

Curcumin alters gene expression-associated DNA damage, cell cycle, cell survival and cell migration and invasion in NCI-H460 human lung cancer cells *in vitro*

I-TSANG CHIANG^{1,2}, WEI-SHU WANG³, HSIN-CHUNG LIU⁴, SU-TSO YANG⁵,
NOU-YING TANG⁶ and JING-GUNG CHUNG^{4,7}

¹Department of Radiation Oncology, National Yang-Ming University Hospital, Yilan 260;

²Department of Radiological Technology, Central Taiwan University of Science and Technology, Taichung 40601;

³Department of Internal Medicine, National Yang-Ming University Hospital, Yilan 260;

⁴Department of Biological Science and Technology, China Medical University, Taichung 404;

⁵Department of Radiology, China Medical University Hospital, Taichung 404;

⁶Graduate Institute of Chinese Medicine, China Medical University, Taichung 404;

⁷Department of Biotechnology, Asia University, Taichung 404, Taiwan, R.O.C.

Received March 31, 2015; Accepted June 26, 2015

DOI: 10.3892/or.2015.4159

Abstract. Lung cancer is the most common cause of cancer mortality and new cases are on the increase worldwide. However, the treatment of lung cancer remains unsatisfactory. Curcumin has been shown to induce cell death in many human cancer cells, including human lung cancer cells. However, the effects of curcumin on genetic mechanisms associated with these actions remain unclear. Curcumin (2 μ M) was added to NCI-H460 human lung cancer cells and the cells were incubated for 24 h. Total RNA was extracted from isolated cells for cDNA synthesis, labeling, microarray hybridization and flour-labeled cDNA hybridized on chip. Localized concentrations of fluorescent molecules were detected and quantified using Expression Console software (Affymetrix) with default RMA parameters. GeneGo software was used for the key genes involved and their possible interaction pathways. The results showed that ~170 genes were significantly upregulated and 577 genes were significantly downregulated in curcumin-treated cells. Specifically, the up- and downregulated genes included *CCNE2*, associated with DNA damage; *ID3*, associated with cell survival and 146 genes with a >2- to 3-fold change including the *TP53INP1* gene, associated with DNA damage; *CDC6*, *CDCA5*, *TAKMIP2*, *CDK14*, *CDK5*, *CDCA76*, *CDC25A*, *CDC5L* and *SKP2*, associated with cell cycle; the

CARD6, *ID1* and *ID2* genes, associated with cell survival and the *BRMS1L*, associated with cell migration and invasion. Additionally, 59 downregulated genes exhibited a >4-fold change, including the *DDIT3* gene, associated with DNA damage; while 97 genes had a >3- to 4-fold change including the *DDIT4* gene, associated with DNA damage; the *CCPG1* gene, associated with cell cycle and 321 genes with a >2- to 3-fold change including the *GADD45A* and *CGREF1* genes, associated with DNA damage; the *CCPG1* gene, associated with cell cycle, the *TNFRSF10B*, *GAS5*, *TSSC1* and *TNFRSF11B* gene, associated with cell survival and the *ARHAP29* and *CADM2* genes, associated with cell migration and invasion. In conclusion, gene alterations provide information regarding the cytotoxic mechanism of curcumin at the genetic level and provide additional biomarkers or targets for the treatment of human lung cancer.

Introduction

Lung cancer, a leading cause of cancer-associated mortality worldwide, is one of the most aggressive human cancers and almost 80% of lung cancer-related deaths are non-small-cell lung cancer (NSCLC) (1,2). Despite recent improvements in chemotherapy and molecular-targeted therapy in lung cancer patients, the outcome of lung cancer remains poor. Treatments can also cause serious side effects that affect quality of life of patients (3). Although there has been considerable progress in the diagnosis and treatment of lung cancer, the overall 5-year survival rate of NSCLC patients remains <15% (4). Thus, studies have focused on new anticancer drugs derived from natural products and attempted to improve the disadvantage of the treatment of lung cancer.

Curcumin, a component of turmeric, is derived from the rhizome of *Curcuma longa*. Previous findings have shown that curcumin has anticancer activities for various types of cancer *in vitro* and *in vivo* (5). Curcumin has been found to

Correspondence to: Professor Jing-Gung Chung, Department of Biological Science and Technology, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan, R.O.C.
E-mail: jgchung@mail.cmu.edu.tw

Key words: curcumin, cDNA microarray, DNA damage, cell cycle, apoptosis, NCI-H460 cells

interfere with pathways that are overexpressed in cancer cells, such as NF κ B, STAT3 and PI3K/Akt to induce cell death in tumors (6). Curcumin also acts as a chemoprotective agent (7-9). Intraperitoneally administered curcumin in rats was able to decrease brain-implanted glioblastomas *in vivo* (10). Additionally, curcumin induces autophagy by activating the AMPK signaling pathway in human lung adenocarcinoma cells (11). Curcumin inhibits migration and invasion of human lung cancer cells through inhibition of the Rac1/PAK1 signaling pathway and MMP-2 and MMP-9 (12). Recently, it was reported that curcumin significantly reduced the tumor growth of orthotopic human NSCLC xenografts and increased survival of treated athymic mice (13).

Cells maintain normal functions and survival via genome integrity. However, genome instability of cells causes genetic aberrations and is considered a hallmark of most types of cancer (14). After the presence of DNA damage, p53 protein can be activated to promote important gene expressions that are involved in cell cycle arrest, DNA repair and apoptosis (15). Thus, identification of genetic abnormalities in NSCLC has been used for the development of targeted therapeutic approaches in NSCLC treatment. Targeting tumors carrying mutations in EGFR or a fusion of the *EML4* and *ALK* genes used clinically have been successful as first-line therapies in NSCLC (16-18). Therefore, it is imperative to identify gene expression or inhibition in NSCLC affected by test compounds or drugs to investigate the molecular mechanism to determine the function of these agents. Although curcumin has been demonstrated to induce cytotoxic effects on many human cancer cells, how associated total genes in human lung cancer cells (NSCLC) are affected by curcumin remains to be determined. Thus, we used cDNA microarray to investigate the altered gene expression in NCI-H460 cells following exposure to curcumin and the results indicated that curcumin altered certain gene expression associated with apoptosis.

Materials and methods

Chemicals and reagents. Curcumin and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Culture medium RPMI-1640, fetal bovine serum (FBS), 1% L-glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin were obtained from Gibco-BRL (Grand Island, NY, USA). Curcumin was dissolved in DMSO and stored at -20°C prior to use.

Lung cancer cells. The NCI-H460 human NSCLC cell line was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were cultured in RPMI-1640 medium and supplemented with 10% FBS, 1% L-glutamine and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in a humidified atmosphere of 95% air and 5% CO₂ air. The cells were split every 4 days to maintain exponential growth and were harvested with 0.025% trypsin and 0.52 mM EDTA in phosphate-buffered saline (PBS), plated at required cell numbers and allowed to adhere for ~24 h prior to treatment.

cDNA microarray assay used for gene expression in NCI-H460 cells following exposure to curcumin. NCI-H460

cells (5 \times 10⁵ cells/ml) were kept in 12-well plates with RPMI-1640 medium for 24 h and then incubated with or without 2 μ M of curcumin for 24 h. After treatment, the cells were collected and total RNA from each sample was extracted using a Qiagen RNeasy Mini kit (Qiagen Inc., Valencia, CA, USA) as previously described (19). Total RNA from curcumin-treated and -untreated cells was quantified and used for cDNA synthesis, labeling and microarray hybridization, followed by flour-labeled cDNA hybridizing their complements on the chip (Affymetrix GeneChip Human Gene 1.0 ST array; Affymetrix, Santa Clara, CA, USA) as previously described (19). On the chip, the resulting localized concentrations of fluorescent molecules were detected and quantified (Asia BioInnovations Corporation, Taipei, Taiwan) and data were further analyzed using Expression Console software (Affymetrix) with default RMA parameters (19,20). Up- or downregulated gene expressions was affected by DMC in NCI-H460 cells and a \geq 2-fold change was recorded and identified. Data were representative of three independent assays.

Statistical analysis. The results were representative of three assays. Differences between curcumin-treated and -untreated groups were presented up to 2-fold changes, with + signifying upregulation and -, downregulation.

Results

Curcumin induces the up- and downregulated gene expression in NCI-H460 cells. The NCI-H460 cells were incubated with or without 2 μ M of curcumin and collected to extract total RNA. The cells were then used for cDNA microarray analysis and results are shown in Tables I and II. Table I indicated that 4 genes exhibited >4-fold change, 20 genes were >3- to 4-fold including the *CCNE2* gene, associated with DNA damage; the *ID3* gene, associated with cell survival and 146 genes exhibited a >2- to 3-fold change, including the *TP53INP1* gene, associated with DNA damage; the *CDC6*, *CDCA5*, *TAKMIP2*, *CDK14*, *CDK5*, *CDCA76*, *CDC25A*, *CDC5L* and *SKP2* gene, associated with the cell cycle; the *CARD6*, *ID1* and *ID2* genes, associated with cell survival and the *BRMS1L* gene, associated with cell migration and invasion.

Table II shows that 59 genes were downregulated with >4-fold change including the *DDIT3* gene, associated with DNA damage, while 97 genes had a >3- to 4-fold change, including the *DDIT4* gene, associated with DNA damage; the *CCPG1* gene, associated with the cell cycle and 321 genes exhibited a >2- to 3-fold change including the *GADD45A* and *CGREF1* genes, associated with DNA damage; the *CCPG1* gene, associated with the cell cycle; the *TNFRSF10B*, *GAS5*, *TSSC1* and *TNFRSF11B* genes, associated with cell survival; and the *ARHAP29* and *CADM2* genes, associated with cell migration and invasion.

Curcumin affects the gene expression scores as measured by GeneGo analysis program in NCI-H460 cells by the number of pathway networks. Curcumin-treated or -untreated cells were performed by cDNA microarray and then processed using GeneGo analysis. The results are shown in

Table I. Representative genes of NCI-H460 cells upregulated following curcumin treatment.

