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Gene Expression Profiling Reveals that Paeoniflorin Has an Apoptotic Potential in Human Leukemia U937 Cells

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Abstract

A major source of paeoniflorin (PF) which was from the *Paeonia lactiflora* root, has been used as a herbal medicine in East Asia for its antiallergic, antiinflammatory, and immunoregulatory effects. However, only few details are known about the mechanism of apoptosis induced by this compound. The present study was undertaken to further elucidate the molecular mechanism of apoptosis and the changes of gene expression elicited by PF using DNA microarrays and computational gene-expression analysis tools in human leukemia U937 cells. A comparative global transcription analysis between treatment with PF and anisomycin (AM) that induces apoptosis in U937 cells revealed that c-Jun-NH_2-kinase (JNK) pathway related genes were less expressed in PF-treated cells. Elucidation of the mechanisms by which PF conducts its anti-cancer activities through comparative analysis of the gene expression is necessary to provide a solid foundation for its use as a promising agent in prevention and treatment strategies.

Keywords: Paeoniflorin, Anisomysin, Comparative genomics, U937, Apoptosis

The root of *Paeonia lactiflora pall* (also called *Paeoniae alba*), a major source of paeoniflorin (PF) has been used as a herbal medicine in East Asia for its anti-allergic, anti-inflammatory, and immuno-regulatory effects. PF has also been shown to exert anticancer and anti-proliferative activities in cancer cells[1] (e.g human leukemia Jurkat cells, human gastric carcinoma cells). In this study, this apoptosis was mediated through the reduction of mitochondrial membrane potential, activation of caspase, and fragmentation of DNA. Additionally, PF induced the phosphorylation of three mitogen-activated protein (MAP) family kinases, extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38 MAP kinase.

But recent studies have shown the contradictory results that cell viability was not affected after PF treatment in human leukemia U937 cells[2]. Apoptosis is thought to be an important response to most of the chemotherapeutic agents in leukemia cells and other kinds of cells. Although apoptosis was not detected in this experiment, it was observed that PF can change the apoptotic pathway related gene expression. The recent experiment indicated that the apoptotic mitochondrial caspase pathway is significant for cell death induced by anisomycin (AM) in human lymphoma U937 cells[3]. AM, which is purified from *Streptomyces griseolus*, was first published as an antibiotic for protozoa by Sobin and Tanner[4]. AM is also known as a potent apoptosis inducer through the activation of JNK. It has been reported that JNK activation stimulates apoptosis[5-7].

In this AM experiment, like the PA experiment, gene expression was also analyzed using a GeneChip® system with Human Genome U133A Array which was spotted with 22,283 probe sets. And AM also changed the apoptotic pathway related gene expression. Transcriptome analyses of human cells treated with these therapeutic agents have only recently emerged, and no comparative analysis between different therapeutic agents has been made. So elucidation of the mechanisms by which PF conducts its anti-cancer activities through comparative analysis of the gene expression with AM is necessary to provide a solid foundation for its use as a potential agent in prevention and treatment strategies.

Transcriptome Changes in Response to AM and PA

Gene-expression profiling of U937 cells exposed to 1 μM AM for 0 to 6 h was performed by GeneChip® oligonucleotide expression arrays. Of the 22,283 probe

Table 1. List of significantly up- or down regulated genes based on comparison between experimental (paeoniflorin (PF)-treated for 3 h) and control (non-treated) in human leukemia U937 cells.

