

## Systemic cell-cycle suppression by Apicidin, a histone deacetylase inhibitor, in MDA-MB-435 cells

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**Abstract.** Histone deacetylase (HDAC) inhibitors are emerging as an exciting new class of potential anti-cancer agents for the treatment of solid and hematological malignancies. However, the best characterized HDAC function concerns the control of gene expression via the regulation of transcription activation or repression. To understand the genome-wide effects of HDAC inhibition on gene regulation, we performed serial gene expression analyses from 0 to 48 h after treating MDA-MB-435, a melanoma-derived highly metastatic tumor cell line, with Apicidin, a HDAC inhibitor. Combined-transcriptomic analysis of large-scale molecular changes induced by Apicidin resulted in the identification of 631 outlier genes that were continuously up- or down-regulated during the 48 h study period. When the 631 outlier genes were mapped to known biological processes, cell-cycle suppression emerged as the function most elicited by Apicidin. In addition comprehensive negative cell-cycle regulation by Apicidin was dissected using gene expression data and validated by Western blot analysis. We suggest the 631 outlier genes as a characteristic molecular signature for Apicidin, and propose concurrent transcriptional suppression of major components of cell-cycle regulatory circuit as potent anti-tumor mechanism of Apicidin. Genetic elements identified during this study also provide the possibility of novel therapeutic interventions in tumor metastasis.

### Introduction

Aberrant gene regulation plays an important role in tumor initiation and progression, and methods designed to correct this dysfunction constitute new anti-cancer strategies (1). The acetylation status of lysine residues in nucleosomal histone proteins is known to play a crucial role in chromatin structure and gene transcription (2). Histone deacetylases (HDACs) and the family of histone acetyltransferases (HATs) are involved in the determination of the acetylation of histones, which are known to participate in the regulation of gene expression (3). HDACs are known to play important roles in the regulation of gene transcription, and as such, are involved in key biological processes during cell proliferation, differentiation, and survival (4-6). Although no clear evidence has been presented demonstrating that HDAC alterations are directly associated with human cancer, class I and II HDACs have been reported to be associated with several well-known oncogenes and tumor suppressors (7). For example, the repression of tumor suppressor genes is known to promote carcinogenesis (8), and multi-protein complexes that contain DNA binding protein commonly use HDACs to repress transcription and block the functions of tumor suppressors.

Recently, HDAC inhibitors have emerged as promising chemotherapeutic agents, and the findings of several studies suggest that they can induce a range of anti-tumor activities including the induction of cell-cycle arrest, the stimulation of differentiation, and the provocation of apoptosis in a variety of transformed cells in culture and tumor bearing animals (3,4,7,9). Unlike conventional chemotherapeutic agents that often cause DNA damage in tumor and normal tissues, HDAC inhibitors display strong selectivity and are less toxic to normal tissues (3). However, the mechanisms underlying this tumor selectivity are not fully understood. During recent years, increasing numbers of structurally diverse HDAC inhibitors have been identified that inhibit the proliferation and induce the differentiation and/or apoptosis of tumor cells *in vitro* and *in vivo*. Actually, several natural and synthetic compounds including n-butylate, trapoxin, trichostatin A (TSA), depudecin, and MS-27-275 can inhibit HDAC (10). The efficacies of these

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agents, particularly of TSA and suberoylanilide hydroxamic acid (SAHA), have been established by *in vitro* experiments and ongoing clinical trials (9,11). Furthermore, some HDAC inhibitors, such as valproate and depsipeptide (FR901228) are currently undergoing phase II clinical trials (12,13).

Apicidin [cyclo(N-O-methyl-L-tryptophanyl-L-isoleucinyl-L-2-amino-8-oxodecanoyl)], a recently studied HDAC inhibitor, has been reported to be a potent and universal inhibitor of HDACs and to exhibit anti-tumor effects (e.g. inhibition of cell proliferation). In addition, these anti-tumor activities have also been observed *in vitro* for different cancer cell lines (14,15). Apicidin was first described as anti-cancer agent that suppressed HeLa cell growth by inducing p21<sup>WAF1/Cip1</sup> (15). However, no attempt has been made to portray the molecular circuit responsible for anti-tumor mechanism via HDAC inhibition in tumor cells. The present study describes large-scale molecular changes at the transcriptomic level and provides insight into the systemic anti-neoplastic mechanisms responsible of inhibiting HDACs, based on a study of the effects of Apicidin, a potent HDAC inhibitor, on MDA-MB-435 cells.

## Materials and methods

**Cell culture.** MDA-MB-435 cell line derived from the melanoma cell line M14 was obtained from the American Type Culture Collection (Manassas, VA, USA) and grown in appropriate culture media supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT, USA), and 1% penicillin/streptomycin (Gibco Industries Inc., Carlsland, CA, USA). The cultures were maintained in a 5% CO<sub>2</sub> humidified atmosphere at 37°C.

**Apicidin treatment.** Apicidin [cyclo(N-O-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)] was purchased from Calbiochem® (Merck KGaA, Darmstadt, Germany). MDA-MB-435 cells were harvested and seeded at 1.5x10<sup>5</sup> cells in a 60-mm dish, and allowed to grow in complete growth media (DMEM/10% FBS) overnight at 37°C in a 5% CO<sub>2</sub> humidified incubator. Cells were then treated with the indicated amounts of Apicidin for cell growth and apoptosis experiments. For comprehensive genomic analysis, Apicidin (1 µg/ml) was added at the above-mentioned time points into MDA-MB-435 cells and, RNAs of cells treated with Apicidin for the above-mentioned times were subjected to DNA microarray analysis.

**Antibodies.** Antibodies against PARP, p21, CDK2, CDK4, were purchased from Cell Signaling (Cell Signaling Technology, Danver, MA, USA). Antibodies against DP-1, E2F1, HDAC1, β-actin were obtained from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and antibodies against histone H4, α-tubulin, and acetyl-histone H4 were purchased from Upstate (Upstate Biotechnology, Lake Placid, NY, USA).

**Apoptosis assays.** MDA-MB-435 cells were plated at a density of 1.5x10<sup>5</sup> cells/plate and treated with Apicidin at the above-mentioned concentrations for 4 days in a 60-mm dish. Annexin V-FITC Apoptosis Detection Kit I (BD Biosciences,

San Diego, CA, USA) was used to quantify levels of apoptosis induced by Apicidin. Briefly, cells were trypsinized, centrifuged and resuspended in binding buffer at 1x10<sup>6</sup> cells/ml, and 5 µl of Annexin V-FITC and 5 µl of PI were added to 100 µl of the suspension (~1x10<sup>5</sup> cells). Cells were then incubated at RT for 15 min in the dark, and analyzed by flow cytometry (FACS Vantage SE Flow Cytometric system; Becton-Dickinson, Franklin Lakes, NJ, USA).

**Cell growth assays.** Cells were seeded at 1x10<sup>5</sup> cells/well in 6-well plates, and 24 h later, a stock solution of Apicidin in ethanol was added to concentrations of 0, 0.5, 1, or 2 µg/ml. At the above-mentioned time points, viable cells were counted using the Trypan blue exclusion method. All experiments were conducted in triplicate.

**Western blot analysis.** After being treated with Apicidin, whole cells were washed twice in ice-cold PBS and lysed with 200 µl of RIPA buffer [50 mM Tris-HCl, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 tablet/50 ml of protease inhibitor cocktail (Roche, Mannheim, Germany)]. Protein concentrations in whole cell lysates were determined using BCA protein assay reagent (Pierce, Rockford, IL, USA), and 20 µg aliquots of proteins were subjected to SDS-polyacrylamide gel electrophoresis, and then transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Piscataway, NJ, USA). Membranes were blocked with Tris-buffered saline (TBS) containing 0.1% Tween-20 and 5% (w/v) dried skimmed milk powder and incubated in fresh blocking solution containing primary antibodies at appropriate dilutions for 1 h at RT. Blots were then washed three times for 5 min in TBS-T, incubated with a 1:5000 dilution of horseradish peroxidase-conjugated 2nd antibody (Pierce) for 1 h at RT, and rewash (3x5 min) in TBS-T. The protein-antibody reactions were detected using ECL detection kits (Amersham Biosciences).

**Large-scale analysis of gene expression profiling using oligo-nucleotide arrays.** Microarray analysis was performed using human DNA microarrays manufactured by the Microdissection Genomics Research Center, College of Medicine, The Catholic University of Korea. Microarrays contained approximately 19000 genetic elements (16). In brief, total cellular RNA was extracted from Apicidin-treated cells using TRIzol® reagent (Gibco Industries Inc.). Total RNA was quantified with NanoDrop (NanoDrop Technologies, Wilmington, DE, USA). RNA qualities were checked using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and intact RNA samples (260/280 ratio >1.8) were used for hybridization. Human universal reference RNA (Stratagene, La Jolla, CA, USA) was used as a reference RNA. For each RNA sample, 20 µg of total RNA was primed with Oligo(dT) and labeled with Cy3-dUTP or Cy5-dUTP (NEN, Boston, MA, USA) during reverse transcription. Labeled cDNA targets were concentrated to ~15 µl using a Microcon-YM30 (Amicon, Bedford, MA, USA). Hybridization was carried out at 42°C for 16 h using the MAUI Hybridization System (BioMicro Systems, Salt Lake City, UT, USA). After hybridization, microarrays were washed with the following buffers; 2X standard sodium citrate (SSC) and 0.1% SDS for 2 min (twice), 1X SSC and

