1).

Effect of Il-18 on gene expression in KG-1 cells

As shown in Fig. 1, secretion of human INF-γ in IL-18-treated

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Table 1. List of relatively up- and down-regulated genes in IL-18 treated KG-1 cells

	Up and down regulated gene	Genbank ID number	Fold ratio
Up	Crystallin, gamma C	NM_020989	29.71459886
	Protein kinase H11; small stress protein-like protein HSP22	NM_014365	24.57296432
	Histone deacetylase 3	NM-003883	5.028750583
	Glutamate receptor, metabotropic 7	NM_181874	4.789582818
	Zinc finger protein 143 (clone pHZ-1)	NM_003442	4.006104348
	Stomatin (EBP72)-like 1	NM_004809	3.993628126
	v-fos FBJ murine osteosarcoma viral oncogene homolog	NM-005252	3.791319396
	Palmitoyl-protein thioesterase 2	NM_138717	3.761218189
	Spondin 1, (f-spondin) extracellular matrix protein	NM_006108	3.650261954
	Checkpoint suppressor 1	NM_005197	3.500351979
	Beta-2-microglobulin	NM_004048	3.373859744
	Regulatory factor X, 5 (influences HLA class II expression)	NM_000449	3.324644308
	Leukemia-associated phosphoprotein p18 (stathmin)	NM_203399	3.263228472
	Ribosomal protein S11	NM_001015	3.26300229
	Neuritin	NM_016588	3.035007328
Down	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NM_020529	0.482165389
	Fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase, Bombay phenotype included)	NM_000148	0.478270927
	Carbonic anhydrase XI	NM_001217	0.354338467
	Papillary renal cell carcinoma (translocation-associated)	NM_005973	0.422463908
	Epidermal growth factor receptor pathway substrate 8	NM_004447	0.448972815
	COX17 (yeast) homolog, cytochrome c oxidase assembly protein	NM_005694	0.380323766
	Syntrophin, beta 1 (dystrophin-associated protein A1, 59kD, basic component 1)	NM_021021	0.340658946
	CD81 antigen (target of antiproliferative antibody 1)	NM_004356	0.490117648
	Homeo box D8	NM_019558	0.476550167
	DEK oncogene (DNA binding)	NM_003472	0.471490301
	Enoyl Coenzyme A hydratase 1, peroxisomal	NM_001398	0.400035517
	Keratin, hair, acidic,1	NM_002277	0.363946930
	Small inducible cytokine subfamily A (Cys-Cys), member 18, pulmonary and activation-regulated	NM_002988	0.478005790
	Transmembrane 7 superfamily member 1 (upregulated in kidney)	XM_514290	0.401229615
	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	NM_005084	0.472046210
	Phospholipase D1, phophatidylcholine-specific	NM_002662	0.416734371
	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1 (6kD, KFYI)	NM_002494	0.444821985
	Nascent-polypeptide-associated complex alpha polypeptide	NM_005594	0.468753056

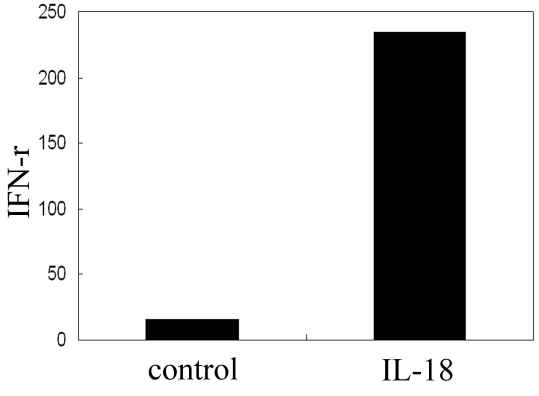

Fig. 1. Confirmation of IL-18 treatment using human INF-γ ELISA. The secretion of human INF-γ in IL-18-treated KG-1 cells increased 15 times more than that of IL-18-untreated KG-1 cells.

KG-1 cells increased 15 times more than that of untreated KG-1 cells. From the up-regulated and down-regulated genes in microarray analyses, three particularly responsive genes were selected (CRYGC, NFκBIA and NACA). Their expression was confirmed using RT-PCR. In IL-18-treated KG-1 cells, expression of CRYGC increased three times more than that of untreated cells, while expressions of NFκBIA and NACA decreased (Fig. 2).

