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Demethoxycurcumin Alters Gene Expression Associated with DNA Damage, Cell Cycle and Apoptosis in Human Lung Cancer NCI-H460 Cells *In Vitro*

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Abstract. *Lung cancer is the leading cause of cancer-related deaths and new lung cancer cases are continuously emerging around the globe; however, treatment of lung cancer remains unsatisfactory. Demethoxycurcumin (DMC) has been shown to exert cytotoxic effects in human cancer cells via induction of apoptosis. However, the effects of DMC on genetic mechanisms associated with these actions have not been yet elucidated. Human lung cancer NCI-H460 cells were incubated with or without 35 μM of DMC for 24 h and total RNA was extracted for cDNA synthesis labeling and microarray hybridization, followed by fluor-labeled cDNA hybridization on chip. Expression Console software with default Robust Multichip Analysis (RMA) parameters were used for detecting and quantitating the localized concentrations of fluorescent molecules. The GeneGo software was used for investigating key genes involved and their possible interaction pathways. Genes associated with DNA damage and repair, cell-cycle check point and apoptosis could be altered by DMC; in particular, 144 genes were found up-regulated and 179 genes down-regulated in NCI-H460 cells after exposure to DMC. In general, DMC-altered genes may offer information to understand the cytotoxic mechanism of this agent at the genetic level since gene alterations can be useful biomarkers or targets for the diagnosis and treatment of human lung cancer in the future.*

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Lung cancer, the leading cause of malignancy-related death globally and is one of the most aggressive human cancers worldwide. Lung cancer most commonly gives rise to metastases in the brain (1) and peripheral tissues before being diagnosed in many clinical cases (2). In lung cancer, about 80% of the cases are non-small cell lung cancer (NSCLC) (3) and early detection is possible (4). The major treatment options for lung cancer are surgical resection, chemotherapy and radiation therapy; however, the survival rate remains very low (5) and serious side-effects impairing quality of life have been registered (6). Currently, numerous studies have focused on personalized modes of therapy for assessing the efficacy and safety of agents used as molecular targets (7). Thus, many investigators are searching for new drugs from natural products to improve the disadvantages of the current treatments, especially in lung cancer.

Curcumin, a component of turmeric, is derived from the rhizome of Curcuma longa. Curcumin, acting as a chemoprotective agent (8-10), has been shown to inhibit cancer cell proliferation, induce cell apoptosis in many cancer cell lines (11-15) and modulate multiple cell signaling pathways, including apoptosis, proliferation, angiogenesis and inflammation (16). Demethoxycurcumin (DMC) is a synthetic curcumin analogue with increased metabolic stability compared to curcumin (17). DMC has been reported to induce apoptosis in human HCT116 colon cancer cells (17), human renal carcinoma Caki cells (18) and in human

breast carcinoma MCF7 cells (19). It has been reported that DMC inhibits NO production, inducible NO synthase expression and NF-kB activation in RAW264.7 macrophages activated with LPS (20). Furthermore, DMC induces heme oxygenase-1 expression through Nrf2 activation in RAW264.7 macrophages (21). However, the effects of DMC on human lung cancer cells have not yet been studied. We, thus, investigated the role and action of DMC on gene expression *in vitro* using the human lung cancer NCI-H460 cell line.

It is well-known that genome's instability, recognized to be a hallmark of most cancers, can cause genetic aberrations as genetic mutations play an important role for oncogenes (22). Typical examples are offered by the *p53* mutation and dysfunction found in over 50% of all types of human cancers (23) and the high incidence of oncogenic *K-RAS* mutations found in human lung adenocarcinomas and carcinogen-induced animal models (24). Thus, finding which gene is affected by certain anticancer drugs, is necessary for exploring the molecular mechanism of these drugs' function. Although DMC has been shown to induce cell apoptosis in various cancer types, there is no report to show how this agent affects gene expression in human lung cancer cells. To answer this question, we used cDNA microarrays to investigate the altered gene expression in NCI-H460 cells and our results indicated that DMC could act on the expression of some genes associated with DNA damage, cell cycle and apoptosis. These findings may, thus, form the basis for future studies about anticancer development by DMC.

Materials and Methods

Chemicals and reagents. Demethoxycurcumin (DMC) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Culture medium RPMI-1640, fetal bovine serum, L-glutamine, penicillin and streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). DMC was dissolved in DMSO and stocked at –20˚C.

Lung cancer cells. The NCI-H460 human non-small cell lung cancer (NSCLC) cell line was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan) and cultured in RPMI-1640 medium containing 10% fetal bovine serum, 1% L-glutamine, 100 units/ml of penicillin G and 100 microgram/ml of streptomycin at 37˚C in a humidified atmosphere of a 5% CO_2 and 95% air. Cells were split every 4 days to maintain exponential growth before the experiments.

cDNA microarray assay for gene expression in NCI-H460 cells after exposure to DMC. NCI-H460 cells (1×10^5 cells/ml) were maintained in RPMI-1640 medium in a 12-well plate for 24 h. Cells were treated with or without 35 μM of DMC for 48 h and then harvested for extracting the total RNAs by using the Qiagen RNeasy Mini Kit (Qiagen, Inc., Valencia, CA, USA), as described previously (25). Quantitatively isolated total RNA from control and DMC treated cells was used for cDNA synthesis, labeling and microarray hybridization, followed by flour-labeled cDNA hybridizing of the cell complements on the chip (Affymetrix GeneChip Human Gene 1.0 ST array; Affymetrix, Santa Clara, CA, USA). Finally, the resulting localized concentrations of fluorescent molecules on the chip were detected and quantitated (Asia BioInnovations Corporation, Taipei, Taiwan). The resulting data were further analyzed by Expression Console software (Affymetrix) with default Robust Multichip Analysis (RMA) parameters (25-27). Down- or up-regulated gene expressions at least at two-fold change by DMC were recorded and identified.

Statistical analysis. Data are representative of three seperate assays. Differences between control and DMC-treated groups were presented for up to a 2-fold-change. +, up-regulation; –, down-regulation.