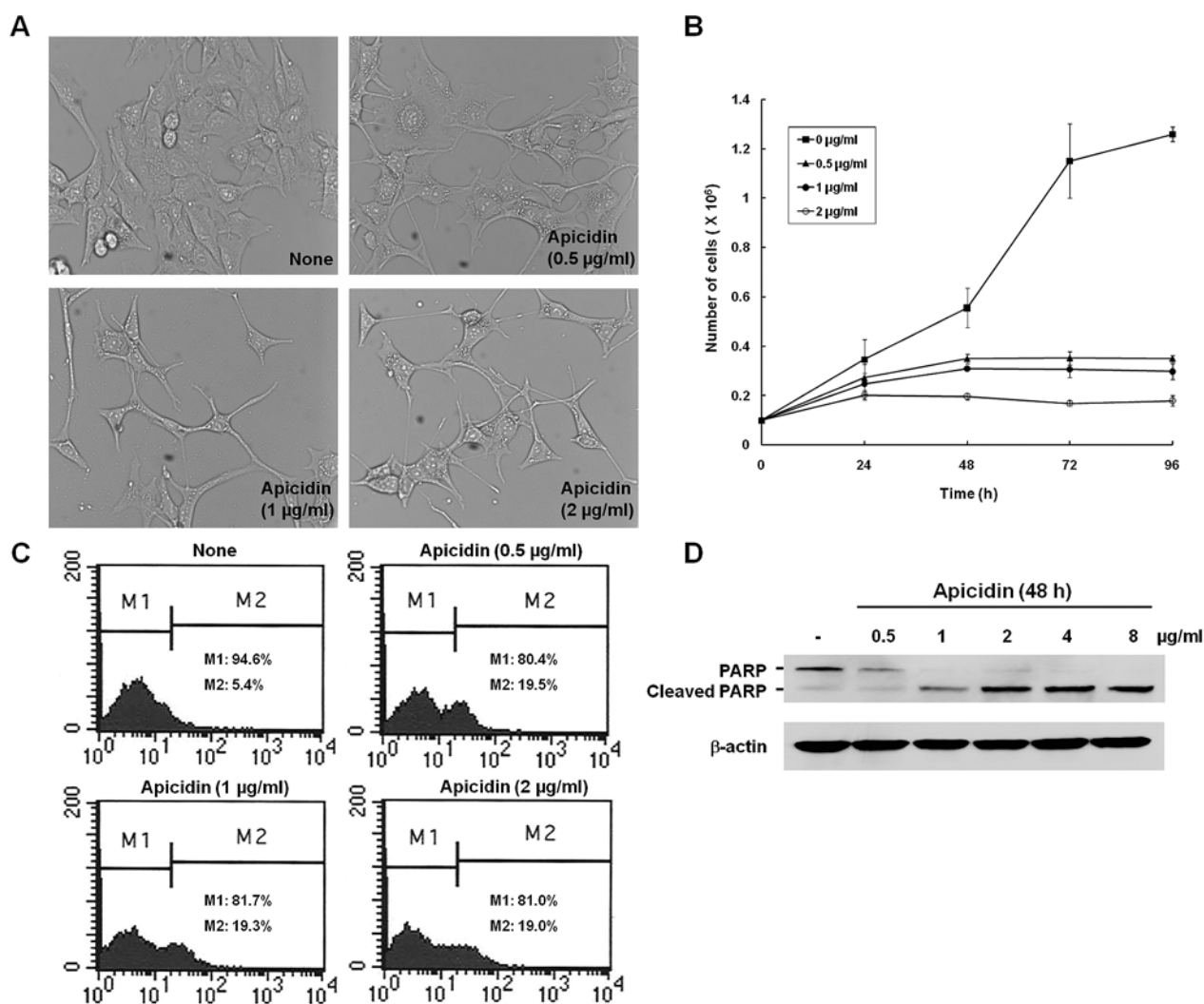


Figure 1. *In vitro* anti-tumor activity of Apicidin on MDA-MB-435 cancer cells. (A), Morphological changes of MDA-MB-435 cells induced by Apicidin. MDA-MB-435 cells were treated with DMSO (control) or with increasing concentrations of Apicidin (0.5-2  $\mu\text{g/ml}$ ) for 48 h. (B), The effect of Apicidin on cell growth. MDA-MB-435 cells were cultured in the presence of different concentrations of Apicidin for 4 days. Numbers of viable cells were counted at each time point by Trypan blue staining. Cell counting was performed in triplicate. (C), Apicidin elicited the apoptosis of MDA-MB-435 cells. Histograms of FACS analysis results indicated that the proportion of cells in the M2 phase, which reflected the population of Annexin-V stained cells undergoing apoptotic events, was increased by Apicidin. (D), Cellular apoptosis in the presence of Apicidin was confirmed by detecting PARP cleavage by Western blot analysis.

0.1% SDS for 3 min, 0.2X SSC for 3 min, 0.05X SSC for 2 min, and finally rinsed with distilled water for 2 min.

**Scanning and data analysis.** The arrays with hybridized targets were scanned using an Axon scanner and images and analyzed using GenePix<sup>®</sup> Pro 4.1 software (Axon Instruments, Foster City, CA, USA). Spots of poor quality, as determined by visual inspection, were excluded from further analysis. Resulting array data were submitted to the BioArray Software Environment (BASE) database at the microarray core facility at the Department of Pathology, College of Medicine at The Catholic University of Korea (<http://genomics.catholic.ac.kr/>). Data were normalized using the LIMMA package (Linear Models for Microarray Data) and the R-package of SMA (Statistics for Microarray Analysis). Spots of less than 50  $\mu\text{m}$  were excluded from analysis, if none are specified. Pearson's correlation coefficients were calculated using S-PLU, and Cluster and TreeView were used for data visualization (17). Pathway analysis was performed with ArrayXPath (<http://www.snubi.org/software/ArrayXPath/>).

## Results

**Anti-mitogenic effects of Apicidin on MDA-MB-435 cells.** Previous studies have shown that Apicidin strongly inhibits HDAC activity in cell lines from various tissues, and that it induces the accumulation of acetylated histones *in vivo* (15,18,19). The MDA-MB-435 cell line was derived from the melanoma cell line M14, which showed highly metastatic potential in animal model experiments (20). To determine whether Apicidin has similar effects on highly metastatic cancer cells, we initially assessed intracellular HDAC levels in MDA-MB-435 cells. Eight different classes of HDACs (HDAC1-8) were found to be expressed in MDA-MB-435 cell lines (data not shown).

The morphological change induced by Apicidin *in vitro* is a hallmark of its anti-tumor effect on cancer cells (15). As shown in Fig. 1A, MDA-MB-435 cells became elongated with filamentous protrusion when they were treated with increasing concentrations of Apicidin (0.5-2  $\mu\text{g/ml}$ ). When cellular growth rate was assessed with the same concentration

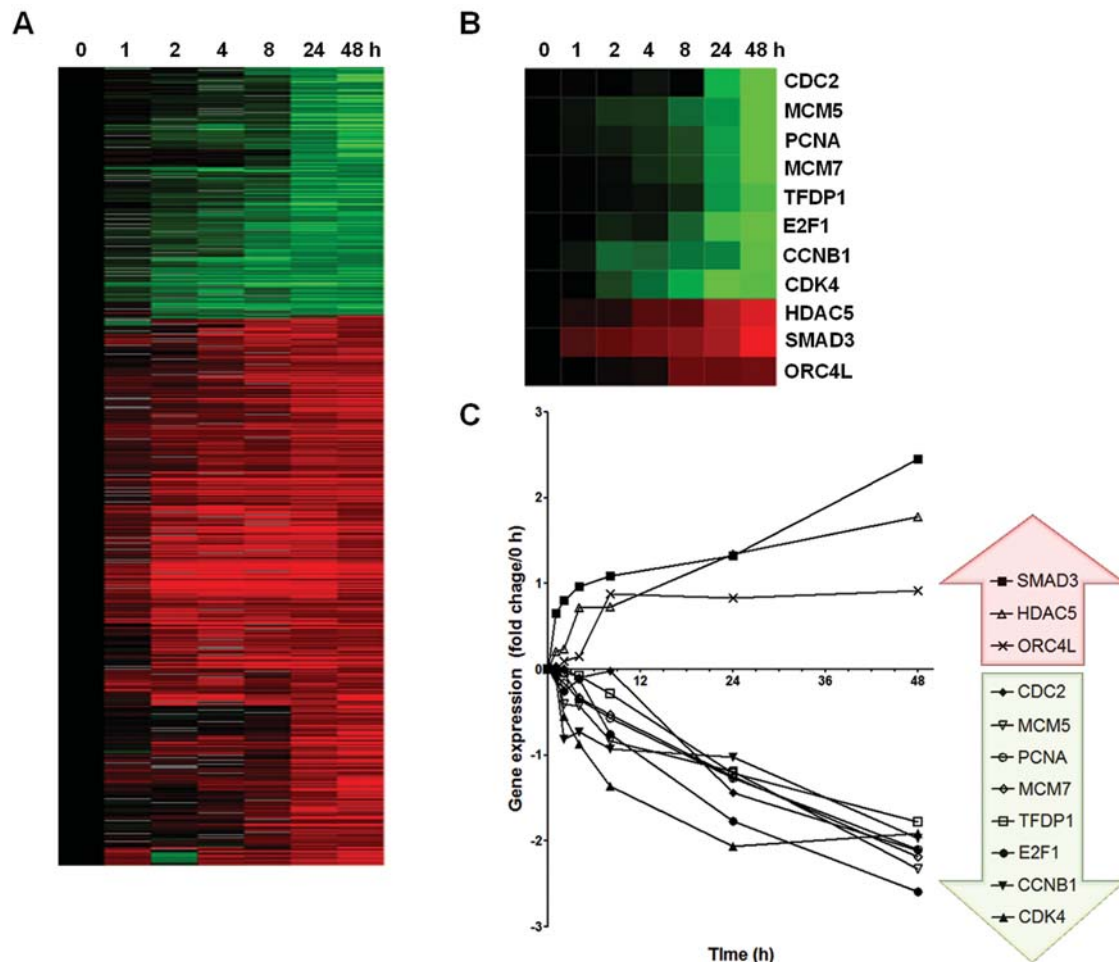


Figure 2. Differential gene expression profiling and the identification of large-scale molecular changes in Apicidin-treated MDA-MB-435 cells. Genes expressively altered by more than 2-fold in Apicidin-treated cells (48 h) versus non-treated controls (0 h) were selected. (A), 631 differentially expressed genes (Wilcoxon  $p < 0.05$ ) were identified and shown as heatmap. (B), Genes related to G1/S transition were selected by ArrayXPath (<http://www.snubi.org/software/ArrayXPath/>). (C), Fold-changes in the expressions of these 11 genes were plotted at each time point. Fold differences were calculated after subtracting mean values of controls (0 h) from those of samples (1–48 h).

of Apicidin, it was dramatically reduced by Apicidin treatment (Fig. 1B). The functional significance of this cell growth inhibition was confirmed by FACS analysis after staining Apicidin treated cells with Annexin V-FITC. As shown in Fig. 1C, the proportion of apoptotic cells was increased from 5.4% to 19% at 48 h after treatment of Apicidin (2  $\mu\text{g}/\text{ml}$ ). Previous reports have suggested that Apicidin induces apoptosis via cytosolic cytochrome c release by selectively inducing Fas/Fas ligand and the activations of caspase-9 and caspase-3 (15,21). To confirm the mechanism of apoptotic cell death by Apicidin, PARP cleavage was analyzed. As shown in Fig. 1D, endogenous PARP cleavage fragments (85 kDa) were detected by treatment with Apicidin at  $< 1.0 \mu\text{g}/\text{ml}$  for 48 h, and accumulated in a dose-dependent manner.