Probe ID	Gene symbol	Gene title	Control expression	Treat expression	Fold change
p53 pathway-related genes					
down					
208711_s_at	CCND1	cyclin D1	25.65	2.49	0.10
208712_at	CCND1	cyclin D1	8.66	1.18	0.14
206083_at	BAI1	brain-specific angiogenesis inhibitor 1	34.77	6.03	0.17
219370_at	RPRM	reprimo, TP53 dependent G2 arrest mediator candidate	22.33	4.97	0.22
222176_at	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	7.81	2.32	0.30
209260_at	SFN	stratifin	2.89	1.12	0.39
205655_at	MDM4	Mdm4, transformed 3T3 cell double minute 4, p53 binding protein (mouse)	22.35	10.99	0.49
202770_s_at	CCNG2	cyclin G2	76.50	37.87	0.50
up					
209542_x_at	IGF1	insulin-like growth factor 1 (somatomedin C)	3.44	16.00	4.65
202627_s_at	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	3.57	10.62	2.97
220804_s_at	TP73	tumor protein p73	3.99	11.53	2.89
203904_x_at	CD82	CD82 molecule	31.27	77.64	2.48
211577_s_at	IGF1	insulin-like growth factor 1 (somatomedin C)	17.26	35.47	2.06
211156_at	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	4.74	9.73	2.05
220402_at	P53AIP1	p53-regulated apoptosis-inducing protein 1	3.06	6.27	2.05
209540_at	IGF1	insulin-like growth factor 1 (somatomedin C)	5.50	11.20	2.04
Apoptosis-related genes					
down					
207993_s_at	CHP	calcium binding protein P22	37.81	6.53	0.17
215561_s_at	IL1R1	interleukin 1 receptor, type I	2.65	0.51	0.19
210538_s_at	BIRC3	baculoviral IAP repeat-containing 3	6.66	2.03	0.30
211524_at	NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	2.79	1.07	0.38
213950_s_at	PPP3CC	Protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	3.82	1.56	0.41
202688_at	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	57.40	26.58	0.46
up					
210955_at	CASP10	caspase 10, apoptosis-related cysteine peptidase	4.49	33.24	7.40
207228_at	PRKACG	protein kinase, cAMP-dependent, catalytic, gamma	2.52	14.64	5.80
204413_at	TRAF2	TNF receptor-associated factor 2	5.50	26.62	4.84
214618_at	CFLAR	CASP8 and FADD-like apoptosis regulator	1.14	2.43	2.13
212559_at	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta	44.33	90.49	2.04
MAPK signaling pathway-related genes					
down					
209908_s_at	TGFB2	transforming growth factor, beta 2	22.75	1.36	0.06
211078_s_at	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)	7.55	0.59	0.08
216139_s_at	MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3	49.35	4.34	0.09
216435_at	MAP2K5	Mitogen-activated protein kinase kinase 5	10.78	1.32	0.12
206249_at	MAP3K13	mitogen-activated protein kinase kinase kinase 13	14.42	2.15	0.15
207993_s_at	CHP	calcium binding protein P22	37.81	6.53	0.17
205447_s_at	MAP3K12	mitogen-activated protein kinase kinase kinase 12	37.22	6.64	0.18
206382_s_at	BDNF	brain-derived neurotrophic factor	7.62	1.65	0.22
204878_s_at	TAOK2	TAO kinase 2	26.35	6.07	0.23
209747_at	TGFB3	transforming growth factor, beta 3	20.22	6.02	0.30
215305_at	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	9.60	3.05	0.32
205801_s_at	RASGRP3	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	3.54	1.16	0.33
213177_at	MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3	22.03	7.38	0.34
204380_s_at	FGFR3	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	7.87	2.72	0.35

Table 1. Continued.

Probe ID	Gene symbol	Gene title	Control expression	Treat expression	Fold change
206178_at	PLA2G5	phospholipase A2, group V	3.59	1.24	0.35
205699_at	MAP2K6	mitogen-activated protein kinase kinase 6	11.57	4.07	0.35
214786_at	MAP3K1	mitogen-activated protein kinase kinase kinase 1	34.64	12.20	0.35
214571_at	FGF3	fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2) oncogene homolog)	4.53	1.62	0.36
210059_s_at	MAPK13	mitogen-activated protein kinase 13	6.78	2.57	0.38
211524_at	NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	2.79	1.07	0.38
206103_at	RAC3	ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)	5.76	2.22	0.39
209189_at	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	185.89	72.01	0.39
201465_s_at	JUN	jun oncogene	53.59	21.36	0.40
211371_at	MAP2K5	mitogen-activated protein kinase kinase 5	15.30	6.14	0.40
209951_s_at	MAP2K7	mitogen-activated protein kinase kinase 7	17.83	7.18	0.40
208893_s_at	DUSP6	dual specificity phosphatase 6	72.41	29.29	0.40
213950_s_at	PPP3CC	Protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	3.82	1.56	0.41
210477_x_at	MAPK8	mitogen-activated protein kinase 8	19.80	8.46	0.43
222164_at	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	33.50	14.43	0.43
205463_s_at	PDGFA	platelet-derived growth factor alpha polypeptide	18.54	8.15	0.44
201041_s_at	DUSP1	dual specificity phosphatase 1	94.48	43.20	0.46
203930_s_at	MAPT	microtubule-associated protein tau	13.81	6.38	0.46
215498_s_at	MAP2K3	mitogen-activated protein kinase kinase 3	94.64	44.52	0.47
207822_at	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	22.92	10.80	0.47
219714_s_at	CACNA2D3	calcium channel, voltage-dependent, alpha 2/delta 3 subunit	47.61	22.77	0.48
212912_at	RPS6KA2	ribosomal protein S6 kinase, 90 kDa, polypeptide 2	70.07	33.73	0.48
214367_at	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	8.33	4.09	0.49
215992_s_at	RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	2.98	1.47	0.49
215050_x_at	MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2	52.30	26.09	0.50
up					
214368_at	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	2.86	58.46	20.43
211485_s_at	FGF18	fibroblast growth factor 18	1.77	29.62	16.69
205590_at	RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	0.97	13.88	14.28
203131_at	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	1.74	12.53	7.20
217515_s_at	CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	4.26	28.83	6.76
203649_s_at	PLA2G2A	phospholipase A2, group IIA (platelets, synovial fluid)	3.66	23.29	6.37
221310_at	FGF14	fibroblast growth factor 14	2.28	14.12	6.20
207228_at	PRKACG	protein kinase, cAMP-dependent, catalytic, gamma	2.52	14.64	5.80
205558_at	TRAF6	TNF receptor-associated factor 6	9.45	53.48	5.66
204421_s_at	FGF2	fibroblast growth factor 2 (basic)	2.97	16.17	5.44
204413_at	TRAF2	TNF receptor-associated factor 2	5.50	26.62	4.84
215195_at	PRKCA	protein kinase C, alpha	1.06	5.08	4.78
201743_at	CD14	CD14 molecule	1.49	6.98	4.68
204200_s_at	PDGFB	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	3.36	14.14	4.21
208432_s_at	CACNA1E	calcium channel, voltage-dependent, R type, alpha 1E subunit	2.11	8.65	4.09
210226_at	NR4A1	nuclear receptor subfamily 4, group A, member 1	9.21	36.44	3.96
211143_x_at	NR4A1	nuclear receptor subfamily 4, group A, member 1	3.18	12.45	3.92
220780_at	PLA2G3	phospholipase A2, group III	2.88	10.49	3.64
207222_at	PLA2G10	phospholipase A2, group X	4.78	17.34	3.63
206311_s_at	PLA2G1B	phospholipase A2, group IB (pancreas)	0.95	3.33	3.52
210675_s_at	PTPRR	protein tyrosine phosphatase, receptor type, R	1.61	5.59	3.47
211499_s_at	MAPK11	mitogen-activated protein kinase 11	12.70	42.89	3.38
207050_at	CACNA2D1	calcium channel, voltage-dependent, alpha 2/delta subunit 1	2.55	8.22	3.22
62987_r_at	CACNG4	calcium channel, voltage-dependent, gamma subunit 4	28.76	90.44	3.14
211533_at	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	1.16	3.43	2.97
211372_s_at	IL1R2	interleukin 1 receptor, type II	1.02	3.02	2.95
214284_s_at	FGF18	Fibroblast growth factor 18	3.66	9.89	2.70