Results

DMC up- and down-regulated gene expression in NCI-H460 cells. Table I indicates that several genes were up-regulated after treatment with 35 μM of DMC. In particular, 2 genes were up-regulated over 4-fold, one being the *TIPARP* (TCDD-inducible poly(ADP-ribose) polymerase) gene, associated with apoptosis; 13 genes were over from 3- to 4-fold, like, for instance, *CCNE2* (cyclin E2), associated with cell cycle and 129 genes were up-regulated from 2- to 3-fold, with *ERCC6L* (excision repair cross-complementing rodent repair deficiency, complementation group 6-like), *TP53INP1* (tumor protein p53 inducible nuclear protein 1) and *BRCC3* (BRCA1/BRCA2-containing complex, subunit 3) associated with DNA damage and repair, *TUBB4B* (tubulin, beta 4B class IVb), *CCNE1* (cyclin E1), *CDC6* (cell division cycle 6 homolog (*S. cerevisiae*), *MAP9* (microtubule-associated protein 9) and *CDC25A* [cell division cycle 25 homolog A (*S. pombe*)], associated with cell cycle distribution and *CARD6* (caspase recruitment domain family, member 6), associated with cell apoptosis, to name a few.

Table II shows that the expression of 28 genes was down-regulated over 4-fold, such as *DDIT3* (DNA-damage-inducible transcript 3), associated with DNA damage, *CHL1* [cell adhesion molecule with homology to *L1CAM* (close homolog of L1)] associated with cell apoptosis; expression of expression of 33 genes was decreased 3- to 4-fold, such as *CLK1* (CDC-like kinase 1) associated with cell cycle, *PDCD1LG2* (programmed cell death 1 ligand 2), associated with cell apoptosis. The expression of 118 genes was decreased 2- to 3-fold, such as *ERCC3* (excision repair cross-complementing rodent repair deficiency, complementation group 3), *DDIT4* (DNA-damage-inducible transcript 4) associated with DNA damage and repair, *CCNL* (cyclin L2), *TUBB4B* (tubulin, beta 4B class IVb), CCNE1 (cyclin E1), *CDC6* (cell division cycle 6 homolog (*S. cerevisiae*)) and *CCPG1* (cell cycle progression 1; *DYX1C1-CCPG1*) readthrough (non-protein coding) associated with cell cycle, *MCL1* [myeloid cell leukemia sequence 1 (*BCL2*-related)], *HYOU1* (hypoxia up-regulated 1), *SLC25A37*

Table I. *Representative genes of NCI-H460 cells that were up-regulated by DMC treatment*.

Fold change	Gene symbol	mRNA description
5.44	*TIPARP*	TCDD-inducible poly(ADP-ribose) polymerase
4.10	*ID3*	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
3.83	*DLX2*	distal-less homeobox 2
3.67	*CYP1A1*	cytochrome P450, family 1, subfamily A, polypeptide 1
3.54	*SNRPN*	small nuclear ribonucleoprotein polypeptide N; uncharacterized LOC100506948; 116-28; 115-26; 115-13; 115-7; 107; 115-5; 115-9; 115-11; 115-12; 115-29; 115-36; 115-43
3.32	*CCNE2*	cyclin E2
3.25	*ID1*	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
3.18	*LIMD1-AS1*	LIMD1 antisense RNA 1 (non-protein coding)
3.11	*LIMD1-AS1*	LIMD1 antisense RNA 1 (non-protein coding)
3.05	*CDRT1*	CMT1A duplicated region transcript 1; F-box and WD repeat domain containing 10
3.04	*ID2*	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
3.00	*C3orf14*	chromosome 3 open reading frame 14
2.94	*POLR2A*	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
2.93	*SFN*	stratifin
2.86	*DHFR*	dihydrofolate reductase
2.86	*PDGFD*	platelet derived growth factor D
2.85	*ERCC6L*	excision repair cross-complementing rodent repair deficiency, complementation group 6-like
2.84	*NAP1L2*	nucleosome assembly protein 1-like 2
2.84	*DHFR*	dihydrofolate reductase
2.84	*RNU5D-1*	RNA, U5D small nuclear 1
2.74	*FGD6*	FYVE, RhoGEF and PH domain containing 6
2.74	*HSPH1*	heat shock 105kDa/110kDa protein 1
2.73	*CLSPN*	claspin
2.71	*HECW1*	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
2.65	*CROT*	carnitine O-octanoyltransferase
2.64	*DHFR*	dihydrofolate reductase; dihydrofolate reductase pseudogene
2.62	*HSPA1A*	heat shock 70kDa protein 1A; 1B
2.62	*HSPA1B*	heat shock 70kDa protein 1B; 1A
2.56	*GTF2I*	general transcription factor IIi
2.56	*TDO2*	tryptophan 2,3-dioxygenase
2.56	*NAP1L3*	nucleosome assembly protein 1-like 3
2.55	*NAV1*	neuron navigator 1
2.55	*TUBB4B*	tubulin, beta 4B class IVb
2.54	*HSPA1A*	heat shock 70kDa protein 1A; heat shock 70kDa protein 1B
2.52	*PIK3R3*	phosphoinositide-3-kinase, regulatory subunit 3 (gamma)
2.51	*METTL7B*	methyltransferase like 7B
2.49	*EML1*	echinoderm microtubule associated protein like 1
2.49	*RNU1-10P*	RNA, U1 small nuclear 10, pseudogene
2.49	*CCNE1*	cyclin E1
2.48	*RN5S63*	RNA, 5S ribosomal 63
2.47	*CLSPN*	claspin
2.47	*TMEM14A*	transmembrane protein 14A
2.45	*SNORD115-32*	small nucleolar RNA, C/D box 115-32
2.43	*VDR*	vitamin D (1,25- dihydroxyvitamin D3) receptor
2.43	*CRYZ*	crystallin, zeta (quinone reductase)
2.42	*ADI1*	acireductone dioxygenase 1
2.42	*CCL2*	chemokine (C-C motif) ligand 2
2.41	*IQCG*	IQ motif containing G
2.41	*TSPAN12*	tetraspanin 12
2.38	*ZNF382*	zinc finger protein 382
2.38	*CEP78*	centrosomal protein 78kDa
2.36	*COMMD9*	COMM domain containing 9
2.36	*RN5S55*	RNA, 5S ribosomal 55
2.35	*RALGPS2*	Ral GEF with PH domain and SH3 binding motif 2
2.35	*SNORA23*	small nucleolar RNA, H/ACA box 23
2.34	*GLULP4*	glutamate-ammonia ligase (glutamine synthetase) pseudogene 4
2.34	*LOC646813*	DEAH (Asp-Glu-Ala-His) box polypeptide 9 pseudogene
2.33	*MAGEE1*	melanoma antigen family E, 1