*Identification of the characteristic molecular signature of Apicidin.* To understand transcriptomic regulation by Apicidin in MDA-MB-435 cells, cells were treated with Apicidin (1  $\mu\text{g}/\text{ml}$ ) for different time points and each cell lysate was subjected to DNA microarray containing approximately 19,000 human genetic elements (see Materials and methods). Next, we designed the following algorithms for the large-scale gene expression analysis to recapitulate specific molecular

signature induced by Apicidin. Essentially, the algorithm was subtracting gene expression level of non-treated control (0 h) from that of experiment at each time point. Genes having the value greater than two in fold change were selected. As a result, the 631 genes were selected and then displayed as a heatmap to view a molecular signature of Apicidin (Fig. 2A). Our heatmap result showed that the expressions of 198 genes continuously decreased, and the expressions of 433 genes continuously increased after Apicidin treatment (Table I). In addition, the expressions of some genes were changed rapidly after exposure to Apicidin (Fig. 2A).

We attempted to determine the functional nature of the 631 selected genes using a pathway mining tool, ArrayXPath, which maps the selected 631 outlier genes into intracellular molecular pathways. Application of this software revealed that genes affected by Apicidin treatment were mostly involved in cell-cycle signaling pathways (data not shown). Eleven of the 631 genes (*SMAD3*, *HDAC5*, *ORC4L*, *CDC2*, *MCM5*, *PCNA*, *MCM7*, *TFDP1*, *E2F1*, *CCNB1*, *CDK4*) were mapped to cell-cycle pathways, and their expression changes with time are depicted as a heatmap and a line-graph in Fig. 2B and C, respectively. *SMAD3*, a downstream effector molecule in TGF- $\beta$  receptor signaling, was highly activated by Apicidin,

Table I. The genes regulated by Apicidin treatment.

GeneBank™ accession	Gene symbol	Gene name	Fold change
Up-regulated (433)			
D17028	PSAP	Human HepG2 partial cDNA clone hmd3c07m5	1.003
NM_018364	FLJ11220	Hypothetical protein FLJ11220 (FLJ11220) mRNA	1.007
NM_004457	FACL3	Fatty-acid-Coenzyme A ligase long-chain 3 (FACL3) mRNA	1.008
AY007114		Clone TCCCTA00151 mRNA sequence	1.011
D86974	KIAA0220	Human mRNA for KIAA0220 gene partial cds	1.012
S66168		Sterol regulatory element 1 binding protein (alternatively spliced clone pCY22) (human HeLa S3 cells mRNA partial 547 nt segment 2 of 2)	1.013
AK026886	FLJ23233	cDNA: FLJ23233 fis clone CAS00458	1.014
AB028986		mRNA for KIAA1063 protein partial cds	1.015
NM_002555	SLC22A1L	Solute carrier family 22 (organic cation transporter) member 1-like (SLC22A1L) mRNA	1.017
U66879	BAD	Human Bcl-2 binding component 6 (bbc6) mRNA complete cds	1.033
NM_003561	PLA2G10	Phospholipase A2 group X (PLA2G10) mRNA	1.034
NM_000097	CPO	Coproporphyrinogen oxidase (coproporphyrin harderoporphyrin) (CPO) mRNA	1.034
AK025268	FLJ21615	cDNA: FLJ21615 fis clone COL07393	1.034
NM_003710	SPINT1	Serine protease inhibitor Kunitz type 1 (SPINT1) mRNA	1.035
NM_005570	LMAN1	Lectin mannose-binding 1 (LMAN1) mRNA	1.038
NM_004037	AMPD2	Adenosine monophosphate deaminase 2 (isoform L) (AMPD2) mRNA	1.038
NM_018239	FLJ10751	Hypothetical protein FLJ10751 (FLJ10751) mRNA	1.041
NM_007081	RABL2B	RAB member of RAS oncogene family-like 2B (RABL2B) mRNA	1.045
AF089897	TRF4-2	Topoisomerase-related function protein (TRF4-2) mRNA partial cds	1.048
NM_006651	CPLX1	Complexin 1 (CPLX1) mRNA	1.048
AF045229	RGS10	Regulator of G protein signaling 10 mRNA complete cds	1.049
AF263547	DAB1	Disabled-1 (DAB1) mRNA complete cds	1.052
NM_018264	FLJ10900	Hypothetical protein FLJ10900 (FLJ10900) mRNA	1.053
NM_006457	LIM	LIM protein (similar to rat protein kinase C-binding enigma) (LIM) mRNA	1.054
Y09836		H. sapiens mRNA for 3' UTR of unknown protein	1.056
AK001647	FLJ10785	cDNA FLJ10785 fis clone NT2RP4000457 weakly similar to ubiquitin carboxyl-terminal hydrolase 15 (EC 3.1.2.15)	1.058
AF209198	ZNF277	Zinc finger protein 277 (ZNF277) mRNA complete cds	1.062
L40027	GSK3A	Glycogen synthase kinase 3 mRNA complete cds	1.063
NM_002133	HMOX1	Heme oxygenase (decycling) 1 (HMOX1) mRNA	1.064
AK000655	LOC142678	cDNA FLJ20648 fis clone KAT02130	1.065
NM_004424	E4F1	E4F transcription factor 1 (E4F1) mRNA	1.066
NM_014503	DRIM	Down-regulated in metastasis (DRIM) mRNA	1.068
NM_003139	SRPR	Signal recognition particle receptor ('docking protein') (SRPR) mRNA	1.069
AK002211	KIAA1272	cDNA FLJ11349 fis clone PLACE4000650 weakly similar to tuberin	1.071
NM_016318	P2RX2	Purinergic receptor P2X ligand-gated ion channel 2 (P2RX2) mRNA	1.081
AB037861	DKFZP586J0619	mRNA for KIAA1440 protein partial cds	1.087
NM_014500	HTATSF1	HIV TAT specific factor 1 (HTATSF1) mRNA	1.089
NM_003607	PK428	Ser-Thr protein kinase related to the myotonic dystrophy protein kinase (PK428) mRNA	1.091
NM_006568	CGR19	Cell growth regulatory with ring finger domain (CGR19) mRNA	1.092
AK026682		cDNA: FLJ23029 fis clone LNG01883	1.092
AB037759	EIF2AK4	mRNA for KIAA1338 protein partial cds	1.095
NM_003443	ZNF151	Zinc finger protein 151 (pHZ-67) (ZNF151) mRNA	1.097
NM_001643	APOA2	Apolipoprotein A-II (APOA2) mRNA	1.097
AK023199		cDNA FLJ13137 fis clone NT2RP3003150	1.104

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
NM_014390	p100	EBNA-2 co-activator (100 kDa) (p100) mRNA	1.107
NM_003718	CDC2L5	Cell division cycle 2-like 5 (cholinesterase-related cell division controller) (CDC2L5) transcript variant 1 mRNA	1.107
NM_012109	C19orf4	Chromosome 19 open reading frame 4 (C19orf4) mRNA	1.107
AK023196	ASB13	cDNA FLJ13134 fis clone NT2RP3003061 weakly similar to ankyrin	1.108
AF211966	LENG1	LENG1 protein (LENG1) mRNA partial cds	1.108
NM_003088	FSCN1	Fascin homolog 1 actin-bundling protein (Strongylocentrotus purpuratus) (FSCN1) mRNA	1.113
NM_012118	CCRN4L	CCR4 carbon catabolite repression 4-like ( <i>S. cerevisiae</i> ) (CCRN4L) mRNA	1.113
AF227899	BCAA	Breast carcinoma-associated antigen isoform I mRNA partial cds	1.114
NM_001497	B4GALT1	UDP-Gal:betaGlcNAc beta 14-galactosyltransferase polypeptide 1 (B4GALT1) mRNA	1.115
D17184		Human HepG2 3' region MboI cDNA clone hmd2h09m3	1.117
AF086254	FLJ35961	Full length insert cDNA clone ZD40H03	1.118
NM_007245	A2LP	Ataxin 2 related protein (A2LP) transcript variant 1 mRNA	1.119
AB014514	KIAA0614	mRNA for KIAA0614 protein partial cds	1.122
AB033029	KIAA1203	mRNA for KIAA1203 protein partial cds	1.129
NM_003525	H2BFBK	H2B histone family member K (H2BFBK) mRNA	1.131
NM_001684	ATP2B4	ATPase Ca <sup>++</sup> transporting plasma membrane 4 (ATP2B4) mRNA	1.134
NM_001478	GALGT	UDP-N-acetyl-alpha-D-galactosamine: (N-acetylneuraminy)-galactosyl-glucosylceramide N-acetylgalactosaminyltransferase (GalNAc-T) (GALGT) mRNA	1.134
NM_014324	AMACR	Alpha-methylacyl-CoA racemase (AMACR) mRNA	1.136
NM_006676	USP20	Ubiquitin specific protease 20 (USP20) mRNA	1.138
NM_018992	FLJ20040	Hypothetical protein FLJ20040 (FLJ20040) mRNA	1.141
NM_000244	MEN1	Multiple endocrine neoplasia I (MEN1) transcript variant 1 mRNA	1.142
NM_004103	PTK2B	Protein tyrosine kinase 2 beta (PTK2B) mRNA	1.146
NM_006440	TXNRD2	Thioredoxin reductase 2 (TXNRD2) transcript variant 1 nuclear gene encoding mitochondrial protein mRNA	1.147
AF085874		Full length insert cDNA clone YP08F12	1.148
AK024167		cDNA FLJ14105 fis clone MAMMA1001202	1.148
U66561	ZNF184	Human kruppel-related zinc finger protein (ZNF184) mRNA partial cds	1.149
NM_000889	ITGB7	Integrin beta 7 (ITGB7) mRNA	1.149
X64177	MT1H	<i>H. sapiens</i> mRNA for metallothionein	1.15
NM_017668	NUDE1	LIS1-interacting protein NUDE1 rat homolog (NUDE1) mRNA	1.151
X15880	COL6A1	Human mRNA for collagen VI alpha-1 C-terminal globular domain	1.156
NM_001127	AP1B1	Adaptor-related protein complex 1 beta 1 subunit (AP1B1) transcript variant 1 mRNA	1.156
NM_014638	KIAA0450	KIAA0450 gene product (KIAA0450) mRNA	1.159
NM_012433	SF3B1	Splicing factor 3b subunit 1 155 kDa (SF3B1) mRNA	1.164
NM_001271	CHD2	Chromodomain helicase DNA binding protein 2 (CHD2) mRNA	1.165
NM_014741	KIAA0652	KIAA0652 gene product (KIAA0652) mRNA	1.169
NM_002840	PTPRF	Protein tyrosine phosphatase receptor type F (PTPRF) transcript variant 1 mRNA	1.17
U66621	RAB1B	Human small GTP-binding protein (rab1b) mRNA partial cds	1.17
AK024487		mRNA for FLJ00086 protein partial cds	1.175
NM_145342	SMBP	Mitogen-activated protein kinase kinase kinase 7 interacting protein 2 (MAP3K7IP2) transcript variant 2 mRNA	1.176
NM_145904		High mobility group AT-hook 1 (HMGA1) transcript variant 6 mRNA	1.177
AF249267	SLC2A4RG	GLUT4 enhancer factor mRNA complete cds	1.18
AK023015	FLJ12953	cDNA FLJ12953 fis clone NT2RP2005490 moderately similar to <i>Mus musculus</i> D3Mm3e (D3Mm3e) mRNA	1.18