Table 1. Continued.

Probe ID	Gene symbol	Gene title	Control expression	Treat expression	Fold change
201983_s_at	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	2.88	7.30	2.53
212647_at	RRAS	related RAS viral (r-ras) oncogene homolog	20.00	48.72	2.44
215365_at	CACNB2	calcium channel, voltage-dependent, beta 2 subunit	1.31	3.10	2.37
206706_at	NTF3	neurotrophin 3	6.10	14.03	2.30
208449_s_at	FGF8	fibroblast growth factor 8 (androgen-induced)	0.64	1.40	2.18
202340_x_at	NR4A1	nuclear receptor subfamily 4, group A, member 1	10.04	21.58	2.15
215688_at	RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1	12.05	24.16	2.01

sets analyzed and identified for exposure periods of 0, 1, 2, 3 and 6 h, respectively[3]. To identify genes responsive to PF treatment in U937 cells, they carried out global-scale DNA microarray analysis of cells cultured at 0, 1, and 3 h after PF treatment (160 mg/mL, 30 min)[2]. These results indicated that the number of expressed probe sets was approximately constant among the different samples. Complete lists of probe sets from all samples are available on the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE8229 and http://www.ncbi.nlm.nih.gov/geo/query/ acc.cgi?acc=GSE8228).

Functional Classifications Analysis

A comparative global transcription analysis between treatment with PF and AM that induces apoptosis in U937 cells revealed that functional classifications of the responding genes are provided in Figure 1 (the hypothetical, unclassified, unknown is omitted). Functional classes are taken from Gene Ontology category in GenPlex v3.0 software.

Metabolic Pathway Analysis

The pathways from the Kyoto Encyclopaedia of Genes and Genomes (KEGG)[8] were downloaded and imported in to the GenPlex v3.0 software and visually inspected for changes based on the 2,159 genes from the *t*-test analysis. These metabolic pathways were compiled into tables to organize the data based on metabolic pathways. Striking features were revealed by inspection. First, the p53 dependent pathway genes were compiled and organized in to Table 1. All genes were significantly upregulated and downregulated. Second, the apoptosis pathway was organized in to Table 2. Third, MAPK-related genes were compiled and organized in to supplementary Table 1.