Fold-change	Gene symbol	mRNA description
5.64	LOC100506948	Uncharacterized LOC100506948; small nucleolar RNA, C/D box 116-28; 115-26; 115-13; 115-7; 115-9; 115-11; 115-12; 115-29; 115-36; 115-39; 115-43
4.57	CYP1A1	Cytochrome P450, family 1, subfamily A
4.46	TIPARP	TCDD-inducible poly(ADP-ribose) polymerase
4.03	NAPIL2	Nucleosome assembly protein 1-like 2
3.98	METTL7B	Methyltransferase-like 7B
3.79	CCNE2	Cyclin E2
3.74	DLX2	Distal-less homeobox 2
3.69	TRNAK37P	Transfer RNA lysine 37 (anticodon CUU) pseudogene
3.62	POLR2A	Polymerase (RNA) II (DNA directed) polypeptide A, 220 kDa
3.56	ID3	Inhibitor of DNA binding 3, dominant-negative
3.49	RN5S452	RNA, 5S ribosomal 452
3.44	SNRPN	Uncharacterized LOC100506948; small nucleolar RNA, C/D box 116-28; 115-26; 115-13; 115-7; 115-5; 115-9; 115-11; 115-12; 115-29
3.40	SNORD115-4	Small nucleolar RNA, C/D box 115-4; 115-6; 115-42
3.34	RNU1-13P	RNA, U1 small nuclear 12, 13, pseudogene
3.29	CLSPN	Claspin
3.26	SNORD115-5	Small nucleolar RNA, C/D box 115-5; 115-12; 115-9; 115-43; 115-36; 115-29; 115-11; 115-20
3.20	GUCY1B3	Guanylate cyclase 1, soluble, β 3
3.20	HSPA1A	Heat shock 70 kDa protein 1A; 1B
3.19	SNORD115-6	Small nucleolar RNA, C/D box 115-6; 115-42
3.15	SFN	Stratifin
3.09	TUBB2A	Tubulin, β 2A class IIa
3.05	RNY4P15	RNA, Ro-associated Y4 pseudogene 15
3.03	FLJ44896	FLJ44896 protein
3.02	PIK3R3	Phosphoinositide-3-kinase, regulatory subunit 3 (γ)
2.99	CARD6	Caspase recruitment domain family, member 6
2.99	TRNAU2	Transfer RNA selenocysteine 2 (anticodon UCA)
2.96	FOSB	FBJ murine osteosarcoma viral oncogene homolog B
2.94	RNU1-10P	RNA, U1 small nuclear 10, pseudogene
2.93	SAMD15	Sterile α motif domain containing 15
2.91	FGD6	FYVE, RhoGEF and PH domain containing 6
2.87	NAV1	Neuron navigator 1
2.85	FAM171B	Family with sequence similarity 171, member B
2.82	VDR	Vitamin D (1,25-dihydroxyvitamin D3) receptor
2.81	ORC1	Origin recognition complex, subunit 1
2.81	DNAH14	Dynein, axonemal, heavy chain 14
2.77	SNORD115-44	Small nucleolar RNA, C/D box 115-44
2.77	GLULP4	Glutamate-ammonia ligase (glutamine synthetase) pseudogene 4
2.76	LPCAT2	Lysophosphatidylcholine acyltransferase 2
2.75	GTF2I	General transcription factor Iii
2.70	ID1	Inhibitor of DNA binding 1, dominant-negative helix-loop-helix protein
2.67	HSPH1	Heat shock 105/110 kDa protein 1
2.58	HIST1H3A	Histone cluster 1, H3a; H3f; H3b; H3h; H3j; H3g; H3i; H3e; H3c; H3d
2.57	STOX1	Storkhead box 1
2.57	RN5S183	RNA, 5S ribosomal 183
2.56	CROT	Carnitine <i>O</i> -octanoyltransferase
2.56	CDC6	Cell division cycle 6 homolog (<i>S. cerevisiae</i>)
2.55	IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
2.54	FOS	FBJ murine osteosarcoma viral oncogene homolog
2.52	SLC29A3	Solute carrier family 29 (nucleoside transporters), member 3

Table I. Continued.

Fold-change	Gene symbol	mRNA description
2.51	PKI55	DKFZp434H1419
2.49	HMGCS1	3-Hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
2.45	TACC1	Transforming, acidic coiled-coil containing protein 1
2.45	DHFR	Dihydrofolate reductase
2.44	ZNF480	Zinc finger protein 480
2.40	KIF5C	Kinesin family member 5C
2.39	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1
2.38	PPP2R3A	Protein phosphatase 2, regulatory subunit B', α
2.37	HMGN3P1	High mobility group nucleosomal binding domain 3 pseudogene 1
2.37	EML1	Echinoderm microtubule-associated protein like 1
2.33	SRRT	Serrate RNA effector molecule homolog (<i>Arabidopsis</i>)
2.32	DBR1	Debranching enzyme homolog 1 (<i>S. cerevisiae</i>)
2.31	JAKMIP2	Janus kinase and microtubule interacting protein 2
2.31	CASC5	Cancer susceptibility candidate 5
2.30	MFSD5	Major facilitator superfamily domain containing 5
2.30	MCM4	Minichromosome maintenance complex component 4
2.29	NEFL	Neurofilament, light polypeptide
2.29	SGK3	Serum/glucocorticoid-regulated kinase family, member 3; C8orf44-SGK3 readthrough
2.28	NCOA5	Nuclear receptor coactivator 5
2.27	MSRB1	Methionine sulfoxide reductase B1
2.27	BRMS1L	Breast cancer metastasis-suppressor 1-like
2.26	NRG4	Neuregulin 4
2.26	LOC100653168	Formin-1-like; formin 1
2.26	CDCA5	Cell division cycle-associated 5
2.26	GIN53	GIN5 complex subunit 3 (Psf3 homolog)
2.25	LOC100653119	Coiled-coil and C2 domain-containing protein 2A-like; coiled-coil and C2 domain containing 2B
2.25	LPHN2	Latrophilin 2
2.25	KIF20A	Kinesin family member 20A
2.25	TMEM14A	Transmembrane protein 14A
2.25	HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5
2.25	TUBB4B	Tubulin, β 4B class IVb
2.25	ANLN	Anillin, actin binding protein
2.24	CKS2	CDC28 protein kinase regulatory subunit 2
2.23	TSPYL2	TSPY-like 2
2.23	RPA2	Replication protein A2, 32 kDa
2.22	RNF38	Ring finger protein 38
2.22	RFWD3	Ring finger and WD repeat domain 3
2.21	GRK5	G protein-coupled receptor kinase 5
2.21	ZNF107	Zinc finger protein 107
2.21	SKA3	Spindle and kinetochore-associated complex subunit 3
2.21	NAGK	N-acetylglucosamine kinase
2.21	CTR9	Ctr9, Paf1/RNA polymerase II complex component, homolog (<i>S. cerevisiae</i>)
2.19	DIO2	Deiodinase, iodothyronine, type II
2.19	AHSA1	AHA1, activator of heat shock 90 kDa protein ATPase homolog 1 (yeast)
2.19	C3orf14	Chromosome 3 open reading frame 14
2.18	MARCH8	Membrane-associated ring finger (C3HC4) 8, E3 ubiquitin protein ligase
2.18	LPAL2	Lipoprotein, Lp(a)-like 2, pseudogene
2.18	TUBB4B	Tubulin, β 4B class IVb
2.17	GATA2	GATA binding protein 2
2.17	PAR4	Prader-Willi/Angelman region gene 4; small nucleolar RNA, C/D box 115-23

Table I. Continued.

Fold-change	Gene symbol	mRNA description
2.17	TRIP13	Thyroid hormone receptor interactor 13
2.17	MAPRE2	Microtubule-associated protein, RP/EB family, member 2
2.16	CHST11	Carbohydrate (chondroitin 4) sulfotransferase 11
2.16	SAMD9	Sterile α motif domain containing 9
2.16	MT1X	Metallothionein 1X
2.16	FNIP2	Folliculin-interacting protein 2
2.16	TACC3	Transforming, acidic coiled-coil containing protein 3
2.15	TERF1P2	Telomeric repeat binding factor (NIMA-interacting) 1 pseudogene 2
2.15	ZNF611	Zinc finger protein 611
2.14	C9orf78	Chromosome 9 open reading frame 78
2.14	DHX9	DEAH (Asp-Glu-Ala-His) box polypeptide 9
2.13	DHFR	Dihydrofolate reductase; dihydrofolate reductase pseudogene
2.13	TMEM171	Transmembrane protein 171
2.12	ID2	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
2.12	CDK14	Cyclin-dependent kinase 14
2.12	TRNASUP1	Transfer RNA suppressor 1 (anticodon UUA)
2.11	SMAD9	SMAD family member 9
2.11	MCM2	Minichromosome maintenance complex component 2
2.11	CT45A5	Cancer/testis antigen family 45, member A5; A2; A3; A4 cancer/testis antigen family 45, member A1; A6
2.11	GDAP1	Ganglioside induced differentiation associated protein 1
2.11	MCM10	Minichromosome maintenance complex component 10
2.11	CABLES1	Cdk5 and Abl enzyme substrate 1
2.11	CENPQ	Centromere protein Q
2.11	CKAP2	Cytoskeleton associated protein 2
2.11	FAM19A1	Family with sequence similarity 19 [chemokine (C-C motif)-like], member A1
2.11	MEPCE	Methylphosphate capping enzyme
2.11	REEP1	Receptor accessory protein 1
2.10	SNORD115-32	Small nucleolar RNA, C/D box 115-32
2.10	ATP6V0D1	ATPase, H ⁺ transporting, lysosomal 38 kDa, V0 subunit d1
2.10	WDR6	WD repeat domain 6
2.10	SNX10	Sorting nexin 10
2.10	BRPF3	Bromodomain and PHD finger containing, 3
2.10	FAM217B	Family with sequence similarity 217, member B
2.10	EML6	Echinoderm microtubule-associated protein-like 6
2.10	SAFB2	Scaffold attachment factor B2
2.09	TP53INP1	Tumor protein p53 inducible nuclear protein 1
2.09	CPM	Carboxypeptidase M
2.09	CDRT1	CMT1A duplicated region transcript 1
2.09	CDCA7L	Cell division cycle associated 7-like
2.08	SMAD6	SMAD family member 6
2.10	SAFB2	Scaffold attachment factor B2
2.09	TP53INP1	Tumor protein p53 inducible nuclear protein 1
2.09	CPM	Carboxypeptidase M
2.09	CDRT1	CMT1A duplicated region transcript 1
2.09	CDCA7L	Cell division cycle associated 7-like
2.08	SMAD6	SMAD family member 6
2.08	CCDC138	Coiled-coil domain containing 138
2.07	TUBB2A	Tubulin, β 2A class IIa; tubulin, β 2B class IIb
2.07	FEN1	Flap structure-specific endonuclease 1
2.06	CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1
2.06	CDC25A	Cell division cycle 25 homolog A (<i>S. pombe</i>)

Table I. Continued.

Fold-change	Gene symbol	mRNA description
2.05	WNK3	WNK lysine deficient protein kinase 3
2.05	CXorf26	Chromosome X open reading frame 26
2.04	TATDN2	TatD DNase domain containing 2
2.04	ZNF840	Zinc finger protein 840
2.04	EIF4EBP2	Eukaryotic translation initiation factor 4E binding protein 2
2.04	PCYT2	Phosphate cytidylyltransferase 2, ethanolamine
2.03	DUSP2	Dual specificity phosphatase 2
2.03	CEP19	Centrosomal protein 19 kDa
2.03	HMGN2	High mobility group nucleosomal binding domain 2
2.02	DNAH6	Dynein, axonemal, heavy chain 6
2.02	DYNLL1	Dynein, light chain, LC8-type 1
2.02	NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)
2.02	PPP2R5D	Protein phosphatase 2, regulatory subunit B', δ
2.02	DPYSL3	Dihydropyrimidinase-like 3
2.02	PCYT2	Phosphate cytidylyltransferase 2, ethanolamine
2.02	SHCBP1	SHC SH2-domain binding protein 1
2.02	CDC5L	CDC5 cell division cycle 5-like (<i>S. pombe</i>)
2.02	CDKN2C	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)
2.02	FRMD3	FERM domain containing 3
2.02	SKP2	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase
2.01	TRNAP28P	Transfer RNA proline 28 (anticodon AGG) pseudogene
2.01	LOC100129648	Uncharacterized LOC100129648
2.01	NXF1	Nuclear RNA export factor 1
2.01	ADII	Acireductone dioxygenase 1
2.01	CFB	Complement factor B
2.01	ZNF483	Zinc finger protein 483

Figs. 1-3 for top, second and third scores, respectively. The analysis results were mapped on the processes as presented in possible signal effects. Red indicated upregulated genes and blue, the downregulated genes. Circles indicated different intensities indicating different enhancing or inhibitions in curcumin-treated NCI-H460 cells.