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AF067777		map 2p11.2; 83 cm from GATA85A06 repeat region complete sequence	1.187
NM_003344	UBE2H	Ubiquitin-conjugating enzyme E2H (UBC8 homolog yeast) (UBE2H) mRNA	1.189
NM_005318	H1F0	H1 histone family member 0 (H1F0) mRNA	1.189
NM_000712	BLVRA	Biliverdin reductase A (BLVRA) mRNA	1.196
Z36828		H. sapiens (xs177) mRNA 350 bp	1.198
NM_004742	BAIAP1	BAI1-associated protein 1 (BAIAP1) mRNA	1.2
AF100756	IGLJ3	Coat protein gamma-cop mRNA complete cds	1.201
NM_000818	GAD2	Glutamate decarboxylase 2 (pancreatic islets and brain 65 kDa) (GAD2) mRNA	1.206
NM_015720	PODLX2	Endoglycan (PODLX2) mRNA	1.208
NM_002219		Integral membrane protein 1 (ITM1) mRNA	1.208
NM_005655	TIEG	TGFB inducible early growth response (TIEG) mRNA	1.208
AK025974	MGC2574	cDNA: FLJ22321 fis clone HRC05390	1.214
D83778	KIAA0194	Human mRNA for KIAA0194 gene partial cds	1.216
AK025659		cDNA: FLJ22006 fis clone HEP06915	1.217
NM_020228	PRDM10	PR domain containing 10 (PRDM10) mRNA	1.225
L49054		t(3;5)(q25.1;p34) fusion gene NPM-MLF1 mRNA complete cds	1.228
NM_006107	OA48-18	Acid-inducible phosphoprotein (OA48-18) mRNA	1.231
J03048	HPX	Huma hemopexin mRNA 3' end	1.235
NM_012266	DNAJB5	DnaJ (Hsp40) homolog subfamily B member 5 (DNAJB5) mRNA	1.237
NM_002636	PHF1	PHD finger protein 1 (PHF1) transcript variant 1 mRNA	1.24
NM_004204	PIGQ	Phosphatidylinositol glycan class Q (PIGQ) transcript variant 2 mRNA	1.242
NM_003573	LTBP4	Latent transforming growth factor beta binding protein 4 (LTBP4) mRNA	1.243
AK026683	MOB	cDNA: FLJ23030 fis clone LNG01891 highly similar to HSY14155 mRNA for Hmob33 protein	1.245
X15624	H1RNA	Human H1 RNA	1.251
NM_005561	LAMP1	Lysosomal-associated membrane protein 1 (LAMP1) mRNA	1.252
D87452	IHPK1	mRNA for KIAA0263 protein partial cds	1.253
NM_000700	ANXA1	Annexin A1 (ANXA1) mRNA	1.257
NM_016441	CRIM1	Cysteine-rich motor neuron 1 (CRIM1) mRNA	1.258
NM_016539	SIRT6	Sirtuin silent mating type information regulation 2 homolog 6 (S. cerevisiae) (SIRT6) mRNA	1.259
NM_020196	HCNP	HCNP protein; XPA-binding protein 2 (HCNP) mRNA	1.26
NM_005718	ARPC4	Actin related protein 2/3 complex subunit 4 20 kDa (ARPC4) mRNA	1.26
NM_014256	B3GNT3	UDP-GlcNAc:betaGal beta-13-N-acetylglucosaminyltransferase 3 (B3GNT3) mRNA	1.26
NM_004158	PSPN	Persephin (PSPN) mRNA	1.26
NM_002613	PDPK1	3-phosphoinositide dependent protein kinase-1 (PDPK1) mRNA	1.261
D70832		mRNA for Zinc-finger protein partial cds	1.261
AK024160		cDNA FLJ14098 fis clone MAMMA1000778	1.265
AJ008118		C17orf1 gene exon 3 3'	1.266
NM_004776	B4GALT5	UDP-Gal:betaGlcNAc beta 14-galactosyltransferase polypeptide 5 (B4GALT5) mRNA	1.267
AK026448	FLJ22795	cDNA: FLJ22795 fis clone KAIA2543	1.27
AY007125	UNC93B1	Clone CDABP0025 mRNA sequence	1.27
AF086157	PERQ1	Full length insert cDNA clone ZB56H07	1.271
NM_020156	C1GALT1	Core1 UDP-galactose:N-acetylgalactosamine-alpha-R beta 13-galactosyltransferase (C1GALT1) mRNA	1.273
NM_004910	PITPNM	Phosphatidylinositol transfer protein membrane-associated (PITPNM) mRNA	1.276
AF303888	MAP1LC3B	Microtubule-associated proteins 1A/1B light chain 3 mRNA complete cds	1.278
AK027089	FLJ23436	cDNA: FLJ23436 fis clone HRC12692	1.288
NM_005116	SLC23A1	Solute carrier family 23 (nucleobase transporters) member 1 (SLC23A1) mRNA	1.29

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AK027035	FLJ38628	cDNA: FLJ23382 fis clone HEP16349	1.29
NM_004207	SLC16A3	Solute carrier family 16 (monocarboxylic acid transporters) member 3 (SLC16A3) mRNA	1.295
AB037858	KIAA1437	mRNA for KIAA1437 protein partial cds	1.302
NM_005600	NIT1	Nitrilase 1 (NIT1) mRNA	1.304
AK024452	FLJ12221	mRNA for FLJ00044 protein partial cds	1.304
NM_018318	FLJ11088	Hypothetical protein FLJ11088 (FLJ11088) mRNA	1.305
AK001204	FLJ10342	cDNA FLJ10342 fis clone NT2RM2000837	1.308
AK024288	RFX2	cDNA FLJ14226 fis clone NT2RP3004078 highly similar to H. sapiens HRFX2 mRNA	1.308
AK025585	DKFZp727A071	cDNA: FLJ21932 fis clone HEP04318	1.308
NM_017988	FLJ10074	Hypothetical protein FLJ10074 (FLJ10074) mRNA	1.314
NM_014779	KIAA0669	KIAA0669 gene product (KIAA0669) mRNA	1.316
BE878426	MIG-6	601487825F1 NIH_MGC_69 cDNA clone IMAGE: 3890190 5' mRNA sequence	1.318
AB042646	TGIF2	TGIF2 mRNA complete cds	1.326
NM_016333	SRRM2	Serine/arginine repetitive matrix 2 (SRRM2) mRNA	1.326
NM_005542	INSIG1	Insulin induced gene 1 (INSIG1) mRNA	1.329
NM_006738	AKAP13	A kinase (PRKA) anchor protein 13 (AKAP13) transcript variant 1 mRNA	1.34
NM_014892	KIAA1116	KIAA1116 protein (KIAA1116) mRNA	1.34
NM_001758		Cyclin D1 (PRAD1: parathyroid adenomatosis 1) (CCND1) mRNA	1.341
NM_012329	MMD	Monocyte to macrophage differentiation-associated (MMD) mRNA	1.341
NM_000018	ACADVL	Acyl-Coenzyme A dehydrogenase very long chain (ACADVL) nuclear gene encoding mitochondrial protein mRNA	1.343
NM_016291	IHPK2	Mammalian inositol hexakisphosphate kinase 2 (IP6K2) mRNA	1.344
AB014568	UNC84B	mRNA for KIAA0668 protein partial cds	1.345
NM_006295	VAR2	Valyl-tRNA synthetase 2 (VAR2) mRNA	1.352
M27877	ZNF83	HPF1 protein complete cds	1.353
NM_003516	H2AFO	H2A histone family member O (H2AFO) mRNA	1.354
NM_002552	ORC4L	Origin recognition complex subunit 4-like (yeast) (ORC4L) mRNA	1.358
NM_006500	MCAM	Melanoma adhesion molecule (MCAM) mRNA	1.36
NM_004193	GBF1	Golgi-specific brefeldin A resistance factor 1 (GBF1) mRNA	1.361
NM_016606	LOC51308	SGC32445 protein (LOC51308) mRNA	1.361
U92820		c33.109 unnamed HERV-H protein mRNAs partial cds	1.361
NM_002827	PTPN1	Protein tyrosine phosphatase non-receptor type 1 (PTPN1) mRNA	1.364
NM_004952	EFNA3	Ephrin-A3 (EFNA3) mRNA	1.368
NM_002743	PRKCSH	Protein kinase C substrate 80K-H (PRKCSH) mRNA	1.371
NM_005567	LGALS3BP	Lectin galactoside-binding soluble 3 binding protein (LGALS3BP) mRNA	1.376
AF042838	MAP3K1	MEK kinase 1 (MEKK1) mRNA partial cds	1.377
NM_001187	BAGE	B melanoma antigen (BAGE) mRNA	1.378
L40520	SMA4	(Clone DR3) mRNA fragment	1.38
NM_012295	CABIN1	Calcineurin binding protein 1 (CABIN1) mRNA	1.38
NM_012201	GLG1	Golgi apparatus protein 1 (GLG1) mRNA	1.381
NM_002332	LRP1	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor) (LRP1) mRNA	1.382
AK021863		cDNA FLJ11801 fis clone HEMBA1006253 weakly similar to DNA-damage-repair/toleration protein DRT111 precursor	1.384
NM_007118	TRIO	Triple functional domain (TPRF interacting) (TRIO) mRNA	1.385
NM_016111	KIAA0683	KIAA0683 gene product (KIAA0683) mRNA	1.388
AB037801	DKFZp761F0118	mRNA for KIAA1380 protein partial cds	1.391



Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
NM_001817	CEACAM4	Carcinoembryonic antigen-related cell adhesion molecule 4 (CEACAM4) mRNA	1.392
AF086467		Full length insert cDNA clone ZD86G12	1.393
NM_016531	KLF3	Kruppel-like factor 3 (basic) (KLF3) mRNA	1.393
D17265	ST13	Human HepG2 3' region MboI cDNA clone hmd5g12m3	1.394
U61089		Human NTera2D1 cell line mRNA containing L1 retroposon clone P2	1.396
D70835		mRNA for Zinc-finger protein partial cds	1.398
NM_018384	IAN4L1	Immune associated nucleotide 4 like 1 (mouse) (IAN4L1) mRNA	1.401
AB014559		mRNA for KIAA0659 protein partial cds	1.404
NM_003150	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3) transcript variant 2 mRNA	1.404
NM_002611	PDK2	Pyruvate dehydrogenase kinase isoenzyme 2 (PDK2) mRNA	1.405
NM_001330	CTF1	Cardiotrophin 1 (CTF1) mRNA	1.409
AB036432	AGER	RAGE mRNA for advanced glycation endproducts receptor complete cds	1.421
NM_006026	H1FX	H1 histone family member X (H1FX) mRNA	1.421
NM_018653	GPRC5C	G protein-coupled receptor family C group 5 member C (GPRC5C) transcript variant 2 mRNA	1.425
NM_001728	BSG	Basigin (BSG) mRNA	1.426
NM_014635	KIAA0336	KIAA0336 gene product (KIAA0336) mRNA	1.426
NM_002356	MARCKS	Myristoylated alanine-rich protein kinase C substrate (MARCKS) mRNA	1.429
NM_005796	NUTF2	Nuclear transport factor 2 (NUTF2) mRNA	1.429
NM_000064	C3	Complement component 3 (C3) mRNA	1.431
NM_018489	ASH1	Hypothetical protein ASH1 (ASH1) mRNA	1.436
NM_003565	ULK1	Unc-51-like kinase 1 (C. elegans) (ULK1) mRNA	1.44
AF075103		Full length insert cDNA YR39D03	1.447
NM_018051	FLJ10300	Hypothetical protein FLJ10300 (FLJ10300) mRNA	1.456
BE906107	ITGA3	601497153F1 NIH_MGC_70 cDNA clone IMAGE:3899221 5' mRNA sequence	1.457
D70834		mRNA for Zinc-finger protein partial cds	1.457
NM_018590	MGC40204	Hypothetical protein PRO0082 (PRO0082) mRNA	1.461
NM_005935	MLLT2	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog Drosophila); translocated to 2 (MLLT2) mRNA	1.462
NM_006929	SKIV2L	Superkiller viralicidic activity 2-like (S. cerevisiae) (SKIV2L) mRNA	1.463
L07033	HMGCL	Human hydroxymethylglutaryl-CoA lyase mRNA complete cds	1.469
AB011128	KIAA0556	mRNA for KIAA0556 protein partial cds	1.472
AF263462	CGN	Cingulin mRNA complete cds	1.475
NM_014007	ZNF297B	Zinc finger protein 297B (ZNF297B) mRNA	1.475
AF027142	ZNF234	Clone B7-4 zinc finger protein mRNA partial cds	1.48
AK026264	FLJ22611	cDNA: FLJ22611 fis clone HSI04961	1.481
NM_000094	COL7A1	Collagen type VII alpha 1 (epidermolysis bullosa dystrophic dominant and recessive) (COL7A1) mRNA	1.482
AJ003145	OR1F2	mRNA for olfactory receptor protein pseudogene	1.483
AK024225		cDNA FLJ14163 fis clone NT2RP1000409	1.488
AK021918	FLJ11856	cDNA FLJ11856 fis clone HEMBA1006789	1.503
NM_004040	ARHB	Ras homolog gene family member B (ARHB) mRNA	1.503
NM_005087	FXR1	Fragile X mental retardation autosomal homolog 1 (FXR1) mRNA	1.505
NM_018174	VCY2IP1	VCY2 interacting protein 1 (VCY2IP1) mRNA	1.508
Z36789		H. sapiens (xs138) mRNA 250 bp	1.509
NM_003324	TULP3	Tubby like protein 3 (TULP3) mRNA	1.51
AF293335	DBC-1	p30 DBC mRNA complete cds	1.511
NM_006005	WFS1	Wolfram syndrome 1 (wolframin) (WFS1) mRNA	1.518

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AL357200		EST from clone 109358 5' end	1.518
NM_001855	COL15A1	Collagen type XV alpha 1 (COL15A1) mRNA	1.524
NM_013330	NME7	NME7 (NME7) mRNA	1.529
AK023696		cDNA FLJ13634 fis clone PLACE1011133	1.532
NM_000499	CYP1A1	Cytochrome P450 subfamily I (aromatic compound-inducible) polypeptide 1 (CYP1A1) mRNA	1.539
NM_018474	C20orf19	Chromosome 20 open reading frame 19 (C20orf19) mRNA	1.54
NM_004985	KRAS2	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog (KRAS2) transcript variant b mRNA	1.545
NM_002872	RAC2	Ras-related C3 botulinum toxin substrate 2 (rho family small GTP binding protein Rac2) (RAC2) mRNA	1.546
NM_001627	ALCAM	Activated leukocyte cell adhesion molecule (ALCAM) mRNA	1.547
NM_015889	PCQAP	PC2 (positive cofactor 2 multiprotein complex) glutamine/Q-rich-associated protein (PCQAP) mRNA	1.548
NM_004480	FUT8	Fucosyltransferase 8 [alpha (16) fucosyltransferase] (FUT8) mRNA	1.554
AK023858		cDNA FLJ13796 fis clone THYRO1000111	1.556
AK001362	ESDN	cDNA FLJ10500 fis clone NT2RP2000369	1.561
NM_003900	SQSTM1	Sequestosome 1 (SQSTM1) mRNA	1.566
NM_015001	SHARP	SMART/HDAC1 associated repressor protein (SHARP) mRNA	1.572
AK024750	C11orf14	cDNA: FLJ21097 fis clone CAS03931	1.572
NM_001065	TNFRSF1A	Tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) mRNA	1.575
NM_005956	MTHFD1	Methylenetetrahydrofolate dehydrogenase (NADP <sup>+</sup> dependent) methenyltetrahydrofolate cyclohydrolase formyltetrahydrofolate synthetase (MTHFD1) mRNA	1.575
AK026198		cDNA: FLJ22545 fis clone HSI00239	1.581
NM003897	IER3	Immediate early response 3 (IER3) transcript variant short mRNA	1.588
AF228704	GSR	Mitochondrial glutathione reductase (GRD1) mRNA complete cds; nuclear gene for mitochondrial product	1.59
AF130075		Clone FLB9413 PRO2532 mRNA complete cds	1.596
NM_016151	PSK	Prostate derived STE20-like kinase PSK (PSK) mRNA	1.602
NM_004605	SULT2B1	Sulfotransferase family cytosolic 2B member 1 (SULT2B1) mRNA	1.603
NM_006928	SILV	Silver homolog (mouse) (SILV) mRNA	1.603
AK000860	CORO1B	cDNA FLJ20853 fis clone ADKA01365 highly similar to coro_bovin coronin-like protein P57	1.605
NM_005830	MRPS31	Mitochondrial ribosomal protein S31 (MRPS31) nuclear gene encoding mitochondrial protein mRNA	1.605
NM_020347	LZTFL1	Leucine zipper transcription factor-like 1 (LZTFL1) mRNA	1.61
X06389	SYP	Human mRNA for synaptophysin (p38)	1.61
AF312678		FGF homologous factor receptor (FHFR) mRNA complete cds	1.619
AF155117	KIF21A	NY-REN-62 antigen mRNA partial cds	1.627
NM_005273	GNB2	Guanine nucleotide binding protein (G protein) beta polypeptide 2 (GNB2) mRNA	1.628
AK024007	MGC29761	cDNA FLJ13945 fis clone Y79AA1000969	1.631
NM_002231	KAI1	Kangai 1 [suppression of tumorigenicity 6 prostate; CD82 antigen (R2 leukocyte antigen antigen detected by monoclonal and antibody IA4)] (KAI1) mRNA	1.642
AF085975		Full length insert cDNA clone YT86G11	1.642
NM_018635		Hypothetical protein PRO2900 (PRO2900) mRNA	1.644
AK025981	MGC3169	cDNA: FLJ22328 fis clone HRC05632	1.65
AF164156	MDS1	Clone 25 EVI1 (EVI1) mRNA alternatively spliced partial cds	1.653
AK000315	FLJ20308	cDNA FLJ20308 fis clone HEP07264	1.655
AL117423	CBARA1	mRNA; cDNA DKFZp564C246 (from clone DKFZp564C246); complete cds	1.664