Discussion

As expected, paeoniflorin (PF) treatment triggered the expression of genes involved in antiinflammatory, antiimmuno-regulatory effects, anticancer and antiproliferative activities. PF has been proposed and shown to elicit similar responses to anisomycin (AM) through MAPK signaling pathway and apoptosis which were presumed to account for the major anticancer[9-11] and apoptosis[1,12,13] (Figure 1). Hence, it has been speculated that PF functions by similar mechanisms as other antiinflamation[14-16]. The p53 pathway senses a variety of stress signals which will reduce the fidelity of cell growth and division, and responds by initiating cell cycle arrest, senescence, or apoptosis. Correspondingly, p53 related genes using cyclin D1 (*CCND*1)[17], p53 binding protein (*MDM*4)[18], and phosphatase and tensin homolog (*PTEN*)[19,20] were all downregulated. Cyclin D1 gene *CCND*1 was strongly repressed (0.1-fold). The mRNA level of MDM4 (Murine Double Minute 4) decreased under PF (0.49-fold). MDM4 shares significant structural homology with MDM2 (Murine Double Minute 2) and interacts and regulates transcriptional activity of the tumor suppressor p53. In tumors with wild-type p53, there is often overexpression of MDM2 or MDM4 leading to functional inactivation of p53[18]. This study explores two p53-regulated gene products, PTEN (0.3-fold) and IGF-1 (4.65-fold), each of which negatively regulates the IGF-1-AKT-mTOR pathways after PF.

Exposure to PF, however, induced a variety of genes involved in apoptosis including p53 dependent and p53 independent[21]. Most notably, Caspase-10 activation, in turn, depended on caspase-8, which cleaved caspase-10 directly[22]. The caspase-8 homologous cellular FLICE-like inhibitory protein (cFLIP) can also be recruited to the DISC. cFLIP acts as an anti-apoptotic regulator by interfering with activation of caspases-8 and -10 at the DISC[23]. Caspase-10 were among the most heavily upregulated 6.4-fold (Table 1). CASP8 and FADD-like apoptosis regulator gene (CFLAR) was upregulated by 2.13-fold.

Signaling from transforming growth factor beta (TGFβ stimulates the MAPK pathway[24]. The MAPK

Table 2. List of significantly Up-regulated genes based on comparison between experimental (paeoniflorin (PF)-treated for 3 h) and experimental (anisomycin (AM)-treated for 3 h) in human leukemia U937 cells.

Probe ID	Fold change	*P*-value	Gene symbol	Gene title	Functional classifications
203626_s_at	19.6	0.014	SKP2	S-phase kinase-associated protein 2	Cell cycle; Ubiquitin mediated proteolysis; Small cell lung cancer
207294_at	13.6	0.004	AGTR2	angiotensin II receptor, type 2	Neuroactive ligand-receptor interaction; Renin-angiotensin system
211338_at	13.2	0.017	IFNA2	interferon, alpha	Cytokine-cytokine receptor interaction
204058_at	12.4	0.006	ME1	malate dehydrogenase	Pyruvate metabolism; Carbon fixation; PPAR signaling pathway
212224_at	10.7	0.006	ALDH1A1	retinal dehydrogenase	Retinol metabolism
213856_at	8.9	0.000	CD47	CD47 antigen (Rh-related antigen, integrin-associated	ECM-receptor interaction
205651_x_at	8.8	0.006	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	Leukocyte transendothelial migration
222053_at	8.2	0.019	TAF6L	transcription initiation factor TFIID subunit D5	Basal transcription factors
213816_s_at	7.5	0.031	MET	proto-oncogene tyrosine-protein kinase Met	Cytokine-cytokine receptor interaction; Axon guidance
203084_at	7.2	0.025	TGFB1	transforming growth factor, beta	MAPK signaling pathway; Cytokine-cytokine receptor interaction
210743_s_at	6.0	0.038	CDC14A	protein phosphatase///cell division cycle 14	Cell cycle
210311_at	5.7	0.027	FGF5	fibroblast growth factor	MAPK signaling pathway; Regulation of actin cytoskeleton; Melanoma
220574_at	5.6	0.029	SEMA6D	semaphorin 6	Axon guidance
219300_s_at	5.4	0.021	CNTNAP2	contactin associated protein-like 2	Cell adhesion molecules (CAMs)
212555_at	5.3	0.028	PRKAR1B	cAMP-dependent protein kinase regulator	Apoptosis; Insulin signaling pathway
215957_at	5.1	0.009	UBE2D1	ubiquitin-conjugating enzyme UBE2D/E	Ubiquitin mediated proteolysis
211991_s_at	5.0	0.024	HLA-DPA1	major histocompatibility complex, class II	Cell adhesion molecules (CAMs); Antigen processing and presentation; Type I diabetes mellitus
216055_at	4.7	0.013	PDGFB	platelet derived growth factor A/B	MAPK signaling pathway; Cytokine-cytokine receptor interaction
203863_at	4.6	0.037	ACTN2	actinin alpha	Focal adhesion; Adherens junction
209975_at	4.3	0.044	CYP2E1	cytochrome P450, family 2, subfamily E	Arachidonic acid metabolism
204602_at	4.1	0.006	DKK1	dickkopf	Wnt signaling pathway
202620_s_at	4.0	0.023	PLOD2	procollagen-lysine, 2-oxoglutarate 5-dioxygenase	Lysine degradation
222051_s_at	3.9	0.048	E2F5	E2F transcription factor 4/5	TGF-beta signaling pathway
204936_at	3.5	0.048	MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2	MAPK signaling pathway
214066_x_at	3.4	0.027	NPR2	guanylate cyclase	Purine metabolism; Gap junction; Long-term depression
211488_s_at	3.3	0.008	ITGB8	integrin beta 8	Focal adhesion; ECM-receptor interaction; Cell adhesion molecules (CAMs); Regulation of actin cytoskeleton
208147_s_at	3.2	0.004	CYP2C8	cytochrome P450, family 2, subfamily C	Arachidonic acid metabolism; Linoleic acid metabolism; Metabolism of xenobiotics by cytochrome P450
205874_at	3.2	0.030	ITPKA	1D-myo-inositol-triphosphate 3-kinase	Inositol phosphate metabolism; Calcium signaling pathway
212813_at	3.0	0.042	JAM3	junction adhesion molecule 3	Cell adhesion molecules (CAMs)
217558_at	2.8	0.038	CYP2C9	cytochrome P450, family 2, subfamily C	Metabolism of xenobiotics by cytochrome P450
204343_at	2.8	0.023	ABCA3	ATP-binding cassette, subfamily A (ABC1), member 3	ABC transporters-General
208592_s_at	2.8	0.027	CD1E	CD1 antigen	Hematopoietic cell lineage
217603_at	2.7	0.028	ATP6V0A2	V-type H^+-transporting ATPase subunit I	Oxidative phosphorylation; Cholera-Infection; Epithelial cell signaling in Helicobacter pylori