Discussion

It has been demonstrated that curcumin induced biological activities including anticancer function *in vitro* and *in vivo*. However, how curcumin affects gene expression and the associated signaling pathways in human lung cancer cells remains to be determined. Thus, we investigated the up- and down-regulation of associated genes with cell cycle, DNA damage, cell survival and cell migration and invasion in NCI H460 cells. In the present study, we found several genes involved DNA damage and repair were increased, such as cyclin E2 (CCNE2), which was increased 3.79-fold. It has also been reported that following cell induction DNA damage led to cell cycle arrest (21). The tumor protein p53 inducible nuclear protein 1 (TP53INP1) was increased 2.09-fold and after DNA damage the p53 protein expression was also increased (22).

The results showed that the *TP53INP1* gene, associated with DNA damage was increased 2.09-fold, the *CDC6*, *TAKMIP2*,

CDCA5, *CDK14*, *CDK5*, *CDCA76*, *CDC25A*, *CDC5L* and *SKP2*, associated with cell cycle were increased 2.56-, 2.31-, 2.26-, 2.12-, 2.11-, 2.09-, 2.06-, 2.02- and 2.02-fold, respectively, associated with cell cycle, while the *CARD6*, *ID1* and *ID2* genes, associated with cell survival were increased 2.99-, 2.70- and 2.12-fold. It was also found that the *BRMS1L* gene, associated with cell migration and invasion, was increased 2.27-fold. It was reported that tumor protein 53-induced nuclear protein 1 (TP53INP1) is a stress-induced p53-target gene function that represses tumorigenesis (23). The cell cycle-dependent centrosomal localization of Cdc6 in the S and G2 phases is considered to have a novel function of Cdc6 in centrosomes (24). CDCA5 and CDK5 have been found to play an important role in the cell cycle (25). It was reported that the mitotic CDK14/cyclin Y complex promotes Wnt signaling (26). Moreover, Cdc25 is known to be involved cell cycle regulation (27). Caspase recruitment domain 6 (CARD6), a microtubule-interacting protein, belongs to the CARD family (28). CARD6 may be a novel target for the treatment of pathological cardiac hypertrophy and failure (29). ID (inhibitor of differentiation and DNA binding) comprises the ID1, ID2, ID3 and ID4 proteins, which are involved in cancer progression (30). ID1 (inhibitor of differentiation and DNA binding 1) was reported to strongly correlate with various types of tumors, including lung cancer (31,32).

Table II. Representative genes of NCI-H460 cells those were upregulated by curcumin treatment.

Fold-change	Gene symbol	mRNA description
-2.01	HORMAD1	HORMA domain containing 1
-2.01	EGFR	Epidermal growth factor receptor
-2.01	VTN	Vitronectin; SEBOX homeobox
-2.01	PGAP1	Post-GPI attachment to proteins 1
-2.01	ZAK	Sterile α motif and leucine zipper containing kinase AZK
-2.01	PLOD1	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1
-2.01	HPCAL1	Hippocalcin-like 1
-2.01	ALS2CR8	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8
-2.01	RPS6KA2	Ribosomal protein S6 kinase, 90 kDa, polypeptide 2
-2.01	ITPR3	Inositol 1,4,5-trisphosphate receptor, type 3
-2.01	LEPRE1	Leucine proline-enriched proteoglycan (leprecan) 1
-2.01	FJX1	Four jointed box 1 (<i>Drosophila</i>)
-2.02	GALC	Galactosylceramidase
-2.02	TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b
-2.02	DENND3	DENN/MADD domain containing 3
-2.02	SORT1	Sortilin 1
-2.02	IGFBP7	Insulin-like growth factor binding protein 7
-2.02	IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2
-2.02	TAS2R31	Taste receptor, type 2, member 31; taste receptor, type 2, member 45
-2.02	LMBRD2	LMBR1 domain containing 2
-2.02	SNORD77	Small nucleolar RNA, C/D box 77
-2.02	C6orf120	Chromosome 6 open reading frame 120
-2.02	FAM188A	Family with sequence similarity 188, member A
-2.02	RBCK1	RanBP-type and C3HC4-type zinc finger containing 1
-2.02	FHDC1	FH2 domain containing 1
-2.03	DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1
-2.03	ANKH	Ankylosis, progressive homolog (mouse)
-2.03	TSSC1	Tumor-suppressing subtransferable candidate 1
-2.03	CLYBL	Citrate lyase β -like
-2.03	GAS5	Growth arrest-specific 5 (non-protein coding); small nucleolar RNA, C/D box 80
-2.03	CEBPG	CCAAT/enhancer binding protein (C/EBP), γ
-2.04	BCKDHA	Branched chain keto acid dehydrogenase E1, α polypeptide
-2.04	CCNB1IP1	Cyclin B1 interacting protein 1, E3 ubiquitin protein ligase
-2.04	TMEM67	Transmembrane protein 67
-2.02	RBCK1	RanBP-type and C3HC4-type zinc finger containing 1
-2.02	FAM27E3	Family with sequence similarity 27, member E3; E2
-2.02	FHDC1	FH2 domain containing 1
-2.03	DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1
-2.03	SNORD49B	Small nucleolar RNA, C/D box 49B; box 65; C17orf76 antisense RNA 1 (non-protein coding); small nucleolar RNA, C/D box 49A
-2.03	ANKH	Ankylosis, progressive homolog (mouse)
-2.03	TSSC1	Tumor-suppressing subtransferable candidate 1
-2.03	CLYBL	Citrate lyase β -like
-2.03	GAS5	Growth arrest-specific 5 (non-protein coding); small nucleolar RNA, C/D box 80
-2.02	FHDC1	FH2 domain containing 1
-2.03	DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1
-2.03	SNORD49B	Small nucleolar RNA, C/D box 49B; 65; C17orf76 antisense RNA 1 (non-protein coding); small nucleolar RNA, C/D box 49A
-2.03	TSSC1	Tumor-suppressing subtransferable candidate 1
-2.03	CLYBL	Citrate lyase β -like
-2.03	GAS5	Growth arrest-specific 5 (non-protein coding); small nucleolar RNA, C/D box 80
-2.03	CEBPG	CCAAT/enhancer binding protein (C/EBP), γ

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.04	BCKDHA	Branched chain keto acid dehydrogenase E1, α polypeptide
-2.04	CCNB1IP1	Cyclin B1 interacting protein 1, E3 ubiquitin protein ligase
-2.04	LCN1	Lipocalin 1
-2.04	TMEM67	Transmembrane protein 67
-2.04	PLD3	Phospholipase D family, member 3
-2.04	FAM27E3	Family with sequence similarity 27, member E3; family with sequence similarity 27, member E2
-2.04	ABCA2	ATP-binding cassette, sub-family A (ABC1), member 2
-2.05	PBX1	Pre-B-cell leukemia homeobox 1
-2.05	C11orf54	Chromosome 11 open reading frame 54
-2.05	KRTAP5-5	Keratin-associated protein 5-5
-2.05	RAP2B	RAP2B, member of RAS oncogene family
2.05	PLK2	Polo-like kinase 2
-2.05	SLC9A3	Solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3
-2.05	ARSG	Arylsulfatase G
-2.06	CADM2	Cell adhesion molecule 2
-2.06	SCD	Stearoyl-CoA desaturase (δ -9-desaturase)
2.05	PLK2	Polo-like kinase 2
-2.05	SLC9A3	Solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3
-2.05	ARSG	Arylsulfatase G
-2.06	CADM2	Cell adhesion molecule 2
-2.06	SLPI	Secretory leukocyte peptidase inhibitor
-2.06	RND3	Rho family GTPase 3
-2.06	CD9	CD9 molecule; uncharacterized LOC100653288
-2.06	PLOD2	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2
-2.06	GPC6	Glypican 6
-2.06	RASSF8	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8
-2.06	MEST	Mesoderm-specific transcript homolog (mouse)
-2.06	SIAE	Sialic acid acetyltransferase
-2.06	CSGALNACT2	Chondroitin sulfate N-acetylgalactosaminyltransferase 2
-2.06	JHDM1D	Jumonji C domain containing histone demethylase 1 homolog D (<i>S. cerevisiae</i>)
-2.06	ASAHI	N-acylsphingosine amidohydrolase (acid ceramidase) 1
-2.07	P2RX4	Purinergic receptor P2X, ligand-gated ion channel, 4
-2.07	RBM24	RNA binding motif protein 24
-2.07	GPR108	G protein-coupled receptor 108
-2.07	ACTR1B	ARP1 actin-related protein 1 homolog B, centractin β (yeast)
-2.07	CTPS1	CTP synthase 1
-2.07	RNF41	Ring finger protein 41
-2.07	SLC1A4	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
-2.08	BCHE	Butyrylcholinesterase
-2.08	GPT2	Glutamic pyruvate transaminase (alanine aminotransferase) 2
-2.08	COL12A1	Collagen, type XII, α 1
-2.08	ARG2	Arginase, type II
-2.08	HFM1	HFM1, ATP-dependent DNA helicase homolog (<i>S. cerevisiae</i>)
-2.08	NOX1	NADPH oxidase 1
-2.08	SNX14	Sorting nexin 14
-2.08	CD109	CD109 molecule
-2.09	CNTNAP3	Contactin-associated protein-like 3; contactin-associated protein-like 3B
-2.09	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)
-2.09	L1CAM	L1 cell adhesion molecule
-2.09	PART1	Prostate androgen-regulated transcript 1 (non-protein coding)
-2.09	TCF4	Transcription factor 4