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AL050037	DKFZP566I1024	mRNA; cDNA DKFZp566I1024 (from clone DKFZp566I1024)	1.667
NM_004953	EIF4G1	Eukaryotic translation initiation factor 4 gamma 1 (EIF4G1) mRNA	1.674
NM_003528	H2BFQ	H2B histone family member Q (H2BFQ) mRNA	1.675
NM_003481	USP5	Ubiquitin specific protease 5 (isopeptidase T) (USP5) mRNA	1.68
NM_002149	HPCAL1	Hippocalcin-like 1 (HPCAL1) transcript variant 1 mRNA	1.685
D85131	MAZ	mRNA for Myc-associated zinc-finger protein of human islet complete cds	1.695
AK022059		cDNA FLJ11997 fis clone HEMBB1001458	1.696
NM_015537	DKFZP586J1624	DKFZP586J1624 protein (DKFZP586J1624) mRNA	1.711
U88897		Human endogenous retroviral H D2 leader region protease region and integrase/envelope region mRNA sequence	1.715
AK001838		cDNA FLJ10976 fis clone PLACE1001399	1.717
NM_003744	NUMB	Numb homolog (Drosophila) (NUMB) mRNA	1.717
M94362	LMNB2	Human lamin B2 (LAMB2) mRNA partial cds	1.718
X89668	OR7E19P	H. sapiens mRNA for TPCR110 protein	1.72
NM_014556	EVC	Ellis van Creveld syndrome (EVC) mRNA	1.726
NM_003465	CHIT1	Chitinase 1 (chitotriosidase) (CHIT1) mRNA	1.734
NM_005453	ZNF297	Zinc finger protein 297 (ZNF297) mRNA	1.736
NM_003242	TGFBR2	Transforming growth factor beta receptor II (70/80 kDa) (TGFBR2) mRNA	1.737
NM_003254	TIMP1	Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity collagenase inhibitor) (TIMP1) mRNA	1.737
NM_001975	ENO2	Enolase 2 (gamma neuronal) (ENO2) mRNA	1.745
AF118124	MCL1	Myeloid cell leukemia sequence 1 (MCL1) mRNA complete cds	1.763
AF084560	FADS3	Fatty acid desaturase 3 (FADS3) mRNA complete cds	1.774
NM_005474	HDAC5	Histone deacetylase 5 (HDAC5) transcript variant 1 mRNA	1.781
NM_001191	BCL2L1	BCL2-like 1 (BCL2L1) nuclear gene encoding mitochondrial protein transcript variant 2 mRNA	1.786
AK000899		cDNA FLJ10037 fis clone HEMBA1000968	1.79
NM_014244	ADAMTS2	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 2 (ADAMTS2) transcript variant 1 mRNA	1.793
NM_002730	PRKACA	Protein kinase cAMP-dependent catalytic alpha (PRKACA) mRNA	1.795
X07979		Human mRNA for integrin beta 1 subunit	1.796
NM_018464	MDS029	Uncharacterized hematopoietic stem/progenitor cells protein MDS029 (MDS029) mRNA	1.813
NM_014944	CLSTN1	Calsyntenin 1 (CLSTN1) mRNA	1.815
AF298152	BDP1	RNA polymerase III transcription initiation factor B' short mRNA complete cds	1.815
AF308802	VPS18	Vacuolar protein sorting protein 18 (VPS18) mRNA complete cds	1.819
NM_004421	DVL1	Dishevelled dsh homolog 1 (Drosophila) (DVL1) mRNA	1.819
NM_005137	DGCR2	DiGeorge syndrome critical region gene 2 (DGCR2) mRNA	1.828
AB037797		mRNA for KIAA1376 protein partial cds	1.832
AY007158	FLJ14827	Clone CDABP0113 mRNA sequence	1.833
X65231		H. sapiens mRNA for Zinc-finger protein (ZNFpT3)	1.834
NM_003851	CREG	Cellular repressor of E1A-stimulated genes (CREG) mRNA	1.835
X56692		H. sapiens mRNA for C-reactive protein	1.836
NM_017870	FLJ20539	Hypothetical protein FLJ20539 (FLJ20539) mRNA	1.849
AB037823	CSGlcA-T	mRNA for KIAA1402 protein partial cds	1.85
NM_000033	ABCD1	ATP-binding cassette sub-family D (ALD) member 1 (ABCD1) mRNA	1.85
AB014564	KIAA0664	mRNA for KIAA0664 protein partial cds	1.85
AL360144	B7-H3	EST from clone 943301 3' end	1.864
M62399	RELA	Human NF-kappa-B transcription factor p65 DNA binding subunit mRNA complete cds	1.866

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AF015910	AKAP1	Unknown protein mRNA partial cds	1.884
NM_001909	CTSD	Cathepsin D (lysosomal aspartyl protease) (CTSD) mRNA	1.89
NM_017992		Hypothetical protein FLJ10083 (FLJ10083) mRNA	1.895
AF038192	PNKP	Clone 23808 mRNA sequence	1.903
AK024472	BMF	mRNA for FLJ00065 protein partial cds	1.91
NM_001756	SERPINA6	Serine (or cysteine) proteinase inhibitor clade A (alpha-1 antiproteinase antitrypsin) member 6 (SERPINA6) mRNA	1.912
AL136549	CYFIP2	mRNA; cDNA DKFZp761I12121 (from clone DKFZp761I12121); complete cds	1.913
AL133662	KIAA0913	mRNA; cDNA DKFZp434E0121 (from clone DKFZp434E0121)	1.928
AA084879	7 KD protein	Zn13f02.r1 Stratagene hNT neuron (937233) cDNA clone IMAGE:547323 5' similar to TR:E241761 E241761 hypothetical 10.; mRNA sequence	1.932
AL161977	PCTK3	mRNA; cDNA DKFZp761L1515 (from clone DKFZp761L1515)	1.936
AK022293		cDNA FLJ12231 fis clone MAMMA1001191	1.939
AK024443	FLJ31318	mRNA for FLJ00033 protein partial cds	1.939
X02067		H. sapiens mRNA for 7SL RNA pseudogene	1.943
M62324	MRF-1	Human modulator recognition factor I (MRF-1) mRNA 3' end	1.946
D70833		mRNA for Zinc-finger protein partial cds	1.955
NM_003378	VGF	VGF nerve growth factor inducible (VGF) mRNA	1.956
NM_001999	FBN2	Fibrillin 2 (congenital contractural arachnodactyly) (FBN2) mRNA	1.96
NM_006848	DIPA	Hepatitis delta antigen-interacting protein A (DIPA) mRNA	1.965
NM_004349	CBFA2T1	Core-binding factor runt domain alpha subunit 2; translocated to 1; cyclin D-related (CBFA2T1) mRNA	1.978
AF119868	RBP4	PRO2222 mRNA complete cds	1.978
AF024698		Clone L3-5 zinc finger protein mRNA partial cds	1.986
AJ278476	TTS-2.2	Partial mRNA for transport-secretion protein 2.2 (TTS-2.2 gene)	1.995
NM_005384	NFIL3	Nuclear factor interleukin 3 regulated (NFIL3) mRNA	2.024
AB046797	KIAA1577	mRNA for KIAA1577 protein partial cds	2.032
U12210		Human small nucleolar RNA (E1b) complete sequence	2.033
NM_015193	ARC	Activity-regulated cytoskeleton-associated protein (ARC) mRNA	2.036
NM_014947	KIAA1041	KIAA1041 protein (KIAA1041) mRNA	2.043
AK001102		cDNA FLJ10240 fis clone HEMBB1000591	2.045
NM_003566	EEA1	Early endosome antigen 1 162 kDa (EEA1) mRNA	2.054
NM_019098	CNGB3	Cyclic nucleotide gated channel beta 3 (CNGB3) mRNA	2.054
NM_012410	PSK-1	Type I transmembrane receptor (seizure-related protein) (PSK-1) mRNA	2.057
NM_002826	QSCN6	Quiescin Q6 (QSCN6) mRNA	2.062
NM_001889	CRYZ	Crystallin zeta (quinone reductase) (CRYZ) mRNA	2.075
U27655	RGS3	Human RGP3 mRNA complete cds	2.081
NM_004559	NSEP1	Nuclease sensitive element binding protein 1 (NSEP1) mRNA	2.092
NM_002539	ODC1	Ornithine decarboxylase 1 (ODC1) mRNA	2.106
AF116620	SLC38A2	PRO1068 mRNA complete cds	2.11
D28381		Human mRNA for osteonectin 5' UTR (sequence from the 5' cap to the start codon)	2.114
NM_009590	AOC2	Amine oxidase copper containing 2 (retina-specific) (AOC2) transcript variant 2 mRNA	2.123
NM_004434	EML1	Echinoderm microtubule associated protein like 1 (EML1) mRNA	2.124
M62403	IGFBP4	Human insulin-like growth factor binding protein 4 (IGFBP4) mRNA complete cds	2.153
NM_004419	DUSP5	Dual specificity phosphatase 5 (DUSP5) mRNA	2.157
AK024155		cDNA FLJ14093 fis clone MAMMA1000279	2.169
AB040969	KIAA1536	mRNA for KIAA1536 protein partial cds	2.183
NM_000834	GRIN2B	Glutamate receptor ionotropic N-methyl D-aspartate 2B (GRIN2B) mRNA	2.188