Table 2. Continued.

Probe ID	Fold change	*P*-value	Gene symbol	Gene title	Functional classifications
207578_s_at	2.7	0.002	HTR4	5-hydroxytryptamine (serotonin) receptor 4	Calcium signaling pathway; Neuroactive ligand-receptor interaction
203392_s_at	2.7	0.018	CTBP1	C-terminal binding protein	Wnt signaling pathway; Notch signaling pathway; Chronic myeloid leukemia
221658_s_at	2.7	0.012	IL21R	interleukin 21 receptor	Cytokine-cytokine receptor interaction; Jak-STAT signaling pathway
212843_at	2.6	0.003	NCAM1	neural cell adhesion molecule	Cell adhesion molecules (CAMs)
200831_s_at	2.6	0.017	SCD	stearoyl-CoA desaturase	Polyunsaturated fatty acid biosynthesis; PPAR signaling pathway
209663_s_at	2.6	0.020	ITGA7	integrin alpha 7	Focal adhesion
206665_s_at	2.4	0.017	BCL2L1	BCL2-like 1 (apoptosis regulator Bcl-X)	Neurodegenerative Disorders; Apoptosis myeloid leukemia; Small cell lung cancer
201951_at	2.3	0.034	ALCAM	activated leukocyte cell adhesion molecule	Cell adhesion molecules (CAMs)
211407_at	2.3	0.040	NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 7	Oxidative phosphorylation
209008_x_at	2.3	0.021	KRT8	type II keratin, basic	Cell Communication
203559_s_at	2.3	0.012	ABP1	copper amine oxidase	Urea cycle and metabolism of amino groups; Glycine, serine and threonine metabolism; Histidine metabolism; Tyrosine metabolism; Phenylalanine metabolism; Tryptophan metabolism; beta-Alanine metabolism; Alkaloid biosynthesis II
214006_s_at	2.3	0.018	GGCX	vitamin K-dependent gamma-carboxylase	Biosynthesis of steroids
219222_at	2.3	0.034	RBKS	ribokinase	Pentose phosphate pathway
221892_at	2.3	0.005	H6PD	glucose 1-dehydrogenase/// 6-phosphogluconolactonase	Pentose phosphate pathway
207218_at	2.3	0.016	F9	coagulation factor IX (Christmas factor)	Complement and coagulation cascades
210756_s_at	2.2	0.026	NOTCH2	Notch	Dorso-ventral axis formation; Notch signaling pathway
218927_s_at	2.2	0.011	CHST12	chondroitin 4-sulfotransferase 12	Chondroitin sulfate biosynthesis; Sulfur metabolism; Glycan structures-biosynthesis 1
200623_s_at	2.2	0.010	CALM3	calmodulin	Calcium signaling pathway; Phosphatidylinositol signaling system; Long-term potentiation; Olfactory transduction; Insulin signaling pathway; GnRH signaling pathway; Melanogenesis; Huntington's disease; Glioma
208829_at	2.2	0.037	TAPBP	TAP binding protein (tapasin)	Antigen processing and presentation
203524_s_at	2.2	0.047	MPST	3-mercaptopyruvate sulfurtransferase	Cysteine metabolism
207827_x_at	2.2	0.014	SNCA	synuclein, alpha (non A4 component of amyloid precursor)	Neurodegenerative Disorders; Alzheimer's disease; Parkinson's disease
214982_at	2.2	0.048	ASCC3L1	---	Starch and sucrose metabolism; Folate biosynthesis
219537_x_at	2.2	0.021	DLL3	delta	Notch signaling pathway
216733_s_at	2.2	0.010	GATM	glycine amidinotransferase	Urea cycle and metabolism of amino groups; Glycine, serine and threonine metabolism; Arginine and proline metabolism
217202_s_at	2.1	0.007	GLUL	glutamine synthetase	Glutamate metabolism; Peptidoglycan biosynthesis; Nitrogen metabolism
217523_at	2.1	0.045	CD44	CD44 antigen	ECM-receptor interaction; Hematopoietic cell lineage
216680_s_at	2.1	0.030	EPHB4	Eph receptor B4	Axon guidance