Table I. *continued*

Table I. *continued*

Fold change	Gene symbol	mRNA description
2.32	*PRKAR1B*	protein kinase, cAMP-dependent, regulatory, type I, beta
2.31	*CDC6*	cell division cycle 6 homolog (*S. cerevisiae*)
2.29	*ZNF578*	zinc finger protein 578
2.29	*NRG4*	neuregulin 4
2.29	*LPCAT2*	lysophosphatidylcholine acyltransferase 2
2.29	*PCNA*	proliferating cell nuclear antigen
2.29	*GEMIN6*	gem (nuclear organelle) associated protein 6
2.29	*SNORA3*	small nucleolar RNA, H/ACA box 3; ribosomal protein L27a
2.28	*CARD6*	caspase recruitment domain family, member 6
2.28	*PRKAR2B*	protein kinase, cAMP-dependent, regulatory, type II, beta
2.27	*MYO1D*	myosin ID
2.26	*SMAD9*	SMAD family member 9
2.26	*GPX3*	glutathione peroxidase 3 (plasma)
2.26	*PLCE1*	phospholipase C, epsilon 1
2.24	*TIMM17A*	translocase of inner mitochondrial membrane 17 homolog A (yeast)
2.24	*ZNF117*	zinc finger protein 117
2.24	*UST*	uronyl-2-sulfotransferase
2.23	*AHSA1*	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)
2.23	*ENPEP*	glutamyl aminopeptidase (aminopeptidase A)
2.21	*GATA2*	GATA binding protein 2
2.21	*MYL6*	myosin, light chain 6, alkali, smooth muscle and non-muscle
2.21	*KIT*	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
2.20	*HMGN3P1*	high mobility group nucleosomal binding domain 3 pseudogene 1
2.20	*STS*	steroid sulfatase (microsomal), isozyme S
2.19	*JAKMIP2*	janus kinase and microtubule interacting protein 2
2.19	*UTP3*	UTP3, small subunit (SSU) processome component, homolog (*S. cerevisiae*)
2.18	*TP53INP1*	tumor protein p53 inducible nuclear protein 1
2.18	*CT45A4*	cancer/testis antigen family 45, member A4, A5, A1, A3, A2, A6
2.18	*GDAP1*	ganglioside induced differentiation associated protein 1
2.17	*SLC4A8*	solute carrier family 4, sodium bicarbonate cotransporter, member 8
2.17	*MCM4*	minichromosome maintenance complex component 4
2.15	*TUBB2A*	tubulin, beta 2A class IIa
2.15	*SMAD6*	SMAD family member 6
2.15	*FEN1*	flap structure-specific endonuclease 1
2.14	*MAP9*	microtubule-associated protein 9
2.14	*HMGCS1*	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
2.14	*ATP6V0D1*	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1
2.14	*TUBB3*	tubulin, beta 3 class III
2.13	*BRCC3*	BRCA1/BRCA2-containing complex, subunit 3
2.12	*TMEM171*	transmembrane protein 171
2.12	*RN5S353*	RNA, 5S ribosomal 353
2.11	*CYP1B1*	cytochrome P450, family 1, subfamily B, polypeptide 1
2.10	*GRK5*	G protein-coupled receptor kinase 5
2.10	*FERMT1*	fermitin family member 1
2.09	*TUBB4B*	tubulin, beta 4B class IVb
2.09	*CXorf57*	chromosome X open reading frame 57
2.08	*ZNF480*	zinc finger protein 480
2.06	*POLE2*	polymerase (DNA directed), epsilon 2, accessory subunit
2.06	*GEMIN5*	gem (nuclear organelle) associated protein 5
2.05	*ARHGEF3*	Rho guanine nucleotide exchange factor (GEF) 3
2.04	*NCOA5*	nuclear receptor coactivator 5
2.04	*TRIP13*	thyroid hormone receptor interactor 13
2.04	*UMPS*	uridine monophosphate synthetase
2.04	*MRPL1*	mitochondrial ribosomal protein L1
2.03	*HIST1H3A*	histone cluster 1, H3a; H3f; H3b; H3h; H3g; H3i; h H3e; H3c; H3d
2.02	*BRMS1L*	breast cancer metastasis-suppressor 1-like
2.02	*CDC25A*	cell division cycle 25 homolog A (S. pombe)
2.01	*ARHGEF26*	Rho guanine nucleotide exchange factor (GEF) 26
2.01	CKAP2	cytoskeleton associated protein 2
–2.01	*ITPRIP*	inositol 1,4,5-trisphosphate receptor interacting protein
–2.02	*CCNL2*	cyclin L2
–2.03	*ABCA2*	ATP-binding cassette, sub-family A (ABC1), member 2