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
NM_016459	PACAP	Proapoptotic caspase adaptor protein (FLJ32987) mRNA	2.194
NM_000224	KRT18	Keratin 18 (KRT18) mRNA	2.201
AF086384		Full length insert cDNA clone ZD70H02	2.201
NM_020182	TMEPAI	Transmembrane prostate androgen induced RNA (TMEPAI) mRNA	2.22
NM_000874	IFNAR2	Interferon (alpha beta and omega) receptor 2 (IFNAR2) mRNA	2.223
AF064729	RANBP16	RAN binding protein 16 mRNA complete cds	2.23
X64978	OR2L2	H. sapiens mRNA HTPCRH07 for olfactory receptor	2.234
NM_005415	SLC20A1	Solute carrier family 20 (phosphate transporter) member 1 (SLC20A1) mRNA	2.236
D17045		Human HepG2 partial cDNA clone hmd3f12m5	2.237
NM_006612	KIF1C	Kinesin family member 1C (KIF1C) mRNA	2.247
AL117565	AXUD1	mRNA; cDNA DKFZp566F164 (from clone DKFZp566F164)	2.249
NM_002961	S100A4	S100 calcium binding protein A4 (calcium protein calvasculin metastasin murine placental homolog) (S100A4) transcript variant 1 mRNA	2.264
NM_002205	ITGA5	Integrin alpha 5 (fibronectin receptor alpha polypeptide) (ITGA5) mRNA	2.277
NM_004996	ABCC1	ATP-binding cassette sub-family C (CFTR/MRP) member 1 (ABCC1) transcript variant 1 mRNA	2.282
NM_001887	CRYBB1	Crystallin beta B1 (CRYBB1) mRNA	2.288
M73837	MRF2	Modulator recognition factor 2 (MRF-2) mRNA partial cds	2.29
AF075110		Full length insert cDNA YU41G07	2.307
NM_016507	CRK7	CDC2-related protein kinase 7 (CRK7) mRNA	2.321
Z48633		H. sapiens mRNA for retrotransposon	2.323
Z36817		H. sapiens (xs165) mRNA 400 bp	2.335
NM_003246	THBS1	Thrombospondin 1 (THBS1) mRNA	2.346
NM_006096	NDRG1	N-myc downstream regulated gene 1 (NDRG1) mRNA	2.346
NM_003469	SCG2	Secretogranin II (chromogranin C) (SCG2) mRNA	2.348
S70385		c-myc = Burkitt-like c-myc (exons 1 and 2 oncogene) (human prolymphocytic leukemia patient mRNA partial mutant 80 nt segment 1 of 2)	2.353
NM_005194	CEBPB	CCAAT/enhancer binding protein (C/EBP) beta (CEBPB) mRNA	2.353
AK026747	LOC54103	cDNA: FLJ23094 fis clone LNG07379 highly similar to HST000007 mRNA full length insert cDNA clone EUROIMAGE 293605	2.357
NM_000177	GSN	Gelsolin (amyloidosis Finnish type) (GSN) mRNA	2.361
NM_015492	DKFZP434H132	DKFZP434H132 protein (DKFZP434H132) mRNA	2.378
NM_004638	BAT2	HLA-B associated transcript 2 (BAT2) transcript variant 2 mRNA	2.378
AF117338	FLJ10081	Cell serum inhibited related protein mRNA 3' UTR sequence	2.404
AK025709	FLJ22056	cDNA: FLJ22056 fis clone HEP09916	2.435
AB040959	DKFZP434N014	mRNA for KIAA1526 protein partial cds	2.45
NM_005902	MADH3	MAD mothers against decapentaplegic homolog 3 (Drosophila) (MADH3) mRNA	2.456
NM_006472	TXNIP	Thioredoxin interacting protein (TXNIP) mRNA	2.46
NM_017458	MVP	Major vault protein (MVP) transcript variant 1 mRNA	2.465
S68954		Metallothionein MT-1g isoform (human monocytes mRNA partial 93 nt)	2.467
NM_019063	EML4	Echinoderm microtubule associated protein like 4 (EML4) mRNA	2.467
NM_019618	IL1F9	Interleukin 1 family member 9 (IL1F9) mRNA	2.472
NM_006860	RABL4	RAB member of RAS oncogene family-like 4 (RABL4) mRNA	2.508
NM_016280	CESR	Carboxylesterase-related protein (LOC51716) mRNA	2.522
NM_002856	PVRL2	Poliovirus receptor-related 2 (herpesvirus entry mediator B) (PVRL2) mRNA	2.542
AF224741	CLCN7	Chloride channel protein 7 (CLCN7) mRNA complete cds	2.543
NM_007108	TCEB2	Transcription elongation factor B (SIII) polypeptide 2 (18 kDa elongin B) (TCEB2) mRNA	2.58
AF047002	THOC4	Transcriptional coactivator ALY mRNA partial cds	2.605

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
J02639	SERPINA5	Human plasma serine protease (protein C) inhibitor mRNA complete cds	2.612
NM_003938	AP3D1	Adaptor-related protein complex 3 delta 1 subunit (AP3D1) mRNA	2.62
NM_003330	TXNRD1	Thioredoxin reductase 1 (TXNRD1) mRNA	2.627
AF135960	LTBP3	Latent transforming growth factor beta binding protein 3 mRNA complete cds	2.633
Z70717		H. sapiens mRNA (fetal brain cDNA h6_2g)	2.637
U73377	SHC1	Human p66shc (SHC) mRNA complete cds	2.696
NM_005319	H1F2	H1 histone family member 2 (H1F2) mRNA	2.701
NM_001567	INPPL1	Inositol polyphosphate phosphatase-like 1 (INPPL1) mRNA	2.702
NM_002087	GRN	Granulin (GRN) mRNA	2.704
AB033073	SULF2	mRNA for KIAA1247 protein partial cds	2.763
NM_000402	G6PD	Glucose-6-phosphate dehydrogenase (G6PD) nuclear gene encoding mitochondrial protein mRNA	2.839
M87943		Human carcinoma cell-derived Alu RNA transcript clone ALU496	2.846
NM_002184	IL6ST	Interleukin 6 signal transducer (gp130 oncostatin M receptor) (IL6ST) mRNA	2.852
BE899154	COQ4	601681948F1 NIH_MGC_9 cDNA clone IMAGE: 3952355 5' mRNA sequence	2.863
X04236		Human small cellular 7 SK mRNA	2.916
AK021733		cDNA FLJ11671 fis clone HEMBA1004730	2.967
M30142		Decay-accelerating factor ADAF	2.967
M96843	ID2B	Human striated muscle contraction regulatory protein (Id2B) mRNA complete cds	3.048
AL050210		mRNA; cDNA DKFZp586G1023 (from clone DKFZp586G1023)	3.059
BC001479		Clone MGC: 1160 IMAGE: 2988160 mRNA complete cds	3.087
U92819		c33.42 unnamed HERV-H protein mRNA partial cds	3.106
NM_000042	APOH	Apolipoprotein H (beta-2-glycoprotein I) (APOH) mRNA	3.172
AF438313	RIS1	Ras-induced senescence 1 (RIS1) mRNA complete cds	3.241
NM_004192	ASMTL	Acetylserotonin O-methyltransferase-like (ASMTL) mRNA	3.258
AL133574	TEAD1	mRNA; cDNA DKFZp434N1435 (from clone DKFZp434N1435)	3.304
NM_006165	NFRKB	Nuclear factor related to kappa B binding protein (NFRKB) mRNA	3.337
M11948		Human promyelocytic leukemia cell mRNA clones pHH58 and pHH81	3.337
NM_004403	DFNA5	Deafness autosomal dominant 5 (DFNA5) mRNA	3.368
NM_000295	SERPINA1	Serine (or cysteine) proteinase inhibitor clade A (alpha-1 antiproteinase antitrypsin) member 1 (SERPINA1) mRNA	3.375
AK026068	FLJ22415	cDNA: FLJ22415 fis clone HRC08561	3.44
NM_002229	JUNB	jun B proto-oncogene (JUNB) mRNA	3.542
NM_001353	AKR1C1	Aldo-keto reductase family 1 member C1 [dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase] (AKR1C1) mRNA	3.8
NM_002775	PRSS11	Protease serine 11 (IGF binding) (PRSS11) mRNA	3.804
NM_000184	HBG2	Hemoglobin gamma G (HBG2) mRNA	4.002
NM_001831		Clusterin (complement lysis inhibitor SP-4040 sulfated glycoprotein 2 testosterone-repressed prostate message 2 apolipoprotein J) (CLU) mRNA	4.03
M17017	IL8	Human beta-thromboglobulin-like protein mRNA complete cds	4.286
NM_006931	SLC2A3	Solute carrier family 2 (facilitated glucose transporter) member 3 (SLC2A3) mRNA	4.538
AB019565		mRNA expressed only in placental villi clone SMAP52	4.828
AL049452		mRNA; cDNA DKFZp586C1322 (from clone DKFZp586C1322)	6.376
Down-regulated (198)			
NM_004811	LPXN	Leupaxin (LPXN) mRNA	-4.235
NM_007019	UBE2C	Ubiquitin-conjugating enzyme E2C (UBE2C) mRNA	-3.989
NM_000372	TYR	Tyrosinase (oculocutaneous albinism IA) (TYR) mRNA	-3.643
NM_006082	K-ALPHA-1	Tubulin alpha ubiquitous (K-ALPHA-1) mRNA	-3.024