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.09	IGF1R	Insulin-like growth factor 1 receptor
-2.09	LRP12	Low-density lipoprotein receptor-related protein 12
-2.09	SLC50A1	Solute carrier family 50 (sugar transporter), member 1
-2.09	GLDN	Gliomedin
-2.10	CYFIP2	Cytoplasmic FMR1 interacting protein 2
-2.10	SNORD116@	Small nucleolar RNA, C/D box 116 cluster; small nucleolar RNA, C/D box 116-21
-2.10	TCEA1	Transcription elongation factor A (SII), 1
-2.10	NCOR2	Nuclear receptor corepressor 2
-2.10	EFHC1	EF-hand domain (C-terminal) containing 1
-2.10	SLC11A2	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2
-2.11	CDH11	Cadherin 11, type 2, OB-cadherin (osteoblast)
-2.11	NOSTRIN	Nitric oxide synthase trafficker
-2.11	EDEM1	ER degradation enhancer, mannosidase α -like 1
-2.11	ALDOC	Aldolase C, fructose-bisphosphate
-2.11	NEDD1	Neural precursor cell expressed, developmentally downregulated 1
-2.11	SARS	Seryl-tRNA synthetase
-2.11	RN5S385	RNA, 5S ribosomal 385
-2.11	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4
-2.11	ENOSF1	Enolase superfamily member 1
-2.12	PRKACB	Protein kinase, cAMP-dependent, catalytic, β
-2.12	CSNK1E	Casein kinase 1, ϵ
-2.12	ALG13	Asparagine-linked glycosylation 13 homolog (<i>S. cerevisiae</i>)
-2.12	DEPDC1	DEP domain containing 1
-2.12	IGFBP3	Insulin-like growth factor binding protein 3
-2.12	UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats
-2.12	COL3A1	Collagen, type III, α 1
-2.12	IVNS1ABP	Influenza virus NS1A binding protein
-2.13	EPB41L3	Erythrocyte membrane protein band 4.1-like 3
-2.13	STEAP1B	STEAP family member 1B
-2.13	SOCS3	Suppressor of cytokine signaling 3
-2.13	MIR186	microRNA 186
-2.13	GFPT1	Glutamine-fructose-6-phosphate transaminase 1
-2.14	MORC4	MORC family CW-type zinc finger 4
-2.14	ANKRD28	Ankyrin repeat domain 28
-2.14	MTMR11	Myotubularin-related protein 11
-2.14	HLA-F	Major histocompatibility complex, class I, F
-2.14	PIGK	Phosphatidylinositol glycan anchor biosynthesis, class K
-2.14	NUCB1	Nucleobindin 1
-2.14	ERAP1	Endoplasmic reticulum aminopeptidase 1
-2.14	PAN2	PAN2 poly(A) specific ribonuclease subunit homolog (<i>S. cerevisiae</i>)
-2.14	SYCP2L	Synaptonemal complex protein 2-like
-2.15	ADAMTS1	ADAM metalloproteinase with thrombospondin type 1 motif, 1
-2.15	IL1A	Interleukin 1, α
-2.15	ARHGAP29	Rho GTPase-activating protein 29
-2.15	C18orf54	Chromosome 18 open reading frame 54
-2.15	ENC1	Ectodermal-neural cortex 1 (with BTB-like domain)
-2.15	HSPG2	Heparan sulfate proteoglycan 2
-2.16	MIR492	microRNA 492; keratin 19 pseudogene 2
-2.16	DNASE2	Deoxyribonuclease II, lysosomal
-2.16	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1
-2.16	MAML3	Mastermind-like 3 (<i>Drosophila</i>)
-2.16	SNORA27	Small nucleolar RNA, H/ACA box 27; 102; ribosomal protein L21

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.16	GSTT1	Glutathione S-transferase θ 1
-2.17	DCLK2	Doublecortin-like kinase 2
-2.17	PPP2R3C	Protein phosphatase 2, regulatory subunit B', γ
-2.17	MARCH1	Membrane-associated ring finger (C3HC4) 1, E3 ubiquitin protein ligase
-2.17	SPG11	Spastic paraplegia 11 (autosomal recessive)
-2.17	ANK3	Ankyrin 3, node of Ranvier (ankyrin G)
-2.17	LINGO2	Leucine rich repeat and Ig domain containing 2
-2.17	TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b
-2.17	SRPX2	Sushi-repeat containing protein, X-linked 2
-2.17	ZNF41	Zinc finger protein 41
-2.17	MAN2C1	Mannosidase, α , class 2C, member 1
-2.18	PDE7B	Phosphodiesterase 7B
-2.18	EPAS1	Endothelial PAS domain protein 1; uncharacterized LOC100652809
-2.18	FN1	Fibronectin 1
-2.18	ETV4	Ets variant 4
-2.18	CTSO	Cathepsin O
-2.18	MGAT4C	Mannosyl (α -1,3-)-glycoprotein β -1,4-N-acetylglucosaminyltransferase, isozyme C (putative)
-2.18	R3HDM2	R3H domain containing 2
-2.18	AMDHD1	Amidohydrolase domain containing 1
-2.18	SNORA68	Small nucleolar RNA, H/ACA box 68
-2.18	SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1
-2.19	NID2	Nidogen 2 (osteonidogen)
-2.19	LXN	Latexin
-2.19	CTIF	CBP80/20-dependent translation initiation factor
-2.19	TRNAG36	Transfer RNA glycine 36 (anticodon CCC); transfer RNA valine 34 (anticodon CAC) pseudogene
-2.19	ST6GAL1	ST6 β -galactosamide α -2,6-sialyltransferase 1
-2.19	SCARNA23	Small Cajal body-specific RNA 23
-2.19	DPYD	Dihydropyrimidine dehydrogenase
-2.19	DDR2	Discoidin domain receptor tyrosine kinase 2
-2.18	FN1	Fibronectin 1
-2.18	ETV4	Ets variant 4
-2.18	CTSO	Cathepsin O
-2.18	MGAT4C	Mannosyl (α -1,3-)-glycoprotein β -1,4-N-acetylglucosaminyltransferase, isozyme C (putative)
-2.18	R3HDM2	R3H domain containing 2
-2.18	AMDHD1	Amidohydrolase domain containing 1
-2.18	SNORA68	Small nucleolar RNA, H/ACA box 68
-2.18	SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1
-2.19	NID2	Nidogen 2 (osteonidogen)
-2.19	LXN	Latexin
-2.19	CTIF	CBP80/20-dependent translation initiation factor
-2.19	TRNAG36	Transfer RNA glycine 36 (anticodon CCC); transfer RNA valine 34 (anticodon CAC) pseudogene
-2.19	ST6GAL1	ST6 β -galactosamide α -2,6-sialyltransferase 1
-2.19	SCARNA23	Small Cajal body-specific RNA 23
-2.19	DPYD	Dihydropyrimidine dehydrogenase
-2.19	DDR2	Discoidin domain receptor tyrosine kinase 2
-2.19	LGI1	Leucine-rich, glioma inactivated 1
-2.19	HLA-F	Major histocompatibility complex, class I, F
-2.20	PSPH	Phosphoserine phosphatase

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.20	HBG1	Hemoglobin, γ A; hemoglobin, γ G; uncharacterized LOC100653319; uncharacterized LOC100653006
-2.20	TMEM231	Transmembrane protein 231
-2.20	EML2	Echinoderm microtubule-associated protein like-2
-2.21	IGF2R	Insulin-like growth factor 2 receptor
-2.21	PYGB	Phosphorylase, glycogen; brain
-2.21	WSB1	WD repeat and SOCS box containing 1
-2.22	ITGBL1	Integrin, β -like 1 (with EGF-like repeat domains)
-2.22	PCBP3	Poly(rC) binding protein 3
-2.22	SERPINE2	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
-2.22	TAPBP	TAP binding protein (tapasin)
-2.22	ERGIC1	Endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1
-2.23	PDLIM5	PDZ and LIM domain 5
-2.23	BCAT1	Branched chain amino-acid transaminase 1, cytosolic
-2.23	RN5S198	RNA, 5S ribosomal 198
-2.23	PC	Pyruvate carboxylase
-2.24	BMPER	BMP binding endothelial regulator
-2.24	BACE2	β -site APP-cleaving enzyme 2
-2.24	CTNNAL1	Catenin (cadherin-associated protein), α -like 1
-2.24	ANKRD20A11P	Ankyrin repeat domain 20 family, member A11, pseudogene; ankyrin repeat domain 20 family, member A8, pseudogene
-2.24	SNORD116-29	Small nucleolar RNA, C/D box 116-29
-2.24	IFRD1	Interferon-related developmental regulator 1
-2.25	EDNRB	Endothelin receptor type B
-2.25	VN1R4	Vomerolateral 1 receptor 4
-2.26	NR4A2	Nuclear receptor subfamily 4, group A, member 2
-2.26	DKK1	Dickkopf 1 homolog (<i>Xenopus laevis</i>)
-2.26	OLFML2A	Olfactomedin-like 2A
-2.26	C6orf48	Chromosome 6 open reading frame 48
-2.27	SPRY3	Sprouty homolog 3 (<i>Drosophila</i>)
-2.27	PSAT1	Phosphoserine aminotransferase 1
-2.27	HOXA2	Homeobox A2
-2.28	DUSP1	Dual specificity phosphatase 1
-2.28	SLC44A1	Solute carrier family 44, member 1
-2.28	ITPRIP	Inositol 1,4,5-trisphosphate receptor interacting protein
-2.28	OSMR	Oncostatin M receptor
-2.28	SUMF2	Sulfatase modifying factor 2
-2.28	S100A16	S100 calcium binding protein A16
-2.28	ITGB5	Integrin, β 5
-2.28	SLC33A1	Solute carrier family 33 (acetyl-CoA transporter), member 1
-2.28	FOSL2	FOS-like antigen 2
-2.28	RBMS2	RNA binding motif, single-stranded interacting protein 2
-2.28	SOCS2	Suppressor of cytokine signaling 2
-2.29	EIF4EBP1	Eukaryotic translation initiation factor 4E binding protein 1
-2.29	RN5S449	RNA, 5S ribosomal 449
-2.29	IDH1	Isocitrate dehydrogenase 1 (NADP ⁺), soluble
-2.29	UPP1	Uridine phosphorylase 1
-2.29	DPYSL2	Dihydropyrimidinase-like 2
-2.30	CAMK2D	Calcium/calmodulin-dependent protein kinase II δ
-2.30	SHMT2	Serine hydroxymethyltransferase 2 (mitochondrial)
-2.30	MUC1	Mucin 1, cell surface associated

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.30	ADAM9	ADAM metalloproteinase domain 9
-2.31	LOC100653286	Aldo-keto reductase family 1 member C2-like; aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3- α hydroxysteroid dehydrogenase, type III)
-2.31	CD226	CD226 molecule
-2.31	ZNF449	Zinc finger protein 449
-2.31	PHLDB2	Pleckstrin homology-like domain, family B, member 2
-2.31	PLGRKT	Plasminogen receptor, C-terminal lysine transmembrane protein
-2.31	SPAG4	Sperm-associated antigen 4
-2.31	GADD45A	Growth arrest and DNA-damage-inducible, α
-2.31	CGREF1	Cell growth regulator with EF-hand domain 1
-2.31	DUSP22	Dual specificity phosphatase 22; dual specificity protein phosphatase 22-like
-2.31	PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1
-2.31	LOC100506948	Uncharacterized LOC100506948; small nucleolar RNA, C/D box 116-28; 115-26; 115-13; 115-7; 116-22
-2.31	ECE1	Endothelin converting enzyme 1
-2.32	PAQR5	Progestin and adipoQ receptor family member V
-2.32	LMF1	Lipase maturation factor 1
-2.32	SNORD13P2	Small nucleolar RNA, C/D box 13 pseudogene 2
-2.32	PLEKHG2	Pleckstrin homology domain containing, family G (with RhoGef domain) member 2
-2.32	TMX3	Thioredoxin-related transmembrane protein 3
-2.33	ZNF558	Zinc finger protein 558
-2.33	SOX9	SRY (sex determining region Y)-box 9
-2.34	SIL1	SIL1 homolog, endoplasmic reticulum chaperone (<i>S. cerevisiae</i>)
-2.34	FSD2	Fibronectin type III and SPRY domain containing 2; small Cajal body-specific RNA 15
-2.34	SNORD116-16	Small nucleolar RNA, C/D box 116-16
-2.34	ANGPTL4	Angiopoietin-like 4
-2.34	PABPC1L	Poly(A) binding protein, cytoplasmic 1-like
-2.34	PLCB4	Phospholipase C, β 4
-2.35	BEST1	Bestrophin 1
-2.35	RNU7-35P	RNA, U7 small nuclear 35 pseudogene
-2.36	CDH2	Cadherin 2, type 1, N-cadherin (neuronal)
-2.36	HYOU1	Hypoxia upregulated 1
-2.36	ITGA6	Integrin, α 6
-2.37	THBS3	Thrombospondin 3
-2.37	TM4SF18	Transmembrane 4 L six family member 18
-2.37	MTHFD1L	Methylenetetrahydrofolate dehydrogenase (NADP ⁺ dependent) 1-like
-2.37	PIR	Pirin (iron-binding nuclear protein); PIR-FIGF readthrough
-2.38	MOCOS	Molybdenum cofactor sulfurase
-2.38	RHBDD1	Rhomboid domain containing 1
-2.38	ARL4D	ADP-ribosylation factor-like 4D
-2.38	F2RL1	Coagulation factor II (thrombin) receptor-like 1
-2.39	RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5
-2.39	PCMTD2	Protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2; long intergenic non-protein coding RNA 266-1
-2.39	SREBF1	Sterol regulatory element binding transcription factor 1
-2.40	CPS1	Carbamoyl-phosphate synthase 1, mitochondrial
-2.40	ZEB2	Zinc finger E-box binding homeobox 2
-2.40	NRP2	Neuropilin 2
-2.40	HLCS	Holocarboxylase synthetase [biotin-(propionyl-CoA-carboxylase (ATP-hydrolysing) ligase]