Table 2. Continued.

Probe ID	Fold change	*P*-value	Gene symbol	Gene title	Functional classifications
219956_at	2.1	0.003	GALNT6	polypeptide N-acetylgalactosaminyltransferase	O-Glycan biosynthesis; Glycan structures-biosynthesis 1
212953_x_at	2.1	0.015	CALR	calreticulin	Antigen processing and presentation
210365_at	2.1	0.036	RUNX1	runt-related transcription factor 1	Chronic myeloid leukemia; Acute myeloid leukemia
211708_s_at	2.1	0.010	SCD	stearoyl-CoA desaturase	Polyunsaturated fatty acid biosynthesis; PPAR signaling pathway
200965_s_at	2.0	0.043	ABLIM1	actin-binding LIM protein	Axon guidance
217009_at	2.0	0.035	PGK2	phosphoglycerate kinase	Glycolysis/Gluconeogenesis; Carbon fixation
221609_s_at	2.0	0.006	WNT6	wingless-type MMTV integration site family, member 6	Wnt signaling pathway; Hedgehog signaling pathway; Melanogenesis; Basal cell carcinoma
211048_s_at	2.0	0.005	PDIA4	protein disulfide isomerase family A, member 4	Cholera-Infection
213792_s_at	2.0	0.012	INSR	insulin receptor	Adherens junction; Insulin signaling pathway

pathways are deeply involved in signaling for various immune responses including apoptosis. MAPKs are serine/threonine kinases (STK), which include the extracellular signal-related kinases (ERKs), p38 kinases, and c-Jun N-terminal kinases (JNKs)[25]. Activation of MAPK pathway often occurs in response to growth factor stimulation of receptor tyrosine kinases, which are coupled to the activation of Ras G-proteins, such Shc and Grb2, and quinine nucleotide exchange factors such as SOS[26,27]. In this study, we detected the downregulation of TGFB, STK3, MAPK8IP3, NFKB2, and FOS in Pf-treated human leukemia U937 cells (Table 1).

Fibroblast growth factor (FGF) signals play fundamental roles in development and tumorigenesis. Thyroid cancer is an example of a tumor with nonoverlapping genetic mutations that up-regulate mitogen-activated protein kinase (MAPK). Here, we show that FGF receptors (FGF18, 16.69-fold; FGF14, 6.2-fold; FGF2, 5.44-fold), which are expressed mainly in U937 cells, propagates MAPK activation and promotes tumor progression. These data unmask an epigenetically controlled FGFR signal that imposes precisely on the intragenically modified BRAF/MAPK pathway to modulate human leukemia U937 cells. Interestingly, our data showed that genes associated with the cell cycle, cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, MAPK signaling pathway, and p53 signaling pathway were highly upregulated in AM-treated than PF-treated U937 cells (Figure 1 and Table 3). The genes encoding enzymes responsible for the F-box and leucine-rich repeat protein 1 (SKP2), angiotensin II receptor, type 2, interferon, alpha, Rap guanine nucleotide exchange factor (GEF) 4, TGFβ, and FGF exhibited the highly upregulated genes after PF-exposure than AM-treated. This Study also revealed that PF induced several IGF, caspase-10, Ras, RASGRP and FGF genes, which are essential for MAPK activation and promotes tumor progression.

Taken together, these results indicate that PF may have potential efficacy for the treatment of anticancer and induction of apoptosis. However, it may possible for proliferation and differentiation by activating FGF signaling and MAPK pathway. A comparative global transcription analysis between treatment with PF and AM that induces apoptosis in U937 cell revealed that JNK pathway related genes were less expressed in PF-treated cells. Elucidation of the mechanisms by which PF conducts its anticancer activities through comparative analysis of the gene expression is necessary to provide a solid foundation for its use as an agent in prevention and treatment strategies.