Table I. *continued*

Table I. *continued*

Fold change	Gene symbol	mRNA description
–2.03	*MIR29C*	microRNA 29c
–2.04	*CACNA2D3*	calcium channel, voltage-dependent, alpha 2/delta subunit 3
–2.05	*MCL1*	myeloid cell leukemia sequence 1 (BCL2-related)
–2.05	*PRKACB*	protein kinase, cAMP-dependent, catalytic, beta
–2.05	*ERCC3*	excision repair cross-complementing rodent repair deficiency, complementation group 3
–2.06	*ADAM20P1*	ADAM metallopeptidase domain 20 pseudogene 1
–2.06	*EGF*	epidermal growth factor
–2.07	*CD36*	CD36 molecule (thrombospondin receptor)
–2.08	*ARHGEF10*	Rho guanine nucleotide exchange factor (GEF) 10
–2.08	*CD226*	CD226 molecule
–2.09	*GPT2*	glutamic pyruvate transaminase
–2.09	*TAPBP*	TAP binding protein (tapasin)
–2.09	*ECE1*	endothelin converting enzyme 1
–2.10	*GDF15*	growth differentiation factor 15
–2.10	*EDNRB*	endothelin receptor type B
–2.13	*SHMT2*	serine hydroxymethyltransferase 2 (mitochondrial)
–2.15	*PSPH*	phosphoserine phosphatase
–2.15	*PHGDH*	phosphoglycerate dehydrogenase
–2.17	*DPYD*	dihydropyrimidine dehydrogenase
–2.18	*CALM1*	calmodulin 1 (phosphorylase kinase, delta)
–2.18	*IFRD1*	interferon-related developmental regulator 1
–2.19	*HIST3H2BB*	histone cluster 3, H2bb
–2.21	*TAPBP*	TAP binding protein (tapasin)
–2.21	*PABPC1L*	poly(A) binding protein, cytoplasmic 1-like
–2.22	*MARS*	methionyl-tRNA synthetase
–2.22	*IGF2R*	insulin-like growth factor 2 receptor
–2.24	*CAMK2D*	calcium/calmodulin-dependent protein kinase II delta
–2.24	*ITGA6*	integrin, alpha 6
–2.24	*VEGFC*	vascular endothelial growth factor C
–2.26	*MACC1*	metastasis associated in colon cancer 1
–2.28	*FN1*	fibronectin 1
–2.31	*PTPRH*	protein tyrosine phosphatase, receptor type, H, D
–2.31	*CACNB2*	calcium channel, voltage-dependent, beta 2 subunit
–2.31	*SOCS2*	suppressor of cytokine signaling 2
–2.32	*CRLF2*	cytokine receptor-like factor 2
–2.35	*HYOU1*	hypoxia up-regulated 1
–2.36	*RBMS3*	RNA binding motif, single stranded interacting protein 3
–2.37	*PLAU*	plasminogen activator, urokinase
–2.38	*GABBR2*	gamma-aminobutyric acid (GABA) B receptor, 2
–2.39	*CPS1*	carbamoyl-phosphate synthase 1, mitochondrial
–2.43	*MIRLET7F1*	microRNA let-7f-1
–2.44	*PLD1*	phospholipase D1, phosphatidylcholine-specific
–2.44	*ACSS3*	acyl-CoA synthetase short-chain family member 3
–2.44	*GFPT1*	glutamine-fructose-6-phosphate transaminase 1
–2.45	*TUBE1*	tubulin, epsilon 1
–2.46	*SLC25A37*	solute carrier family 25 (mitochondrial iron transporter), member 37
–2.47	*ASNS*	asparagine synthetase (glutamine-hydrolyzing)
–2.47	*CCPG1*	cell cycle progression 1; DYX1C1-CCPG1 readthrough (non-protein coding)
–2.47	*ZNF724P*	zinc finger protein 724, pseudogene
–2.49	*MIR21*	microRNA 21
–2.50	*IGFBP3*	insulin-like growth factor binding protein 3
–2.50	*ANGPTL4*	angiopoietin-like 4
–2.51	*FRK*	fyn-related kinase
–2.51	*H1F0*	H1 histone family, member 0
–2.53	*CARS*	cysteinyl-tRNA synthetase
–2.55	*DUSP1*	dual specificity phosphatase 1
–2.56	*MIR186*	microRNA 186
–2.57	*CYP20A1*	cytochrome P450, family 20, subfamily A, polypeptide 1
–2.60	*F2R*	coagulation factor II (thrombin) receptor
–2.60	*PTPRB*	protein tyrosine phosphatase, receptor type, B
–2.61	*COL4A6*	collagen, type IV, alpha 6
–2.62	*TMEM100*	transmembrane protein 100

Table I. *continued*

Table I. *continued*

Fold change	Gene symbol	mRNA description
–2.66	*KLRC2*	killer cell lectin-like receptor subfamily C, member 2
–2.66	*GLRXP3*	glutaredoxin (thioltransferase) pseudogene 3
–2.69	*NFE2L3*	nuclear factor (erythroid-derived 2)-like 3
–2.70	*ALPK2*	alpha-kinase 2
–2.73	*COL4A5*	collagen, type IV, alpha 5
–2.77	*IL8*	interleukin 8
–2.79	*EFEMP1*	EGF containing fibulin-like extracellular matrix protein 1
–2.81	*PDE1A*	phosphodiesterase 1A, calmodulin-dependent
–2.83	*PDK1*	pyruvate dehydrogenase kinase, isozyme 1
–2.84	*PLA2G7*	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
–2.85	*LONP1*	ion peptidase 1, mitochondrial
–2.86	*PTPRM*	protein tyrosine phosphatase, receptor type, M
–2.89	*PLA2R1*	phospholipase A2 receptor 1, 180kDa
–2.92	*DDIT4*	DNA-damage-inducible transcript 4
–2.93	*CFH*	complement factor H
–2.93	*PCK2*	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
–2.96	*HSPA13*	heat shock protein 70kDa family, member 13
–2.98	*TFPI*	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
–3.00	*LIF*	leukemia inhibitory factor
–3.00	*TNFRSF19*	tumor necrosis factor receptor superfamily, member 19
–3.02	*SUCNR1*	succinate receptor 1
–3.04	*CLK1*	CDC-like kinase 1
–3.10	*GPR37*	G protein-coupled receptor 37
–3.15	*PDCD1LG2*	programmed cell death 1 ligand 2
–3.16	*IL18R1*	interleukin 18 receptor 1
–3.17	*FSTL1*	follistatin-like 1
–3.17	*TSPAN8*	tetraspanin 8
–3.22	*VEGFA*	vascular endothelial growth factor A
–3.23	*CNTN1*	contactin 1
–3.23	*SAT1*	spermidine/spermine N1-acetyltransferase 1
–3.24	*PDE3A*	phosphodiesterase 3A, cGMP-inhibited
–3.27	*SNORD13P2*	small nucleolar RNA, C/D box 13 pseudogene 2
–3.28	*PLAUR*	plasminogen activator, urokinase receptor
–3.29	*TRPC6*	transient receptor potential cation channel, subfamily C, member 6
–3.31	*B4GALT1*	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1
–3.34	*IFITM1*	interferon induced transmembrane protein 1
–3.42	*PITPNC1*	phosphatidylinositol transfer protein, cytoplasmic 1
–3.43	*SCD*	stearoyl-CoA desaturase (delta-9-desaturase)
–3.56	*TMEM144*	transmembrane protein 144
–3.61	*GTPBP2*	GTP binding protein 2
–3.64	*ABCC3*	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
–3.66	*TNFAIP3*	tumor necrosis factor, alpha-induced protein 3
–3.79	*SMOX*	spermine oxidase
–3.88	*DUSP6*	dual specificity phosphatase 6
–3.95	*ALDH1L2*	aldehyde dehydrogenase 1 family, member L2
–4.28	*IL6*	Interleukin 6 (interferon, beta 2)
–4.37	*CTH*	cystathionase (cystathionine gamma-lyase)
–4.46	*HPGDS*	hematopoietic prostaglandin D synthase
–4.48	*SCARA5*	scavenger receptor class A, member 5 (putative)
–4.62	*ABI3BP*	ABI family, member 3 (NESH) binding protein
–4.68	*WARS*	tryptophanyl-tRNA synthetase
–4.73	*NUPR1*	nuclear protein, transcriptional regulator, 1
–4.75	*RN5S449*	RNA, 5S ribosomal 449
–5.17	*CBS*	cystathionine-beta-synthase
–5.18	*DDIT3*	DNA-damage-inducible transcript 3
–5.61	*ABCC9*	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
–5.70	*ASS1*	argininosuccinate synthase 1
–5.75	*KLRC3*	killer cell lectin-like receptor subfamily C, member 3, 2
–5.83	*PARK2*	parkinson protein 2, E3 ubiquitin protein ligase (parkin)
–6.82	*CHL1*	cell adhesion molecule with homology to L1CAM (close homolog of L1)
–8.53	*TGFB2*	transforming growth factor, beta 2
–12.39	*FST*	follistatin
–12.90	*GABRE*	gamma-aminobutyric acid (GABA) A receptor, epsilon; microRNA 452; microRNA 224
–14.69	*IL1RL1*	interleukin 1 receptor-like 1