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.40	CRIM1	Cysteine rich transmembrane BMP regulator 1 (chordin-like)
-2.41	ZC3H6	Zinc finger CCCH-type containing 6
-2.41	ARRDC4	Arrestin domain containing 4
-2.41	PDIA5	Protein disulfide isomerase family A, member 5
-2.41	WDFY2	WD repeat and FYVE domain containing 2
-2.41	VEGFC	Vascular endothelial growth factor C
-2.42	CA5B	Carbonic anhydrase VB, mitochondrial
-2.42	CLN8	Ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)
-2.42	CYP20A1	Cytochrome P450, family 20, subfamily A, polypeptide 1
-2.43	AKR1B10	Aldo-keto reductase family 1, member B10 (aldose reductase)
-2.43	GPR126	G protein-coupled receptor 126
-2.43	TSPAN4	Tetraspanin 4
-2.43	FGG	Fibrinogen γ chain
-2.44	RORA	RAR-related orphan receptor A
-2.44	UGGT2	UDP-glucose glycoprotein glucosyltransferase 2
-2.44	DUSP5	Dual specificity phosphatase 5
-2.45	P4HA3	Prolyl 4-hydroxylase, α polypeptide III
-2.45	MARS	Methionyl-tRNA synthetase
-2.45	IGF2BP3	Insulin-like growth factor 2 mRNA binding protein 3
-2.45	TTC13	Tetratricopeptide repeat domain 13
-2.45	C16orf58	Chromosome 16 open reading frame 58
-2.46	BMP5	Bone morphogenetic protein 5
-2.46	SREBF2	Sterol regulatory element binding transcription factor 2
-2.46	NEDD9	Neural precursor cell expressed, developmentally downregulated 9
-2.48	OCLN	Occludin; occludin pseudogene
-2.48	PAG1	Phosphoprotein associated with glycosphingolipid microdomains 1
-2.48	PPFIBP1	PTPRF interacting protein, binding protein 1 (liprin β 1)
-2.48	BTG1	B-cell translocation gene 1, antiproliferative
-2.48	XYLT1	Xylosyltransferase I
-2.48	CEP72	Centrosomal protein 72 kDa
-2.48	HNRNPU-AS1	HNRNPU antisense RNA 1 (non-protein coding)
-2.49	CLK1	CDC-like kinase 1
-2.49	VPS13C	Vacuolar protein sorting 13 homolog C (<i>S. cerevisiae</i>)
-2.50	LAMA1	Laminin, α 1
-2.50	PLD1	Phospholipase D1, phosphatidylcholine-specific
-2.50	AGXT2L2	Alanine-glyoxylate aminotransferase 2-like 2
-2.50	SLCO5A1	Solute carrier organic anion transporter family, member 5A1
-2.51	CARS	CysteinyI-tRNA synthetase
-2.51	ACAD11	Acyl-CoA dehydrogenase family, member 11; nephronophthisis 3 (adolescent); NPHP3-ACAD11 readthrough
-2.51	SLC2A14	Solute carrier family 2 (facilitated glucose transporter), member 14
-2.52	SCCPDH	Saccharopine dehydrogenase (putative)
-2.52	NT5E	5'-nucleotidase, ecto (CD73)
-2.53	AADAC	Arylacetamide deacetylase (esterase)
-2.53	TMEM106B	Transmembrane protein 106B
-2.54	GDPD1	Glycerophosphodiester phosphodiesterase domain containing 1
-2.55	DNER	δ /notch-like EGF repeat containing
-2.55	SLITRK4	SLIT and NTRK-like family, member 4
-2.55	ST3GAL1	ST3 β -galactoside α -2,3-sialyltransferase 1
-2.55	SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1
-2.55	SPDYE8P	Speedy homolog E8 (<i>Xenopus laevis</i>), pseudogene

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.55	SLC9B1	Solute carrier family 9, subfamily B (NHA1, cation proton antiporter 1), member 1; solute carrier family 9, subfamily B (NHA1, cation proton antiporter 1), member 1 pseudogene 2; solute carrier family 9, subfamily B (NHA1, cation proton antiporter 1), member 1 pseudogene 3
-2.56	ZP3	Zona pellucida glycoprotein 3 (sperm receptor)
-2.56	BCAR3	Breast cancer anti-estrogen resistance 3
-2.57	GABBR2	γ -aminobutyric acid (GABA) B receptor, 2
-2.57	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
-2.57	GALNT3	UDP- <i>N</i> -acetyl- α -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)
-2.57	THBS1	Thrombospondin 1
-2.57	MIR21	microRNA 21
-2.58	SESN2	Sestrin 2
-2.59	FLNB	Filamin B, β
-2.59	EFNA5	Ephrin-A5
-2.59	PTPRB	Protein tyrosine phosphatase, receptor type, B
-2.59	PION	Pigeon homolog (<i>Drosophila</i>)
-2.60	EFEMP1	EGF containing fibulin-like extracellular matrix protein 1
-2.60	DUSP4	Dual specificity phosphatase 4
-2.61	RTN1	Reticulon 1
-2.61	DGKD	Diacylglycerol kinase, δ 130 kDa
-2.61	ANTXR1	Anthrax toxin receptor 1
-2.61	SUCLG2	Succinate-CoA ligase, GDP-forming, β subunit
-2.61	LOC100506965	Uncharacterized LOC100506965
-2.62	LAMC1	laminin, γ 1 (formerly LAMB2)
-2.63	CEP70	Centrosomal protein 70 kDa
-2.64	ABCA8	ATP-binding cassette, sub-family A (ABC1), member 8
-2.65	SERPINB9	Serpin peptidase inhibitor, clade B (ovalbumin), member 9
-2.65	PDE1C	Phosphodiesterase 1C, calmodulin-dependent 70 kDa
-2.65	ACSS3	Acyl-CoA synthetase short-chain family member 3
-2.66	SCG2	Secretogranin II
-2.67	SLC39A8	Solute carrier family 39 (zinc transporter), member 8
-2.67	F2R	Coagulation factor II (thrombin) receptor
-2.67	MME	Membrane metallo-endopeptidase
-2.68	LCP1	Lymphocyte cytosolic protein 1 (L-plastin)
-2.68	CFH	Complement factor H
-2.68	NCAM2	Neural cell adhesion molecule 2
-2.70	HINT3	Histidine triad nucleotide binding protein 3
-2.70	GPR37	G protein-coupled receptor 37 (endothelin receptor type B-like)
-2.71	PPAP2B	Phosphatidic acid phosphatase type 2B
-2.72	LAMB1	Laminin, β 1
-2.73	GALNTL1	UDP- <i>N</i> -acetyl- α -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1
-2.73	PDK1	Pyruvate dehydrogenase kinase, isozyme 1
-2.74	TRMT2B	tRNA methyltransferase 2 homolog B (<i>S. cerevisiae</i>)
-2.74	SSFA2	Sperm-specific antigen 2
-2.75	TMEM100	Transmembrane protein 100
-2.75	RAB39B	RAB39B, member RAS oncogene family
-2.75	SORBS2	Sorbin and SH3 domain containing 2
-2.76	RGS2	Regulator of G-protein signaling 2, 24 kDa
-2.76	SERPIND1	Serpin peptidase inhibitor, clade D (heparin cofactor), member 1
-2.77	LOC344887	NmrA-like family domain containing 1 pseudogene

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-2.77	PRKAA2	Protein kinase, AMP-activated, α 2 catalytic subunit
-2.77	PYCR1	Pyrroline-5-carboxylate reductase 1
-2.77	MIR32	microRNA 32
-2.77	TTC32	Tetratricopeptide repeat domain 32
-2.78	CCDC68	Coiled-coil domain containing 68
-2.78	GPR22	G protein-coupled receptor 22
-2.79	ULBP1	UL16 binding protein 1
-2.79	AXL	AXL receptor tyrosine kinase
-2.79	ERVK-9	Endogenous retrovirus group K, member 9; endogenous retrovirus group K, member 4; endogenous retrovirus group K, member 19
-2.79	DOCK4	Dedicator of cytokinesis 4
-2.80	PTPRM	Protein tyrosine phosphatase, receptor type, M
-2.80	STC2	Stanniocalcin 2
-2.80	PHLDA1	Pleckstrin homology-like domain, family A, member 1
-2.81	FAM198B	Family with sequence similarity 198, member B
-2.81	NR1D1	Nuclear receptor subfamily 1, group D, member 1
-2.82	NUP210	Nucleoporin 210 kDa
-2.83	PCID2	PCI domain containing 2
-2.83	ZBTB38	Zinc finger and BTB domain containing 38
-2.83	PIGF	Phosphatidylinositol glycan anchor biosynthesis, class F
-2.83	GLRXP3	Glutaredoxin (thioltransferase) pseudogene 3
-2.74	SSFA2	Sperm specific antigen 2
-2.84	HSPA13	Heat shock protein 70 kDa family, member 13
-2.85	RBMS3	RNA binding motif, single stranded interacting protein 3
-2.88	SMYD3	SET and MYND domain containing 3
-2.88	ASNS	Asparagine synthetase (glutamine-hydrolyzing)
-2.89	TFPI2	Tissue factor pathway inhibitor 2
-2.89	TNFRSF19	Tumor necrosis factor receptor superfamily, member 19
-2.90	CRY1	Cryptochrome 1 (photolyase-like)
-2.90	IL1RAP	Interleukin 1 receptor accessory protein
-2.91	AGA	Aspartyl glucose aminidase
-2.93	FBXO32	F-box protein 32
-2.94	LIF	Leukemia inhibitory factor
-2.94	TMEM194B	Transmembrane protein 194B
-2.95	FAM115A	Family with sequence similarity 115, member A
-2.96	PTPRD	Protein tyrosine phosphatase, receptor type, D
-2.98	HERPUD1	Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1
-2.98	ALPK2	α -kinase 2
-3.00	BMP6	Bone morphogenetic protein 6
-3.00	LONP1	Lon peptidase 1, mitochondrial
-3.02	CCPG1	Cell cycle progression 1; DYX1C1-CCPG1 readthrough (non-protein coding)
-3.03	CHAC1	ChaC, cation transport regulator homolog 1 (<i>E. coli</i>)
-3.04	XBP1	X-box binding protein 1
-3.05	FHAD1	Forkhead-associated (FHA) phosphopeptide binding domain 1
-3.07	GREM2	Gremlin 2
-3.07	CXCL2	Chemokine (C-X-C motif) ligand 2
-3.07	KLRC3	Killer cell lectin-like receptor subfamily C, member 3; member 2
-3.08	CD36	CD36 molecule (thrombospondin receptor)
-3.08	LINC00173	Long intergenic non-protein coding RNA 173
-3.08	MIRLET7F1	microRNA let-7f-1
-3.09	GDF15	Growth differentiation factor 15