Materials & Methods

Data Analysis

The MAS5 algorithm was used to evaluate the expression signals generated by the Affymetrix Human Genome U133A Array. Global scaling normalization was then performed and the normalized data were log-transformed with base 2. Next, fold change was applied to select the differentially expressed genes (DEGs) using a fold change threshold of 2.0-fold and a $P < 0.05$ to indicate significance. Each probe set used in the Affymetrix GeneChip produces a detection call, with P (present call) indicating good quality, M (marginal call) indicating intermediate quality and A (abs-

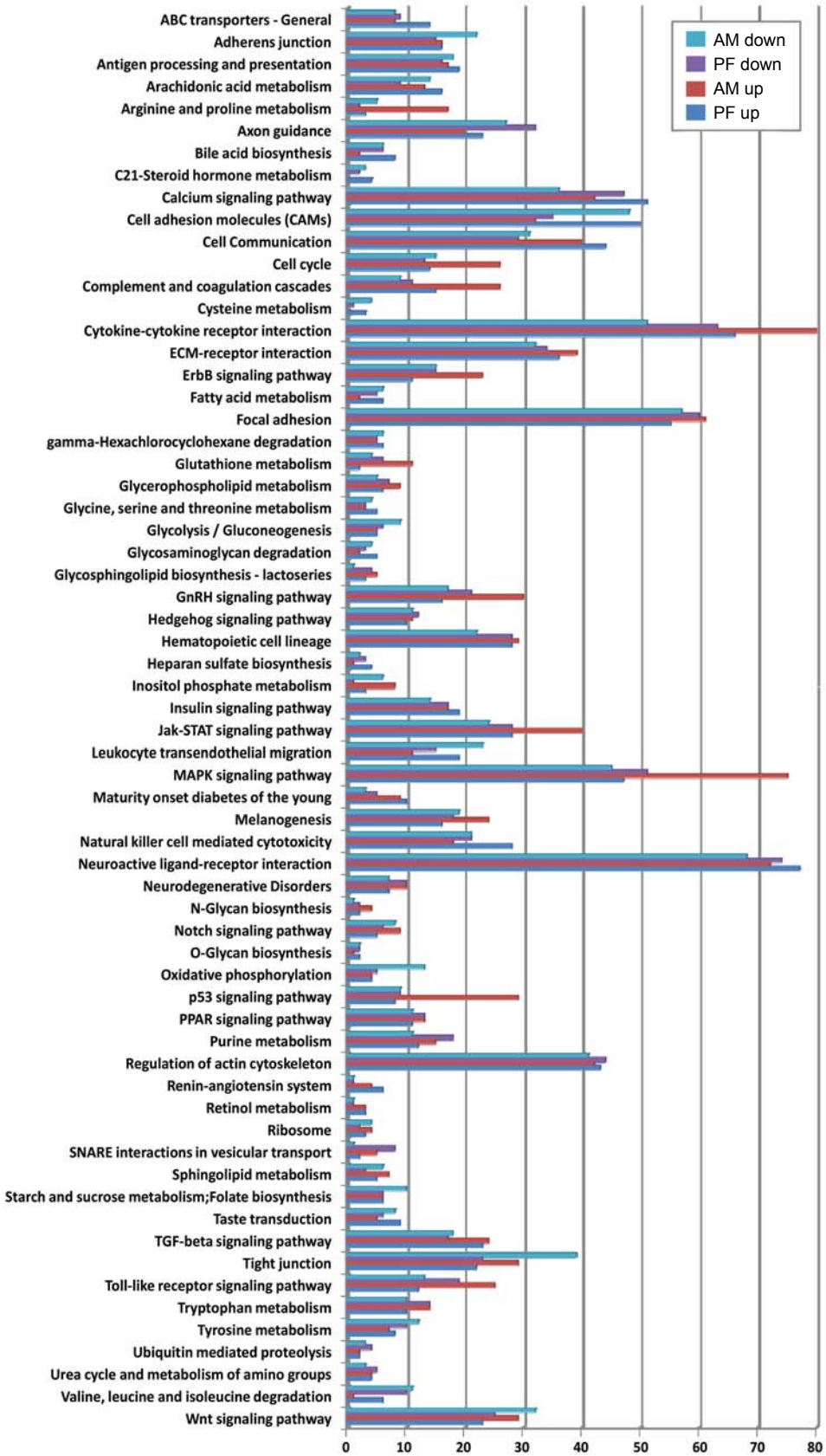

Figure 1. Gene Ontology classifications of genes based on comparison of gene expression between experimental (paeoniflorin (PF)-treated and anisomycin (AM)-treated) and control (non-treated) in human leukemia U937 cells.