Table II. *Representative genes of NCI-H460 cells those were down-regulated by DMC treatment*.

Fold change	Gene symbol	mRNA description
-2.01	*ITPRIP*	inositol 1,4,5-trisphosphate receptor interacting protein
-2.02	*CCNL2*	cyclin L2
-2.03	*ABCA2*	ATP-binding cassette, sub-family A (ABC1), member 2
-2.03	*MIR29C*	microRNA 29c
-2.04	*CACNA2D3*	calcium channel, voltage-dependent, alpha 2/delta subunit 3
-2.05	*MCL1*	myeloid cell leukemia sequence 1 (BCL2-related)
-2.05	*PRKACB*	protein kinase, cAMP-dependent, catalytic, beta
-2.05	*ERCC3*	excision repair cross-complementing rodent repair deficiency, complementation group 3
-2.06	*ADAM20P1*	ADAM metallopeptidase domain 20 pseudogene 1
-2.06	*EGF*	epidermal growth factor
-2.07	*CD36*	CD36 molecule (thrombospondin receptor)
-2.08	*ARHGEF10*	Rho guanine nucleotide exchange factor (GEF) 10
-2.08	*CD226*	CD226 molecule
-2.09	*GPT2*	glutamic pyruvate transaminase
-2.09	*TAPBP*	TAP binding protein (tapasin)
-2.09	*ECE1*	endothelin converting enzyme 1
-2.10	*GDF15*	growth differentiation factor 15
-2.10	*EDNRB*	endothelin receptor type B
-2.13	*SHMT2*	serine hydroxymethyltransferase 2 (mitochondrial)
-2.15	*PSPH*	phosphoserine phosphatase
-2.15	*PHGDH*	phosphoglycerate dehydrogenase
-2.17	*DPYD*	dihydropyrimidine dehydrogenase
-2.18	*CALM1*	calmodulin 1 (phosphorylase kinase, delta)
-2.18	*IFRD1*	interferon-related developmental regulator 1
-2.19	*HIST3H2BB*	histone cluster 3, H2bb
-2.21	*TAPBP*	TAP binding protein (tapasin)
-2.21	*PABPC1L*	poly(A) binding protein, cytoplasmic 1-like
-2.22	*MARS*	methionyl-tRNA synthetase
-2.22	*IGF2R*	insulin-like growth factor 2 receptor
-2.24	*CAMK2D*	calcium/calmodulin-dependent protein kinase II delta
-2.24	*ITGA6*	integrin, alpha 6
-2.24	*VEGFC*	vascular endothelial growth factor C
-2.26	*MACC1*	metastasis associated in colon cancer 1
-2.28	*FN1*	fibronectin 1
-2.31	*PTPRH*	protein tyrosine phosphatase, receptor type, H, D
-2.31	*CACNB2*	calcium channel, voltage-dependent, beta 2 subunit
-2.31	*SOCS2*	suppressor of cytokine signaling 2
-2.32	*CRLF2*	cytokine receptor-like factor 2
-2.35	*HYOU1*	hypoxia up-regulated 1
-2.36	*RBMS3*	RNA binding motif, single stranded interacting protein 3
-2.37	*PLAU*	plasminogen activator, urokinase
-2.38	*GABBR2*	gamma-aminobutyric acid (GABA) B receptor, 2
-2.39	*CPS1*	carbamoyl-phosphate synthase 1, mitochondrial
-2.43	*MIRLET7F1*	microRNA let-7f-1
-2.44	*PLD1*	phospholipase D1, phosphatidylcholine-specific
-2.44	*ACSS3*	acyl-CoA synthetase short-chain family member 3
-2.44	*GFPT1*	glutamine-fructose-6-phosphate transaminase 1
-2.45	*TUBE1*	tubulin, epsilon 1
-2.46	*SLC25A37*	solute carrier family 25 (mitochondrial iron transporter), member 37
-2.47	*ASNS*	asparagine synthetase (glutamine-hydrolyzing)
-2.47	*CCPG1*	cell cycle progression 1; DYX1C1-CCPG1 readthrough (non-protein coding)
-2.47	*ZNF724P*	zinc finger protein 724, pseudogene
-2.49	*MIR21*	microRNA 21
-2.50	*IGFBP3*	insulin-like growth factor binding protein 3
-2.50	*ANGPTL4*	angiopoietin-like 4
-2.51	*FRK*	fyn-related kinase
-2.51	*H1F0*	H1 histone family, member 0
-2.53	*CARS*	cysteinyl-tRNA synthetase
-2.55	*DUSP1*	dual specificity phosphatase 1
-2.56	*MIR186*	microRNA 186
-2.57	*CYP20A1*	cytochrome P450, family 20, subfamily A, polypeptide 1
-2.60	*F2R*	coagulation factor II (thrombin) receptor
-2.60	*PTPRB*	protein tyrosine phosphatase, receptor type, B