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-3.09	VEGFA	Vascular endothelial growth factor A
-3.11	COL4A6	Collagen, type IV, $\alpha 6$
-3.11	ANTXR2	Anthrax toxin receptor 2
-3.12	EDIL3	EGF-like repeats and discoidin I-like domains 3
-3.12	SEPP1	Selenoprotein P, plasma, 1
-3.12	COL4A5	Collagen, type IV, $\alpha 5$
-3.12	RNF19A	Ring finger protein 19A, E3 ubiquitin protein ligase
-3.12	SNRPA1	Small nuclear ribonucleoprotein polypeptide A'
-3.12	P4HA1	Prolyl 4-hydroxylase, α polypeptide I
-3.13	ANK2	Ankyrin 2, neuronal
-3.13	TUBE1	Tubulin, $\epsilon 1$
-3.14	GPR65	G protein-coupled receptor 65
-3.15	FRK	Fyn-related kinase
-3.15	SLC2A13	Solute carrier family 2 (facilitated glucose transporter), member 13
-3.15	CTH	Cystathionase (cystathionine γ -lyase)
-3.15	PLA2G7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
-3.16	PCK2	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)
-3.17	PRSS23	Protease, serine, 23
-3.18	FBXL13	F-box and leucine-rich repeat protein 13
-3.18	B4GALT1	UDP-Gal: β GlcNAc β 1,4-galactosyltransferase, polypeptide 1
-3.19	CXCL5	Chemokine (C-X-C motif) ligand 5
-3.19	PHGDH	Phosphoglycerate dehydrogenase
-3.19	SLC4A7	Solute carrier family 4, sodium bicarbonate cotransporter, member 7
-3.19	DDIT4	DNA-damage-inducible transcript 4
-3.21	RSPO3	R-spondin 3
-3.21	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase
-3.22	FAM129A	Family with sequence similarity 129, member A
-3.22	CDH13	Cadherin 13, H-cadherin (heart)
-3.23	PLAU	Plasminogen activator, urokinase
-3.24	SEMA3C	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C
-3.26	CTBS	Chitinase, di-N-acetyl-
-3.27	SLC4A11	Solute carrier family 4, sodium borate transporter, member 11
-3.28	CSGALNACT1	Chondroitin sulfate N-acetylgalactosaminyltransferase 1
-3.29	PDE1A	Phosphodiesterase 1A, calmodulin-dependent
-3.29	BDNF	Brain-derived neurotrophic factor
-3.29	SAMD13	Sterile α motif domain containing 13
-3.30	KLRC4-KLRK1	KLRC4-KLRK1 readthrough; killer cell lectin-like receptor subfamily K, member 1
-3.31	NRN1	Neuritin 1
-3.31	SEL1L3	Sel-1 suppressor of lin-12-like 3 (<i>C. elegans</i>)
-3.33	PDE3A	Phosphodiesterase 3A, cGMP-inhibited
-3.33	FBN2	Fibrillin 2
-3.33	NCAM1	Neural cell adhesion molecule 1
-3.34	RAB31	RAB31, member RAS oncogene family
-3.35	DEFB1	Defensin, $\beta 1$
-3.36	PLAUR	Plasminogen activator, urokinase receptor
-3.36	TSC22D3	TSC22 domain family, member 3
-3.36	CNTN1	Contactin 1
-3.37	PCSK1	Proprotein convertase subtilisin/kexin type 1
-3.38	ADAMTS6	ADAM metalloproteinase with thrombospondin type 1 motif, 6
-3.40	LOXL2	Lysyl oxidase-like 2

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-3.40	LURAP1L	Leucine rich adaptor protein 1-like
-3.41	SCD	Stearoyl-CoA desaturase (δ -9-desaturase)
-3.42	PSD3	Pleckstrin and Sec7 domain containing 3
-3.43	EPSTI1	Epithelial stromal interaction 1 (breast)
-3.44	PLA2R1	Phospholipase A2 receptor 1, 180 kDa
-3.45	CDR1	Cerebellar degeneration-related protein 1, 34 kDa
-3.46	CYR61	Cysteine-rich, angiogenic inducer, 61
-3.46	KLRC2	Killer cell lectin-like receptor subfamily C, member 2
-3.46	BHLHE40	Basic helix-loop-helix family, member e40
-3.47	FSTL1	Follistatin-like 1
-3.52	SLC7A2	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 2
-3.53	GTPBP2	GTP binding protein 2
-3.53	THSD4	Thrombospondin, type I, domain containing 4
-3.54	CPED1	Cadherin-like and PC-esterase domain containing 1
-3.54	ESM1	Endothelial cell-specific molecule 1
-3.54	SEMA3A	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A
-3.55	ASS1P11	Argininosuccinate synthetase 1 pseudogene 11
-3.56	ITGA3	Integrin, α 3 (antigen CD49C, α 3 subunit of VLA-3 receptor)
-3.57	SAT1	Spermidine/spermine N1-acetyltransferase 1
-3.57	PYROXD1	Pyridine nucleotide-disulphide oxidoreductase domain 1
-3.59	WDR69	WD repeat domain 69
-3.59	SLC6A9	Solute carrier family 6 (neurotransmitter transporter, glycine), member 9
-3.62	PAPPA2	Pappalysin 2
-3.63	MALAT1	Metastasis-associated lung adenocarcinoma transcript 1 (non-protein coding)
-3.64	LAMA5	Laminin, α 5
-3.65	EDN1	Endothelin 1
-3.71	SMOX	Spermine oxidase
-3.72	MIR29A	microRNA 29a
-3.74	TFPI	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
-3.75	GLRX	Glutaredoxin (thioltransferase)
-3.79	GPRIN3	GPRIN family member 3
-3.79	TRPC6	Transient receptor potential cation channel, subfamily C, member 6
-3.81	IL8	Interleukin 8
-3.86	IL18R1	Interleukin 18 receptor 1
-3.89	LAMB3	Laminin, β 3
-3.90	PDCD1LG2	Programmed cell death 1 ligand 2
-3.93	STARD4	StAR-related lipid transfer (START) domain containing 4
-3.93	DLG2	Discs, large homolog 2 (<i>Drosophila</i>)
-3.96	LSAMP	Limbic system-associated membrane protein
-4.00	PITPNC1	Phosphatidylinositol transfer protein, cytoplasmic 1
-4.03	APLN	Apelin
-4.03	CTGF	Connective tissue growth factor
-4.06	LRRC63	Leucine rich repeat containing 63
-4.08	PLAT	Plasminogen activator, tissue
-4.15	SLC25A37	Solute carrier family 25 (mitochondrial iron transporter), member 37
-4.19	NUPR1	Nuclear protein, transcriptional regulator, 1
-4.23	TSPAN8	Tetraspanin 8
-4.24	SPOCK1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1
-4.25	NRCAM	Neuronal cell adhesion molecule
-4.25	C3orf49	Chromosome 3 open reading frame 49
-4.35	TMEM144	Transmembrane protein 144

Table II. Continued.

Fold-change	Gene symbol	mRNA description
-4.41	PARK2	Parkinson protein 2, E3 ubiquitin protein ligase (parkin)
-4.44	SCARA5	Scavenger receptor class A, member 5 (putative)
-4.53	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
-4.60	THSD7A	Thrombospondin, type I, domain containing 7A
-4.66	IL6	Interleukin 6 (interferon, β 2)
-4.67	CDH19	Cadherin 19, type 2
-4.70	VLDLR	Very low density lipoprotein receptor
-4.78	STC1	Stanniocalcin 1
-4.81	ABI3BP	ABI family, member 3 (NESH) binding protein
-4.90	TNFAIP3	Tumor necrosis factor, α -induced protein 3
-4.90	DUSP6	Dual specificity phosphatase 6
-4.90	SEMA3D	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D
-4.93	CBS	Cystathionine- β -synthase
-4.95	DPP10	Dipeptidyl-peptidase 10 (non-functional)
-5.19	SLC16A4	Solute carrier family 16, member 4 (monocarboxylic acid transporter 5)
-5.22	CLDN1	Claudin 1
-5.28	KIAA0825	KIAA0825
-5.35	PLAC4	Placenta-specific 4
-5.38	EPHA4	EPH receptor A4
-5.48	ALDH1L2	Aldehyde dehydrogenase 1 family, member L2
-5.51	DDIT3	DNA-damage-inducible transcript 3
-5.51	TXNIP	Thioredoxin interacting protein
-5.52	WARS	Tryptophanyl-tRNA synthetase
-5.69	PRICKLE1	Prickle homolog 1 (<i>Drosophila</i>)
-5.81	CLGN	Calmegin
-5.81	ERRFI1	ERBB receptor feedback inhibitor 1
-5.94	SMOC1	SPARC related modular calcium binding 1
-6.03	ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
-6.05	ASS1	Argininosuccinate synthase 1
-6.20	KCNE4	Potassium voltage-gated channel, Isk-related family, member 4
-6.23	C12orf39	Chromosome 12 open reading frame 39
-6.24	LCN1	Lipocalin 1
-6.28	PAPPA	Pregnancy-associated plasma protein A, pappalysin 1
-6.44	TMEM27	Transmembrane protein 27
-6.70	CHL1	Cell adhesion molecule with homology to L1CAM (close homolog of L1)
-6.83	SUCNR1	Succinate receptor 1
-7.16	AREG	Amphiregulin
-7.57	HPGDS	Hematopoietic prostaglandin D synthase
-7.62	ANXA3	Annexin A3
-8.16	SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3
-8.34	TGFB2	Transforming growth factor, β 2
-8.40	GABRE	γ -aminobutyric acid (GABA) A receptor, ϵ ; microRNA 452; microRNA 224
-8.88	RNY1P5	RNA, Ro-associated Y1 pseudogene 5
-9.52	RPE65	Retinal pigment epithelium-specific protein 65 kDa
-9.54	CD274	CD274 molecule
-12.33	FST	Follistatin
-12.69	GPR133	G protein-coupled receptor 133
-13.11	SERPINB11	Serpin peptidase inhibitor, clade B (ovalbumin), member 11 (gene/pseudogene)
-15.73	IL1RL1	Interleukin 1 receptor-like 1