Table 3. List of significantly Down-regulated genes based on comparison between experimental (paeoniflorin (PF)-treated for 3 h) and experimental (anisomycin (AM)-treated for 3 h) in human leukemia U937 cells.

Probe ID	Fold change	*P*-value	Gene symbol	Gene title	Functional classifications
211449_at	0.2	0.008	MSH6	DNA mismatch repair protein MSH6	Colorectal cancer
208116_s_at	0.2	0.002	MAN1A1	mannosyl-oligosaccharide alpha-1,2-mannosidase	N-Glycan biosynthesis
211496_s_at	0.2	0.004	PDC	phosducin	Olfactory transduction
212759_s_at	0.2	0.008	TCF7L2	transcription factor 7-like 2 leukemia	Wnt signaling pathway; Acute myeloid
206751_s_at	0.3	0.038	PCYT1B	choline-phosphate cytidylyltransferase	Aminophosphonate metabolism
202669_s_at	0.3	0.004	EFNB2	ephrin-B	Axon guidance
203710_at	0.3	0.022	ITPR1	inositol 1,4,5-triphosphate receptor, type 1	Calcium signaling pathway
214378_at	0.3	0.019	TFPI	tissue factor pathway inhibitor	Complement and coagulation cascades
213055_at	0.3	0.007	CD47	CD47 antigen (Rh-related antigen, integrin-associated	ECM-receptor interaction
204062_s_at	0.3	0.032	ULK2	unc51-like kinase	Regulation of autophagy; mTOR signaling pathway
201334_s_at	0.4	0.000	ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	Axon guidance; Regulation of actin cytoskeleton
202861_at	0.4	0.038	PER1	period circadian protein	Circadian rhythm
209050_s_at	0.4	0.048	RALGDS	ral guanine nucleotide dissociation stimulator	Colorectal cancer; Pancreatic cancer
212067_s_at	0.4	0.043	C1R	complement component 1, r subcomponent	Complement and coagulation cascades
201963_at	0.4	0.038	ACSL1	long-chain fatty-acid-CoA ligase	Fatty acid metabolism
201602_s_at	0.4	0.013	PPP1R12A	protein phosphatase 1, regulatory (inhibitor) subunit 12	Focal adhesion
203544_s_at	0.4	0.019	STAM	signal transducing adaptor molecule	Jak-STAT signaling pathway
204635_at	0.4	0.006	RPS6KA5	mitogen-, stress activated protein kinases	MAPK signaling pathway; Bladder cancer
205105_at	0.4	0.000	MAN2A1	alpha-mannosidase II	N-Glycan biosynthesis
202981_x_at	0.4	0.006	SIAH1	ubiquitin ligase SIAH1	p53 signaling pathway; Wnt signaling pathway
206769_at	0.4	0.014	TMSB4Y	thymosin, beta 4	Regulation of actin cytoskeleton
217028_at	0.5	0.039	CXCR4	chemokine (C-X-C motif) receptor 4	Cytokine-cytokine receptor interaction
210512_s_at	0.5	0.046	VEGFA	vascular endothelial growth factor A/B, PGF	Cytokine-cytokine receptor interaction
212249_at	0.5	0.013	PIK3R1	phosphoinositide-3-kinase, regulatory subunit	ErbB signaling pathway
205448_s_at	0.5	0.010	MAP3K12	mitogen-activated protein kinase kinase kinase 12	MAPK signaling pathway
203096_s_at	0.5	0.010	RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	MAPK signaling pathway
204633_s_at	0.5	0.031	RPS6KA5	mitogen-, stress activated protein kinases	MAPK signaling pathway; Bladder cancer

ent call) indicating relatively low reliability. Therefore, probe sets that resulted in A calls in the compared groups were removed to filter false positives. The 2.0-fold DEGs were clustered using the GenPlex™ v3.0 software (ISTECH Inc., Korea) using hierarchical clustering with Pearson correlation as a similarity measure and complete linkage as the linkage method. In addition, gene ontology significance analysis was conducted to investigate the functional relationships among the 2.0-fold DEGs using high-throughput GoMiner. The 2.0-fold DEGs were then mapped to relevant pathways using GenPlex™ v3.0 software (ISTECH Inc., Korea). The pathway resources were provided by the KEGG database.

Data analysis was also performed with the GeneSpring GX v. X (Agilent Technologies). The following parameters were employed for GCOS expression analysis: $\alpha_1=0.04$, $\alpha_2=0.06$, and $\tau=0.015$; target signal was scaled to 150. Genes that received "absent" calls from 50% or more of the replicates in GeneSpring were not used for the analysis. Finally, gene expression changes with statistical significance were identified by an upper 1-tailed *t*-test (*P* cutoff value, 0.05). "Fold-change" was calculated as the ratio between the signal averages of

four untreated and four treated cultures. That is, results are from biological quadruplicate experiments. Genes with a 2.0-fold or more induction or repression were used in this analysis.

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