Table II. *continued*

Table II. *continued*

Fold change	Gene symbol	mRNA description
-2.61	*COL4A6*	collagen, type IV, alpha 6
-2.62	*TMEM100*	transmembrane protein 100
-2.66	*KLRC2*	killer cell lectin-like receptor subfamily C, member 2
-2.66	*GLRXP3*	glutaredoxin (thioltransferase) pseudogene 3
-2.69	*NFE2L3*	nuclear factor (erythroid-derived 2)-like 3
-2.70	*ALPK2*	alpha-kinase 2
-2.73	*COL4A5*	collagen, type IV, alpha 5
-2.77	*IL8*	interleukin 8
-2.79	*EFEMP1*	EGF containing fibulin-like extracellular matrix protein 1
-2.81	*PDE1A*	phosphodiesterase 1A, calmodulin-dependent
-2.83	*PDK1*	pyruvate dehydrogenase kinase, isozyme 1
-2.84	*PLA2G7*	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
-2.85	*LONP1*	ion peptidase 1, mitochondrial
-2.86	*PTPRM*	protein tyrosine phosphatase, receptor type, M
-2.89	*PLA2R1*	phospholipase A2 receptor 1, 180kDa
-2.92	*DDIT4*	DNA-damage-inducible transcript 4
-2.93	*CFH*	complement factor H
-2.93	*PCK2*	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
-2.96	*HSPA13*	heat shock protein 70kDa family, member 13
-2.98	*TFPI*	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
-3.00	*LIF*	leukemia inhibitory factor
-3.00	*TNFRSF19*	tumor necrosis factor receptor superfamily, member 19
-3.02	*SUCNR1*	succinate receptor 1
-3.04	*CLK1*	CDC-like kinase 1
-3.10	*GPR37*	G protein-coupled receptor 37
-3.15	*PDCD1LG2*	programmed cell death 1 ligand 2
-3.16	*IL18R1*	interleukin 18 receptor 1
-3.17	*FSTL1*	follistatin-like 1
-3.17	*TSPAN8*	tetraspanin 8
-3.22	*VEGFA*	vascular endothelial growth factor A
-3.23	*CNTN1*	contactin 1
-3.23	*SAT1*	spermidine/spermine N1-acetyltransferase 1
-3.24	*PDE3A*	phosphodiesterase 3A, cGMP-inhibited
-3.27	*SNORD13P2*	small nucleolar RNA, C/D box 13 pseudogene 2
-3.28	*PLAUR*	plasminogen activator, urokinase receptor
-3.29	*TRPC6*	transient receptor potential cation channel, subfamily C, member 6
-3.31	*B4GALT1*	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1
-3.34	*IFITM1*	interferon induced transmembrane protein 1
-3.42	*PITPNC1*	phosphatidylinositol transfer protein, cytoplasmic 1
-3.43	*SCD*	stearoyl-CoA desaturase (delta-9-desaturase)
-3.56	*TMEM144*	transmembrane protein 144
-3.61	*GTPBP2*	GTP binding protein 2
-3.64	*ABCC3*	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
-3.66	*TNFAIP3*	tumor necrosis factor, alpha-induced protein 3
-3.79	*SMOX*	spermine oxidase
-3.88	*DUSP6*	dual specificity phosphatase 6
-3.95	*ALDH1L2*	aldehyde dehydrogenase 1 family, member L2
-4.28	*IL6*	Interleukin 6 (interferon, beta 2)
-4.37	*CTH*	cystathionase (cystathionine gamma-lyase)
-4.46	*HPGDS*	hematopoietic prostaglandin D synthase
-4.48	*SCARA5*	scavenger receptor class A, member 5 (putative)
-4.62	*ABI3BP*	ABI family, member 3 (NESH) binding protein
-4.68	*WARS*	tryptophanyl-tRNA synthetase
-4.73	*NUPR1*	nuclear protein, transcriptional regulator, 1
-4.75	*RN5S449*	RNA, 5S ribosomal 449
-5.17	*CBS*	cystathionine-beta-synthase
-5.18	*DDIT3*	DNA-damage-inducible transcript 3
-5.61	*ABCC9*	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
-5.70	*ASS1*	argininosuccinate synthase 1
-5.75	*KLRC3*	killer cell lectin-like receptor subfamily C, member 3, 2
-5.83	*PARK2*	parkinson protein 2, E3 ubiquitin protein ligase (parkin)
-6.82	*CHL1*	cell adhesion molecule with homology to L1CAM (close homolog of L1)
-8.53	*TGFB2*	transforming growth factor, beta 2
-12.39	*FST*	follistatin
-12.90	*GABRE*	gamma-aminobutyric acid (GABA) A receptor, epsilon; microRNA 452; microRNA 224
-14.69	*IL1RL1*	interleukin 1 receptor-like 1