Down-regulation of NFκBIA after IL-18 treatment

We confirmed the expression of NFκBIA at the level of protein production. To monitor the changes in gene expression, we harvested cells at various times following exposure to IL-18. NFκBIA protein production was elevated 24 hrs after IL-18 treatment, but decreased by 48 hrs (Fig. 3), consistent with the RT-PCR results.

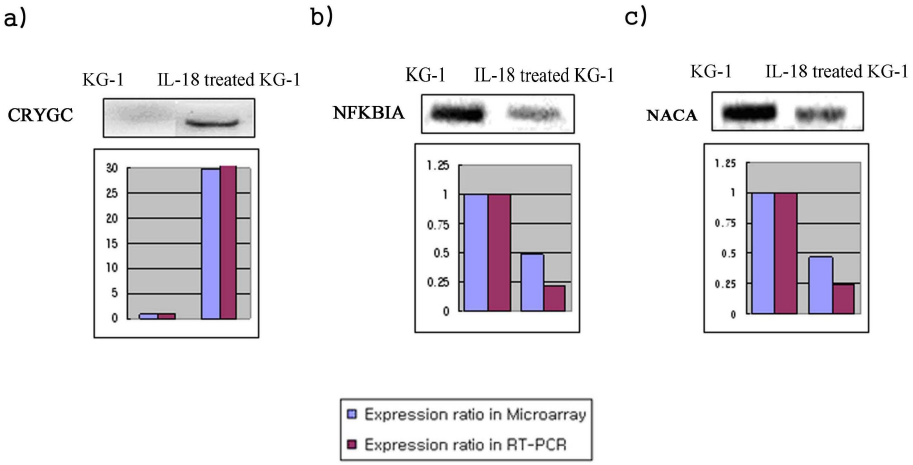

Fig. 2. Confirmation up and down regulated genes in KG-1 by RT-PCR. In IL-18-treated KG-1 cells, expression of CRYGC increased 3 times more than that of IL-18 untreated cells. Expressions of NFκBIA and NACA decreased. (a) Up regulated genes, (b) Down regulated genes, (c) Down regulated genes.

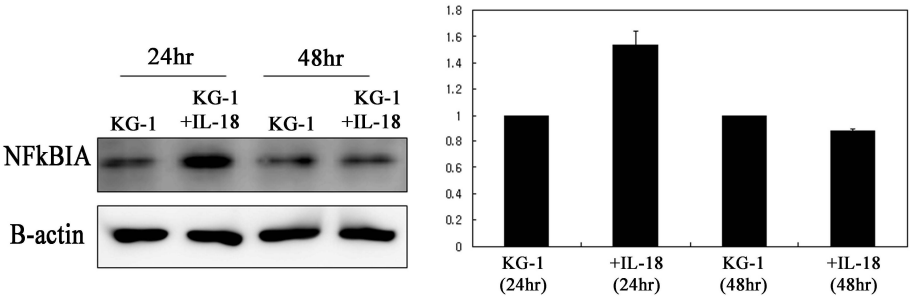

Fig. 3. Protein expression of NFκBIA using Western blot. After IL-18 treatment, the expression level of NFκBIA increased within 24 hours, but decreased by 48 hours.

DISCUSSION

AML is the most common tumor and accompanying disorder of disease specific genes (11). Based on clinical data, we divided the patients in two groups according to their treatment response (good prognosis and poor prognosis) and were able to demonstrate an important difference between the prognostic groups in relation to IL-18 production. NB4 cells, which are associated with optimistic prognosis (12), did not secrete IL-18, while KG-1 cells, which are associated with a poorer outlook (12), did secrete Il-18. To broadly estimate the influence of IL-18 on KG-1 cells, cDNA microarray technology was utilized. Expression levels of 57 genes, including stress related genes, and CRYGC increased at least 2-fold, whereas expression of 48 genes including NFκBIA and NACA decreased at least 2-fold.

IL-18 is a novel cytokine produced from Kupper cells, activated macrophages, keratinocyte, intestinal epitherial cells, osteroblast, adenal cortex cells and murine diencephalon (13). Furthermore, IL-18 has a co-stimulatory factor for production of INF-γ in response to toxic shock (14), and shares functional similarity with IL-12 (14). INF-γ is produced by activated T and NK cells, and plays critical defensive roles against microbial pathogens (14). IL-18 acts on T helper 1 type T cells and strongly combines with IL-12 to produce INF-γ (14).