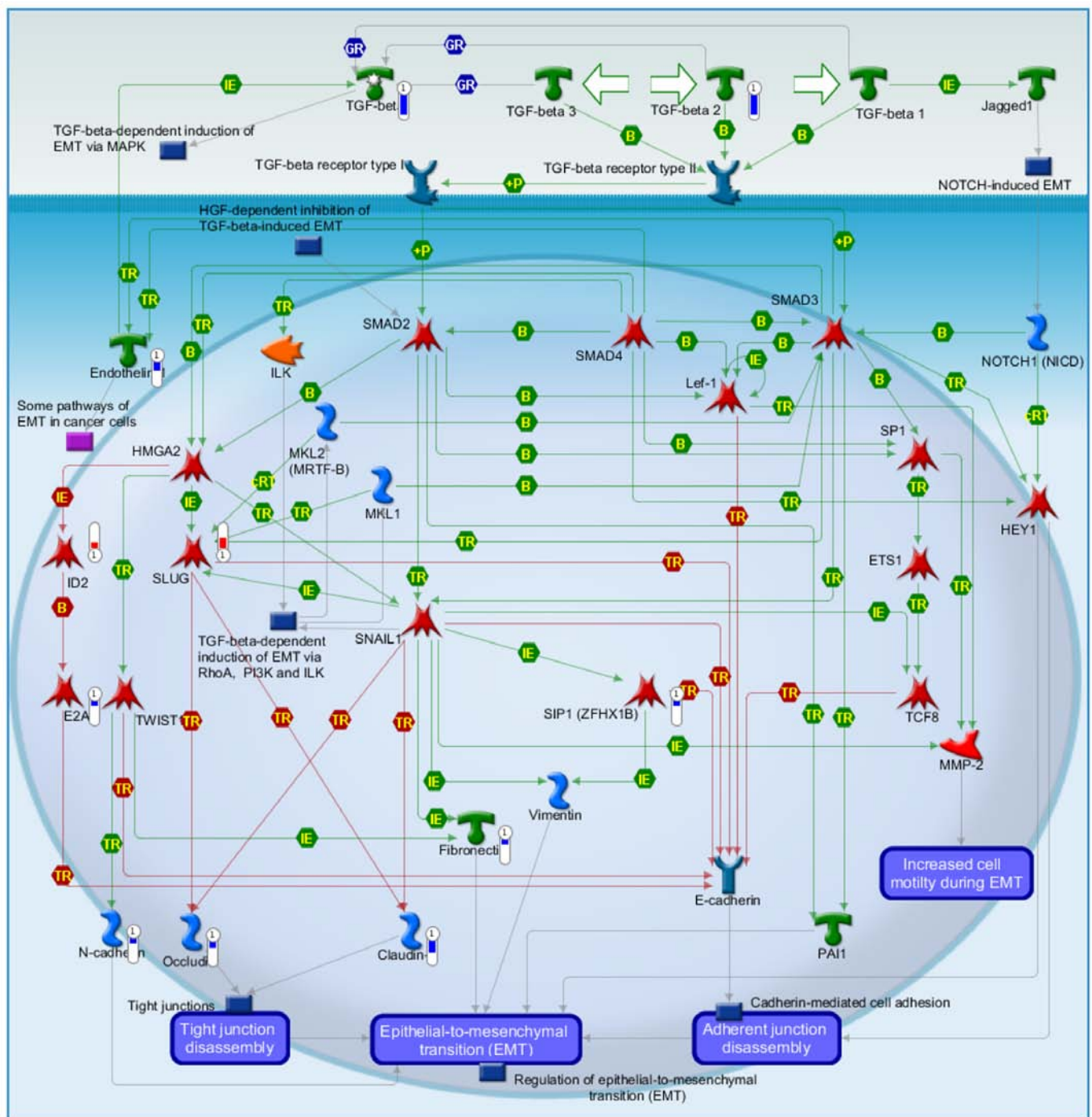


Figure 1. The top scored map (map with the lowest p-value) based on the enrichment distribution sorted by the 'Statistically significant Maps' set. Experimental data from all the files are linked to and visualized on the maps as thermometer-like figures. Upward thermometers (red) indicate upregulated signals and downward (blue) ones indicate downregulated gene expression levels.

The results also show that curcumin decreased several gene expressions associated with DNA damage, cell cycle, cell survival and cell migration and invasion. For example, DDIT3, DDIT4, GADD45A and CGREF1 were decreased 5.51-, 3.19-, 2.31- and 2.31-fold, respectively, which was associated with DNA damage. Curcumin decreased 3.02-fold of the *CCPG1* gene expression which was associated with cell cycle progression and the *TNFRSF10B*, *TSSC1*, *GAS5* and *TNFRSF11B* genes were decreased 2.17-, 2.03-, 2.03- and 2.02-fold, which was associated with cell survival. Furthermore, curcumin

decreased two gene expressions at 2.15- and 2.06-fold, respectively, which associated with cell migration. It was reported that the induction of DNA-damage-inducible transcript 3 (DDIT3) was observed in the liver of mice exposed to coal mining and this gene is frequently upregulated in response to cell stress and DNA damage (33). DNA-damage-inducible transcript 4 (DDIT4) is involved in multiple biological settings such as inhibiting mTORC1 signaling and regulating the production of reactive oxygen species (35,36), which was also associated with DNA damage. It was demonstrated that

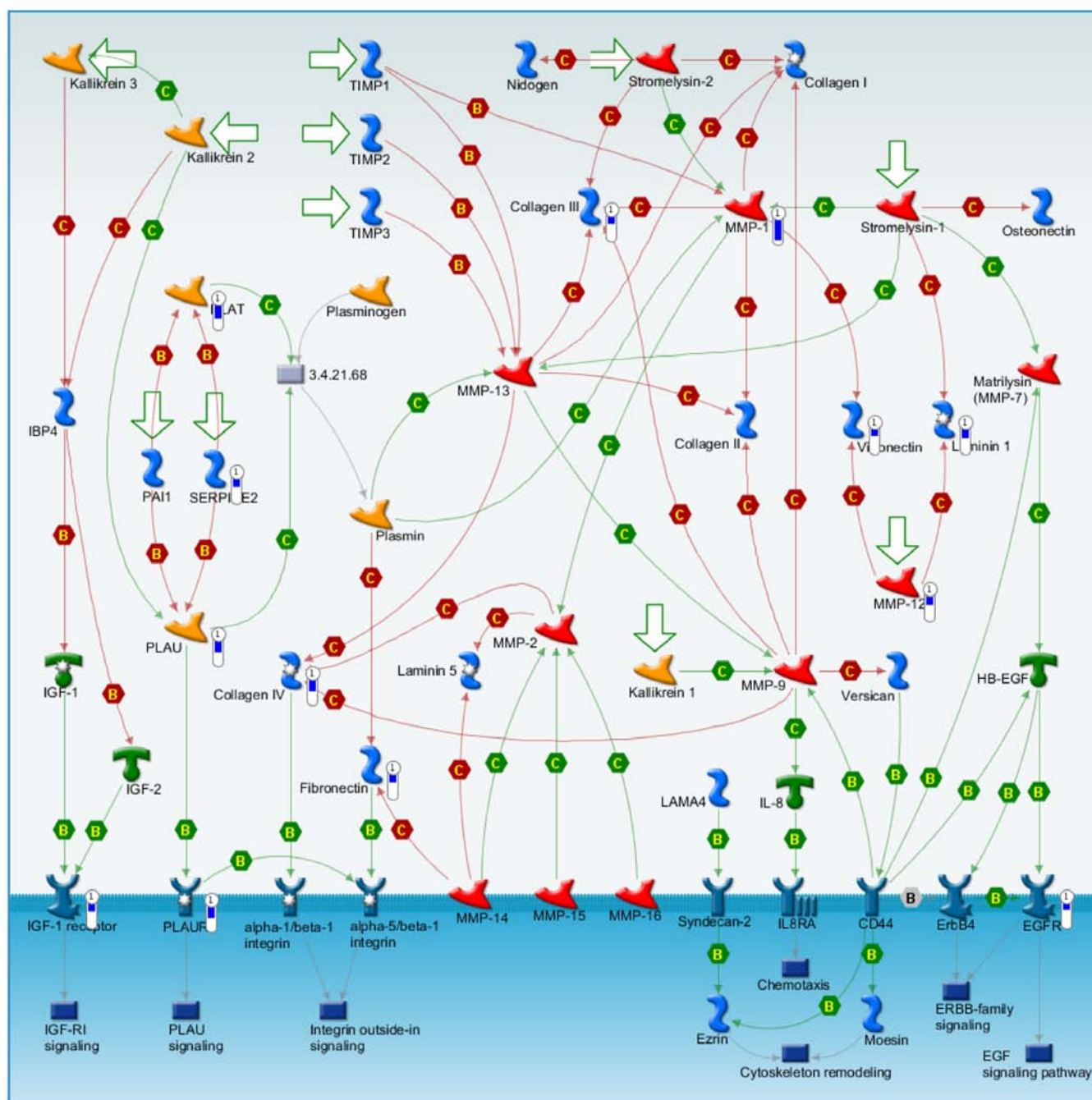


Figure 2. The second scored map (map with the second lowest p-value) based on the enrichment distribution sorted by the 'Statistically significant Maps' set. Experimental data from all the files are linked to and visualized on the maps as thermometer-like images. Upward thermometers (red) and indicate upregulated signals and downward (blue) ones indicate downregulated gene expression levels.

myristicin, an allylbenzene, is a major active component of various spices inducing DNA damage signalling (ATM) and stress response (GADD45A and GADD45G) in leukemia cells (34). The TNFRSF10B gene which encodes the apoptotic death receptor TNFRSF10B was associated with cell death (38). It was also reported that the recurrent amplification of MYC and TNFRSF11B in 8q24 is associated with poor survival in patients with gastric cancer survival (35). Growth arrest-specific 5 (GAS5) is a non-coding gene that hosts a number of small nucleolar RNAs (snoRNAs) and it has been suggested to play a tumor suppressive role (36,37).

The results of the present study indicated that a number of genes were associated with DNA damage and repair, cell cycle check point, cell survival and cell migration and invasion in NCI-H460 cells following exposure to curcumin. Based on these observations, we determined these associated genes with possible signaling complex interactions. Thus, future investigations are needed to expand or append our current findings and possible understandings. These genes were affected by curcumin, which provides more information for the understanding of the cytotoxic mechanism of curcumin at the genetic level. Furthermore, it provides potentially useful