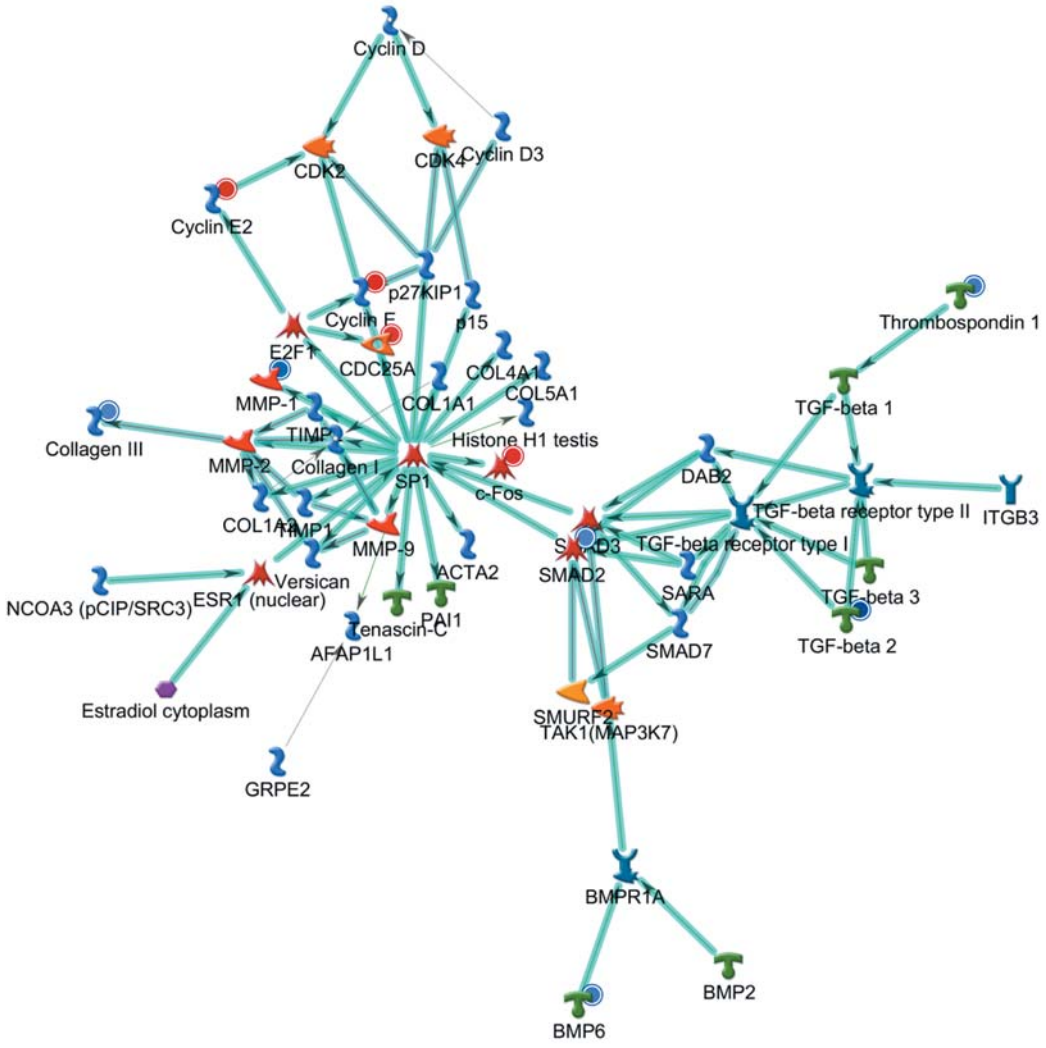

Figure 1. *The top scored (by the number of pathways) network from GeneGo 02. Thick cyan lines were used as fragments of canonical pathways. Red-colored circles represent up-regulated genes and blue-colored circles show down-regulated genes. The 'checkerboard' color indicates mixed expressions for the genes between files or between multiple tags for the same gene.*

[solute carrier family 25 (mitochondrial iron transporter) member 37)] and *TP53INP1* (tumor protein p53 inducible nuclear protein 1).

GeneGo analysis by the number of pathway networks involved for the top scored gene expression alterations on DMC-treated NCI-H460 cells. After cDNA microarray analysis, all samples were further processed by using the GeneGo system to enrich the analysis of significant genes in the context of pathways. The results shown in Figures 1, 2 and 3 map the processes in possible signal outcomes. Red-colored (up-regulation) and blue-colored circles (down-regulation) represent different intensities indicating various enhancing or inhibiting effects in NCI-H460 cell after DMC treatment.

Discussion

In the present study, we demonstrated that several genes involved in DNA damage and repair were up-regulated as was case with the *TP53INP1* (tumor protein p53 inducible nuclear protein 1) gene that was increased 2.18-fold; it is well known that, after DNA damage, p53 protein expression is increased (28). Other examples include *ERCC6L* and BRCC3 (*BRCA1*/*BRCA2*-containing complex, subunit 3) that were up-regulated by 2.85- and 2.13-fold, respectively. These two genes have been reported to be involved in cell responses concerning repair of DNA damage for maintaining survival (29, 30). Similarly, *CCNE2* showed a 3.32-fold increase, and the associated with cell-cycle distribution genes *CCNE1*,