CRYGC is involved in protection against stress, indicating that IL-18 may function as a stress factor in KG-1 cells. When we monitored NFκBIA expression over time, initial increase was observed by 24 hrs, but decreased expression occurred by 48hrs upon exposure to Il-18. NFκBIA, which inhibits NF-κB activity by binding to the Rel domain of NF-kB, contains ankyrin repeats and plays an important role in responses involving growth regulation and apoptosis (15). Apparently, apoptosis is involved in the Il-18-mediated inhibition of NF-κB in KG-1 cells, perhaps in an autoregulatory role. The increased expression of NACA further bolsters the view that IL-18 plays a key role in the enhancement of cell survival.

Furthermore, the observations obtained with CRYGC, NACA and NFκBIA indicate that these genes may constitute reasonable prognostic markers in assessing the progression of AML and treatment responses.

MATERIALS AND METHODS

Cell culture and IL-18 treatment

KG-1 cells were cultured in RPMI-1640 medium (Gibco BRL, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a 5% CO_2 environment in the presence

of 80 ng/ml recombinant human IL-18 (MBL, Woburn, MA) for 48 hrs.

RNA isolation

Freshly isolated cells were lysed in Trizol (Life Technologies, Carlsbad, CA). RNA was extracted from the cell lysate as described previously (2). Briefly, 0.2 volumes of chloroform were added and RNA was precipitated with isopropanol in the aqueous phase and washed in 70% ethanol. The RNA pellet was dissolved in diethylene-pyrocarbonate (DEPC)-treated water. Total RNA was determined spectrophotometrically (Nanodrop Technologies, Wilmington, DE) within an optical density ratio (260/280 nm) of 1.9-2.1

cDNA microarray hybridization

Fluorescence-labeled probes for cDNA microarrays were generated using 100 μg of total RNA by oligo dT, SuperscriptII (Invitrogen, Carlsbad, CA) and Cy3-UTP or Cy5-UTP (Amersham Biosciences, Buckinghamshire, UK). Probes were combined and hybridized to array overnight at 56°C in buffer containing poly dA, yeast transfer RNA and human Cot1 DNA. Slides were sequentially placed in buffers containing 1X sodium chloride-sodium citrate (SSC) and 0.1% sodium dodecyl sulfate (SDS) for 10 min, 0.1X SSC, 0.1% SDS for 10 min, and 0.1X SSC for 10 min. Slides were centrifuged at 1000 rpm for 1 min to dry.

Scanning and data analysis

Hybridized cDNA microarrays were scanned using an Arrayworx scanner (Applied Precision, Issaquah, WA) containing two lasers that excite cyanin dyes at 685 nm for Cy5 and 595 nm for Cy3. Imagene version 5.1 software (Biodiscovery, El Segundo, CA) was used to automate the time consuming process of measuring and visualizing gene expression data from high-density array images. GeneSight version 3.2 software (Biodiscovery) was used for data analysis with normalization using the 'Subtract Mean and Use All Genes' function. The benchmarks for up-regulated and down-regulated genes in each hybridization were 2-fold and 0.5-fold, respectively. Selected genes were clustered using K-means or Hierarchical clustering (12).

RT-PCR

Total RNA (5 ug) was reverse-transcribed at 37°C for 45 min in the presence of oligo dT and SuperscriptII (Invitrogen). RNA was mixed with 0.5 ug oligo dT primer and 10 mM dNTP (Amersham Pharmacia Biotech, Piscataway, NJ), and incubated at 65°C for 5 minutes and placed on ice for 1 min. The reaction solution was added to 20 ul contained 200 units of SuperscriptII reverse transcriptase, four units of RNase inhibitor, 5X reaction buffer and 0.1 M diothiothreitol (DTT). The solution was incubated at 42°C for 50 min and heated at 70°C for 15 min. PCR was performed in a 50 ul reaction mixture containing 10 pmol dNTP (Bionet, Seoul, Korea), and 10X reaction buffer. The solution was incubated at 95°C for 5 min, 35 cycles of 94°C for 1min, 57°C for 50 s and 72°C for 1 minute.

Western blot

Equal amounts of proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were transferred to a nitrocellulose membrane as previously described (16). Following blocking with a 5% solution of skimmed milk and phosphate buffered saline-Tween-20-(PBST), primary antibody against NFκBIA or B-actin was added. After washing the membrane for 1 hr using PBST, the secondary antibody was added and each membrane was washed again for 1 hr using PBST. Finally, bound primary antibody was detected via electrogenerated chemiluminescence (ECL) (GE Healthcare Danmark) kit as previously described (16).

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