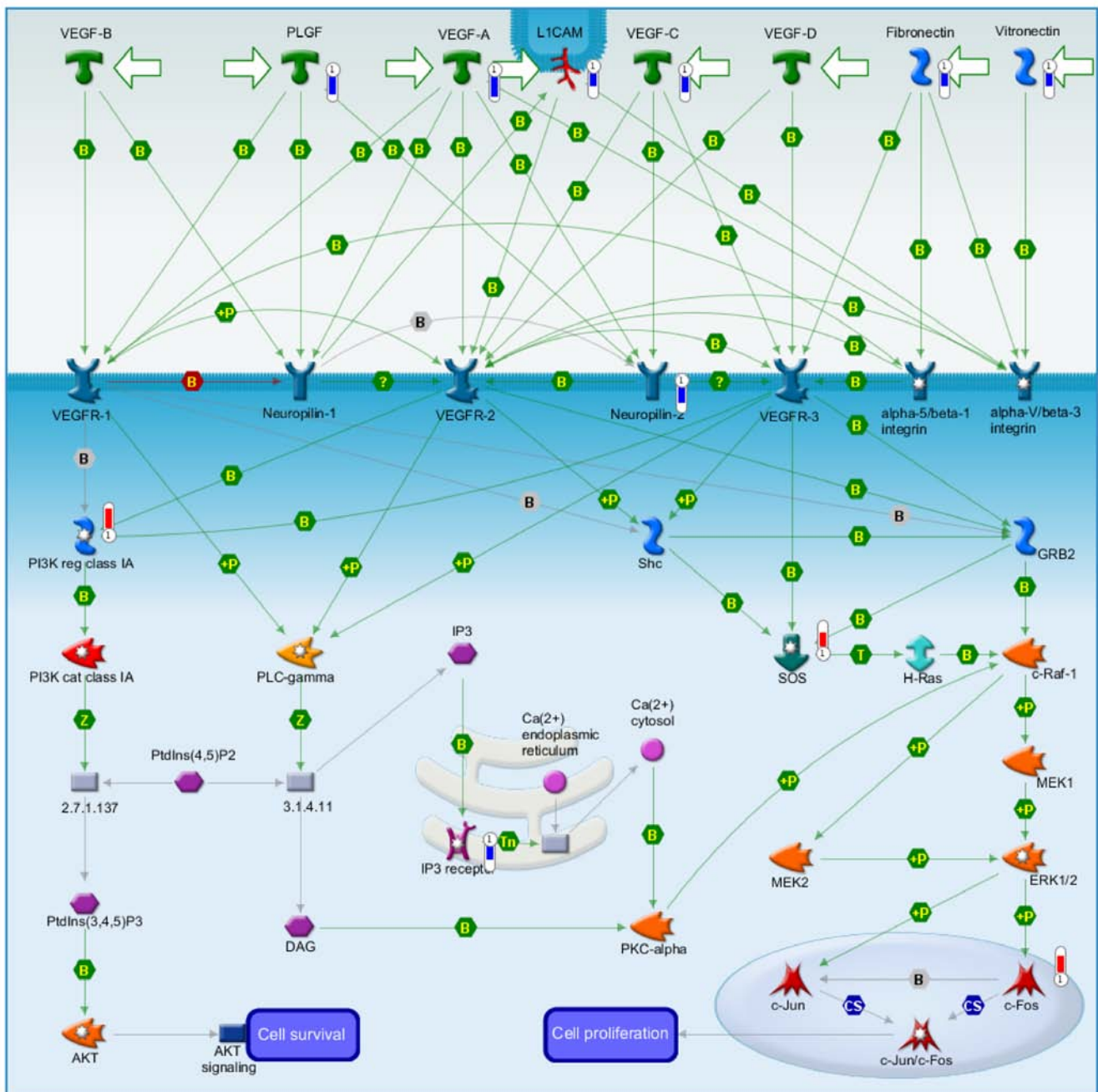


Figure 3. The third scored map (map with the third lowest p-value) based on the enrichment distribution sorted by the 'Statistically significant Maps' set. Experimental data from all the files are linked to and visualized on the maps as thermometer-like figures. Upward thermometers (red) indicate upregulated signals and downward (blue) ones indicate downregulated gene expression levels.

biomarkers or targets in clinic for the diagnosis and treatment of human lung cancer.

Acknowledgements

This study was supported by grant RD2015-032 from the National Yang-Ming University Hospital, Yilan, Taiwan.

References

1. Buyukcelik A, Yalcin B and Utkan G: Multidisciplinary management of lung cancer. *N Engl J Med* 350: 2008-2010, 2004.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. *CA Cancer J Clin* 61: 69-90, 2011.
3. McCulloch M, See C, Shu XJ, Broffman M, Kramer A, Fan WY, Gao J, Lieb W, Shieh K and Colford JM Jr: *Astragalus*-based Chinese herbs and platinum-based chemotherapy for advanced non-small-cell lung cancer: Meta-analysis of randomized trials. *J Clin Oncol* 24: 419-430, 2006.
4. Tsai MF, Wang CC and Chen JJ: Tumour suppressor HLJ1: A potential diagnostic, preventive and therapeutic target in non-small cell lung cancer. *World J Clin Oncol* 5: 865-873, 2014.
5. Prasad S, Tyagi AK and Aggarwal BB: Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. *Cancer Res Treat* 46: 2-18, 2014.
6. Hasima N and Aggarwal BB: Cancer-linked targets modulated by curcumin. *Int J Biochem Mol Biol* 3: 328-351, 2012.

7. Campbell FC and Collett GP: Chemopreventive properties of curcumin. *Future Oncol* 1: 405-414, 2005.
8. Sarkar FH and Li Y: Cell signaling pathways altered by natural chemopreventive agents. *Mutat Res* 555: 53-64, 2004.
9. Shishodia S, Sethi G and Aggarwal BB: Curcumin: Getting back to the roots. *Ann NY Acad Sci* 1056: 206-217, 2005.
10. Zanutto-Filho A, Braganhol E, Edelweiss MI, Behr GA, Zanin R, Schröder R, Simões-Pires A, Battastini AM and Moreira JC: The curry spice curcumin selectively inhibits cancer cells growth in vitro and in preclinical model of glioblastoma. *J Nutr Biochem* 23: 591-601, 2012.
11. Xiao K, Jiang J, Guan C, Dong C, Wang G, Bai L, Sun J, Hu C and Bai C: Curcumin induces autophagy via activating the AMPK signaling pathway in lung adenocarcinoma cells. *J Pharmacol Sci* 123: 102-109, 2013.
12. Chen QY, Zheng Y, Jiao DM, Chen FY, Hu HZ, Wu YQ, Song J, Yan J, Wu LJ and Lv GY: Curcumin inhibits lung cancer cell migration and invasion through Rac1-dependent signaling pathway. *J Nutr Biochem* 25: 177-185, 2014.
13. Lev-Ari S, Starr A, Katzburg S, Berkovich L, Rimmon A, Ben-Yosef R, Vexler A, Ron I and Earon G: Curcumin induces apoptosis and inhibits growth of orthotopic human non-small cell lung cancer xenografts. *J Nutr Biochem* 25: 843-850, 2014.
14. Shen Y, Zhang S, Huang X, Chen K, Shen J and Wang Z: Involvement of p53 mutation and mismatch repair proteins dysregulation in NNK-induced malignant transformation of human bronchial epithelial cells. *Biomed Res Int* 2014: 920275, 2014.
15. Muller PA and Vousden KH: p53 mutations in cancer. *Nat Cell Biol* 15: 2-8, 2013.
16. Bunn PA Jr: Worldwide overview of the current status of lung cancer diagnosis and treatment. *Arch Pathol Lab Med* 136: 1478-1481, 2012.
17. Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, Ince WL, Jänne PA, Januario T, Johnson DH, *et al*: Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 23: 5900-5909, 2005.
18. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Jänne PA, Costa DB, *et al*: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 363: 1693-1703, 2010.
19. Wu SH, Hsiao YT, Chen JC, Lin JH, Hsu SC, Hsia TC, Yang ST, Hsu WH and Chung JG: Bufalin alters gene expressions associated DNA damage, cell cycle, and apoptosis in human lung cancer NCI-H460 cells in vitro. *Molecules* 19: 6047-6057, 2014.
20. Gardina PJ, Clark TA, Shimada B, Staples MK, Yang Q, Veitch J, Schweitzer A, Awad T, Sugnet C, Dee S, *et al*: Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. *BMC Genomics* 7: 325, 2006.
21. Li Z, Meng Q, Yu Q, Zhou Z and Li L: Evaluation of c-myc and CCNE2 amplification in breast cancer with quantitative multi-gene fluorescence in-situ hybridization. *Zhonghua Bing Li Xue Za Zhi* 43: 455-458, 2014 (In Chinese).
22. Kawase T, Ichikawa H, Ohta T, Nozaki N, Tashiro F, Ohki R and Taya Y: p53 target gene *AEN* is a nuclear exonuclease required for p53-dependent apoptosis. *Oncogene* 27: 3797-3810, 2008.
23. Shahbazi J, Lock R and Liu T: Tumor protein 53-induced nuclear protein 1 enhances p53 function and represses tumorigenesis. *Front Genet* 4: 80, 2013.
24. Kim GS, Kang J, Bang SW and Hwang DS: Cdc6 localizes to S- and G2-phase centrosomes in a cell cycle-dependent manner. *Biochem Biophys Res Commun* 456: 763-767, 2015.
25. Bidkhorji G, Narimani Z, Hosseini Ashtiani S, Moeini A, Nowzari-Dalini A and Masoudi-Nejad A: Reconstruction of an integrated genome-scale co-expression network reveals key modules involved in lung adenocarcinoma. *PLoS One* 8: e67552, 2013.
26. Davidson G and Niehrs C: Emerging links between CDK cell cycle regulators and Wnt signaling. *Trends Cell Biol* 20: 453-460, 2010.
27. Ding Y, Zhu W, Sun R, Yuan G, Zhang D, Fan Y and Sun J: Diphenylene iodonium interferes with cell cycle progression and induces apoptosis by modulating NAD(P)H oxidase/ROS/cell cycle regulatory pathways in Burkitt's lymphoma cells. *Oncol Rep* 33: 1434-1442, 2015.
28. Jiang DS, Wei X, Zhang XF, Liu Y, Zhang Y, Chen K, Gao L, Zhou H, Zhu XH, Liu PP, *et al*: IRF8 suppresses pathological cardiac remodelling by inhibiting calcineurin signalling. *Nat Commun* 5: 3303, 2014.
29. Li L, Chen W, Zhu Y, Wang X, Jiang DS, Huang F, Wang L, Xiang F, Qin W, Wang Q, *et al*: Caspase recruitment domain 6 protects against cardiac hypertrophy in response to pressure overload. *Hypertension* 64: 94-102, 2014.
30. Norton JD: ID helix-loop-helix proteins in cell growth, differentiation and tumorigenesis. *J Cell Sci* 113: 3897-3905, 2000.
31. Lasorella A, Uo T and Iavarone A: Id proteins at the cross-road of development and cancer. *Oncogene* 20: 8326-8333, 2001.
32. Sikder HA, Devlin MK, Dunlap S, Ryu B and Alani RM: Id proteins in cell growth and tumorigenesis. *Cancer Cell* 3: 525-530, 2003.
33. Sun LY, Bokov AF, Richardson A and Miller RA: Hepatic response to oxidative injury in long-lived Ames dwarf mice. *FASEB J* 25: 398-408, 2011.
34. Martins C, Doran C, Silva IC, Miranda C, Rueff J and Rodrigues AS: Myristicin from nutmeg induces apoptosis via the mitochondrial pathway and down regulates genes of the DNA damage response pathways in human leukaemia K562 cells. *Chem Biol Interact* 218: 1-9, 2014.
35. Wang X, Liu Y, Shao D, Qian Z, Dong Z, Sun Y, Xing X, Cheng X, Du H, Hu Y, *et al*: Recurrent amplification of *MYC* and *TNFRSF11B* in 8q24 is associated with poor survival in patients with gastric cancer. *Gastric Cancer*: Jan 25, 2015 (Epub ahead of print). <http://dx.doi.org/10.1007/s10120-015-0467-2>.
36. Kino T, Hurt DE, Ichijo T, Nader N and Chrousos GP: Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal* 3: ra8, 2010.
37. Mourtada-Maarabouni M, Hasan AM, Farzaneh F and Williams GT: Inhibition of human T-cell proliferation by mammalian target of rapamycin (mTOR) antagonists requires noncoding RNA growth-arrest-specific transcript s5 (GAS5). *Mol Pharmacol* 78: 19-28, 2010.