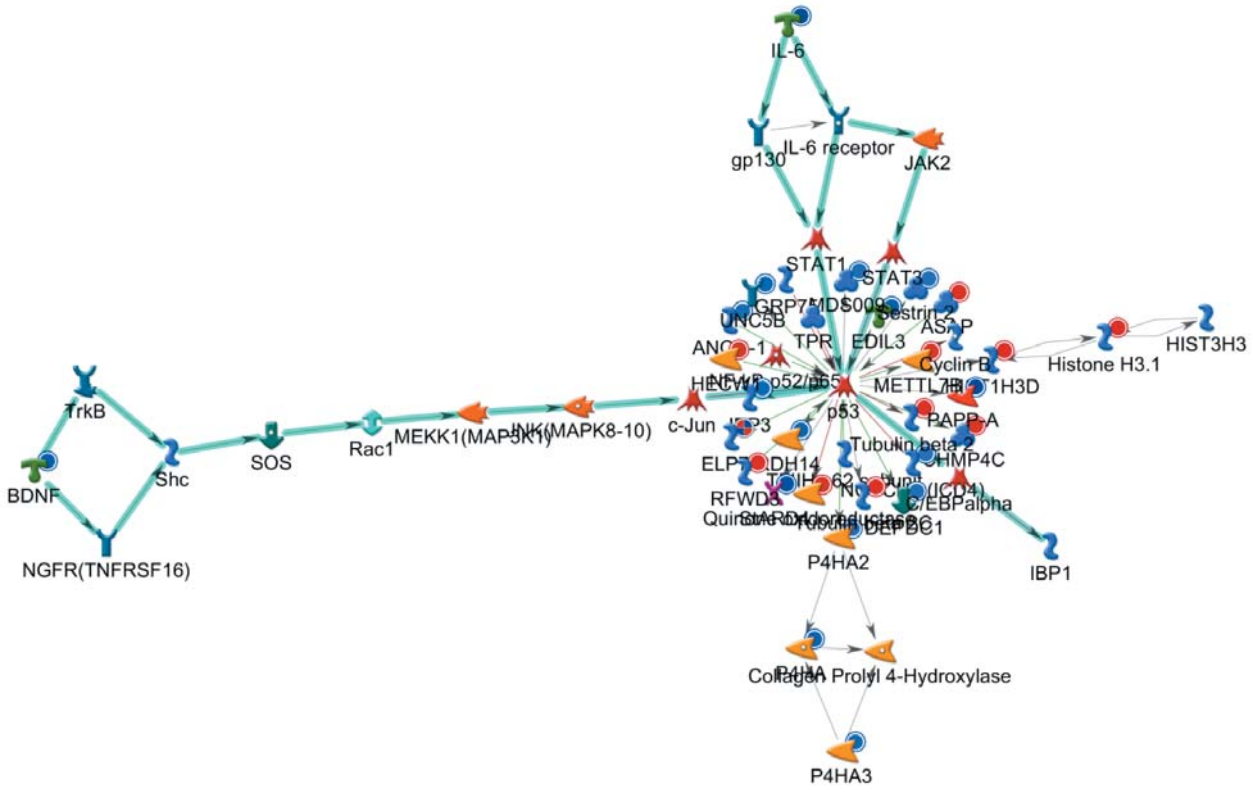

Figure 2. *The second scored (by the number of pathways) network from GeneGo 02. Thick cyan lines were used as fragments of canonical pathways. Red-colored circles represent up-regulated genes and blue-colored circles show down-regulated genes. The 'checkerboard' color indicates mixed expressions for the genes between files or between multiple tags for the same gene.*

CDC6, *MAP9* and *CDC25A* exhibited a 2.49-, 2.31-, 2.14- and 2.02-fold up-regulation, respectively (Table I). Also, Table I shows that *TIPARP* gene expression was increased by 5.44-fold; it is well known that cells under apoptosis may go through an increase of poly(ADP-ribose) polymerase levels (31). Moreover, the associated with cell apoptosis gene *CARD6* was up-regulated by 2.28-fold. In general, it is well-documented that some anticancer drugs induce cancer cell apoptosis through the activation of the caspase pathway, especially the caspase-8, -9 and -3 (32).

Table II shows that DMC down-regulated the gene levels of several genes like, *DDIT3* and *DDIT4* that were suppressed by 5.18- and 2.92-fold, respectively; DDIT3 has been reported to be involved in DNA damage and repair mechanisms (33). DMC also inhibited the levels of genes associated with cell cycle like *CLK1* by 3.04-fold, *CCNL1* by 2.02-fold, *TUBE1* (tubulin, epsilon 1) by 2.45-fold, *CHL1* by 6.82-fold and *CCPG1* by 2.47-fold.

It is also well-known that anticancer drugs induce cancer cell apoptosis through the arrest of cell cycle at the G_0/G_1 or G_2/M phase (34, 35). Indeed, DMC inhibited the levels of genes associated with cell apoptosis, like *CHL1* by 6.82-fold, *PDCD1LG2* by 3.15-fold, *MCL1* by 2.05-fold and *SLC25A37* by 2.46-fold.

Conclusion

Tables I and II indicate that numerous genes that are associated with DNA damage and repair, cell-cycle check point and cell apoptosis in NCI-H460 cells after exposure to DMC can be up- or down-regulated. These results were further confirmed by using the GeneGo analysis program, as depicted in Figures 1 to 3, showing their possible signaling complex interactions. The noted changes provide information for understanding the cytotoxic action of DMC at the genetic level. Gene alterations may be proven as useful biomarkers or targets for the diagnosis and treatment of human lung cancer in the future. However, further studies are necessary in order to expand or append our current knowledge.

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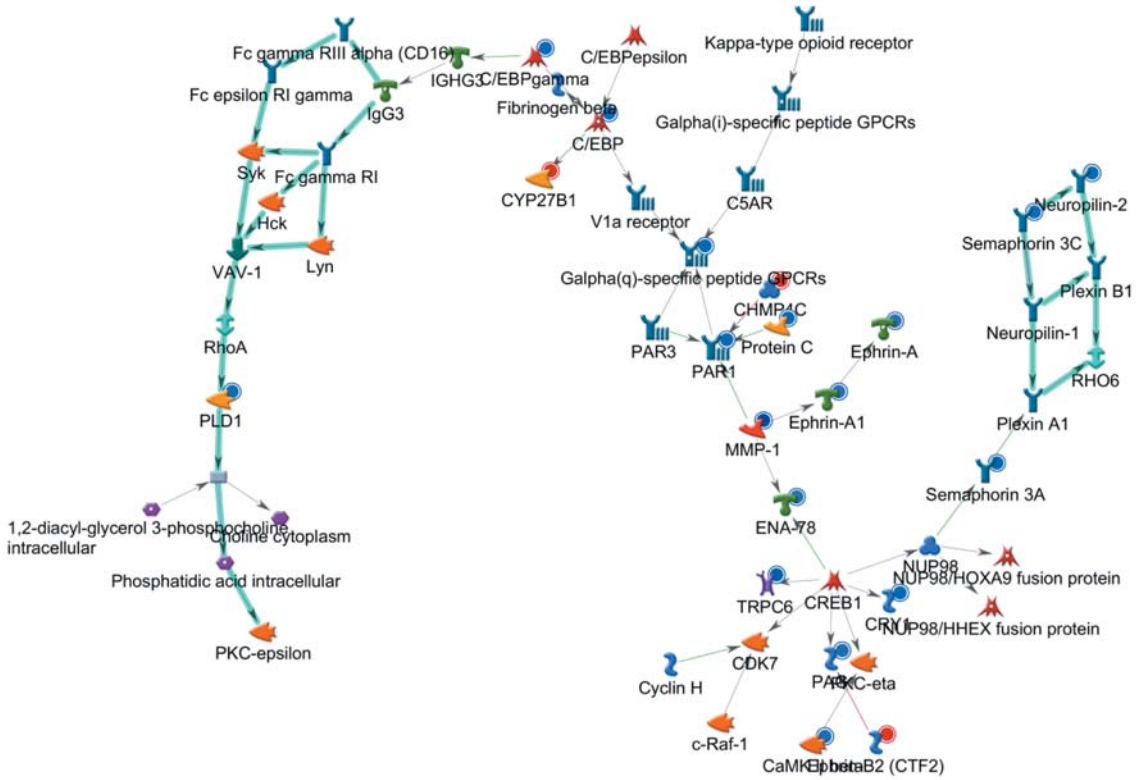

Figure 3. *The third scored (by the number of pathways) network from GeneGo 02. Thick cyan lines were used as fragments of canonical pathways. Red-colored circles represent up-regulated genes and blue-colored circles show down-regulated genes. The 'checkerboard' color indicates mixed expressions for the genes between files or between multiple tags for the same gene.*

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