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
NM_005563	STMN1	Stathmin 1/oncoprotein 18 (STMN1) mRNA	-2.936
NM_000281	PCBD	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) (PCBD) mRNA	-2.922
NM_018492	TOPK	T-LAK cell-originated protein kinase (TOPK) mRNA	-2.821
AL160131	MGC861	Novel human gene mapping to chromosome 22	-2.645
NM_018269	SIPL	SIPL protein (SIPL) mRNA	-2.615
D28450	H2AFZ	Human mRNA for histone H2A.Z 5' UTR (sequence from the 5' cap to the start codon)	-2.577
AK000992		cDNA FLJ10130 fis clone HEMBA1003035	-2.382
NM_019020	FLJ20748	Hypothetical protein FLJ20748 (FLJ20748) mRNA	-2.379
M96577	E2F1	(E2F-1) pRB-binding protein mRNA complete cds	-2.345
NM_006739	MCM5	MCM5 minichromosome maintenance deficient 5 cell division cycle 46 ( <i>S. cerevisiae</i> ) (MCM5) mRNA	-2.329
NM_015954	CGI-26	CGI-26 protein (LOC51071) mRNA	-2.328
NM_012145	DTYMK	Deoxythymidylate kinase (thymidylate kinase) (DTYMK) mRNA	-2.323
NM_018298	MCOLN3	Mcolipin-3 (MCOLN3) mRNA	-2.296
NM_004217	STK12	Serine/threonine kinase 12 (STK12) mRNA	-2.256
NM_001071	TYMS	Thymidylate synthetase (TYMS) mRNA	-2.254
U96131	TRIP13	HPV16 E1 protein binding protein mRNA complete cds	-2.239
NM_000714	BZRP	Benzodiazapine receptor (peripheral) (BZRP) nuclear gene encoding mitochondrial protein transcript variant PBR mRNA	-2.195
NM_002882	RANBP1	RAN binding protein 1 (RANBP1) mRNA	-2.193
D55716	MCM7	Human mRNA for P1cdc47 complete cds	-2.181
NM_005720	ARPC1B	Actin related protein 2/3 complex subunit 1B 41 kDa (ARPC1B) mRNA	-2.18
NM_006936	SMT3H1	SMT3 suppressor of mif two 3 homolog 1 (yeast) (SMT3H1) mRNA	-2.177
AK000770	B3GNT7	cDNA FLJ20763 fis clone COL09911	-2.149
AL137479	TPC2	mRNA; cDNA DKFZp434M0223 (from clone DKFZp434M0223); partial cds	-2.127
NM_001316	CSE1L	CSE1 chromosome segregation 1-like (yeast) (CSE1L) mRNA	-2.11
NM_014096	EEG1	Likely ortholog of mouse embryonic epithelial gene 1 (EEG1) mRNA	-2.11
NM_001786	CDC2	Cell division cycle 2 G1 to S and G2 to M (CDC2) transcript variant 1 mRNA	-2.106
NM_002592	PCNA	Proliferating cell nuclear antigen (PCNA) mRNA	-2.098
AK022250		cDNA FLJ12188 fis clone MAMMA1000839	-2.085
AL365411	LOC56931	mRNA full length insert cDNA clone EUROIMAGE 1967720	-2.075
NM_001948	DUT	dUTP pyrophosphatase (DUT) mRNA	-2.044
D38553	BRRN1	HCAP-H mRNA partial cds	-2.041
AK026447	FLJ22794	cDNA: FLJ22794 fis clone KAIA2442	-2.007
NM_001179	ART3	ADP-ribosyltransferase 3 (ART3) mRNA	-1.987
NM_014026	DCPS	mRNA decapping enzyme (DCPS) mRNA	-1.983
NM_004425	ECM1	Extracellular matrix protein 1 (ECM1) transcript variant 1 mRNA	-1.975
M25753	CCNB1	Human cyclin B mRNA 3' end	-1.967
NM_001363	DKC1	Dyskeratosis congenita 1 dyskerin (DKC1) mRNA	-1.939
NM_005511	MLANA	Melan-A (MLANA) mRNA	-1.927
NM_000337	SGCD	Sarcoglycan delta (35 kDa dystrophin-associated glycoprotein) (SGCD) mRNA	-1.921
NM_000075	CDK4	Cyclin-dependent kinase 4 (CDK4) transcript variant 1 mRNA	-1.911
NM_000248	MITF	Microphthalmia-associated transcription factor (MITF) mRNA	-1.902
NM_005009	NME4	Non-metastatic cells 4 protein expressed in (NME4) mRNA	-1.897
NM_004411	DNCI1	Dynein cytoplasmic intermediate polypeptide 1 (DNCI1) mRNA	-1.894
NM_003712	PPAP2C	Phosphatidic acid phosphatase type 2C (PPAP2C) mRNA	-1.886
NM_015927	TGFB1I1	Transforming growth factor beta 1 induced transcript 1 (TGFB1I1) mRNA	-1.874
NM_016613	DKFZp434L142	Hypothetical protein DKFZp434L142 (DKFZp434L142) mRNA	-1.849

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
NM_000900	MGP	Matrix Gla protein (MGP) mRNA	-1.827
NM_001152	SLC25A5	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator) member 5 (SLC25A5) nuclear gene encoding mitochondrial protein mRNA	-1.818
NM_001814	CTSC	Cathepsin C (CTSC) transcript variant 1 mRNA	-1.816
NM_002823	PTMA	Prothymosin alpha (gene sequence 28) (PTMA) mRNA	-1.792
AK025627	LOC91768	cDNA: FLJ21974 fis clone HEP05861	-1.787
NM_002719	PPP2R5C	Protein phosphatase 2 regulatory subunit B (B56) gamma isoform (PPP2R5C) mRNA	-1.766
NM_002633	PGM1	Phosphoglucomutase 1 (PGM1) mRNA	-1.765
NM_013322	SNX10	Sorting nexin 10 (SNX10) mRNA	-1.757
NM_014791	MELK	Maternal embryonic leucine zipper kinase (MELK) mRNA	-1.753
D87076	KIAA0239	Human mRNA for KIAA0239 gene partial cds	-1.752
NM_003287	TPD52L1	Tumor protein D52-like 1 (TPD52L1) mRNA	-1.747
NM_019896	POLE4	Polymerase (DNA-directed) epsilon 4 (p12 subunit) (POLE4) mRNA	-1.741
NM_012296	GAB2	GRB2-associated binding protein 2 (GAB2) transcript variant 2 mRNA	-1.736
AF054589	HIC	HIC protein isoform p40 and HIC protein isoform p32 mRNAs complete cds	-1.725
AF086473		Full length insert cDNA clone ZD88D08	-1.72
NM_005775	SCAM-1	Vinexin beta (SH3-containing adaptor molecule-1) (SCAM-1) mRNA	-1.714
AF085956		Full length insert cDNA clone YR87F08	-1.705
NM_018436	ALLC	Allantoicase (ALLC) mRNA	-1.684
NM_001767	CD2	CD2 antigen (p50) sheep red blood cell receptor (CD2) mRNA	-1.682
NM_000819	GART	Phosphoribosylglycinamide formyltransferase phosphoribosylglycinamide synthetase phosphoribosylaminoimidazole synthetase (GART) mRNA	-1.665
AF086235		Full length insert cDNA clone ZD20D09	-1.653
NM_014129	PRO0478	PRO0478 protein (PRO0478) mRNA	-1.638
NM_007111	TFDP1	Transcription factor Dp-1 (TFDP1) mRNA	-1.631
NM_018108	FLJ10483	Hypothetical protein FLJ10483 (FLJ10483) mRNA	-1.626
NM_002048	GAS1	Growth arrest-specific 1 (GAS1) mRNA	-1.605
AK022667	FLJ11712	cDNA FLJ12605 fis clone NT2RM4001455	-1.601
AL049270		mRNA; cDNA DKFZp564G223 (from clone DKFZp564G223)	-1.592
NM_020371	AVEN	Apoptosis caspase activation inhibitor (AVEN) mRNA	-1.591
NM_012413	QPCT	Glutaminyl-peptide cyclotransferase (glutaminyl cyclase) (QPCT) mRNA	-1.587
NM_003124	SPR	Sepiapterin reductase (78-dihydrobiopterin: NADP+ oxidoreductase) (SPR) mRNA	-1.577
NM_014248	RBX1	Ring-box 1 (RBX1) mRNA	-1.57
AK023684	FLJ13433	cDNA FLJ13622 fis clone PLACE1010960 weakly similar to actin-like protein 13E	-1.557
M58603	NFKB1	Human nuclear factor kappa-B DNA binding subunit (NF-kappa-B) mRNA complete cds	-1.546
NM_003016	SFRS2	Splicing factor arginine/serine-rich 2 (SFRS2) mRNA	-1.539
NM_003627	POV1	Prostate cancer overexpressed gene 1 (POV1) mRNA	-1.539
NM_004672	MAP3K6	Mitogen-activated protein kinase kinase kinase 6 (MAP3K6) mRNA	-1.534
NM_015917	LOC51064	Glutathione S-transferase subunit 13 homolog (LOC51064) mRNA	-1.531
NM_012183	FOXD3	Forkhead box D3 (FOXD3) mRNA	-1.523
NM_002668	PLP2	Proteolipid protein 2 (colonic epithelium-enriched) (PLP2) mRNA	-1.518
AK024449	PP2135	mRNA for FLJ00041 protein partial cds	-1.516
AK023326		cDNA FLJ13264 fis clone OVARC1000936 weakly similar to coat protein GP37	-1.499
AF069307	SLC5A6	Sodium-dependent multivitamin transporter (SMVT) mRNA complete cds	-1.493
NM_003032	SIAT1	Sialyltransferase 1 (beta-galactoside alpha-26-sialyltransferase) (SIAT1) mRNA	-1.493
NM_006761	YWHAE	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon polypeptide (YWHAE) mRNA	-1.492
BC005946	TUBA6	Clone MGC: 14580 IMAGE: 4134187 mRNA complete cds	-1.491
Z36811		H. sapiens (xs159) mRNA 300 bp	-1.49



Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AK023945		cDNA FLJ13883 fis clone THYRO1001487	-1.489
AL110152		mRNA; cDNA DKFZp586E1624 (from clone DKFZp586E1624)	-1.486
NM_003073	SMARCB1	SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily b member 1 (SMARCB1) mRNA	-1.485
NM_016388	TRIM	T-cell receptor interacting molecule (TRIM) mRNA	-1.484
AB002351	DMN	Human mRNA for KIAA0353 gene partial cds	-1.471
NM_015535	DKFZP564A2416	DKFZP564A2416 protein (DKFZP564A2416) mRNA	-1.464
NM_005672	PSCA	Prostate stem cell antigen (PSCA) mRNA	-1.451
AF235022	RAB38	Rab-related GTP-binding protein mRNA complete cds	-1.444
NM_000049	ASPA	Aspartoacylase (aminoacylase 2 Canavan disease) (ASPA) mRNA	-1.442
NM_013327	PARVB	Parvin beta (PARVB) mRNA	-1.432
AJ008139		NIK gene exon 3 3'	-1.431
NM_014481	APEX2	APEX nuclease (apurinic/aprimidinic endonuclease) 2 (APEX2) nuclear gene encoding mitochondrial protein mRNA	-1.425
L27479	X123	Human X123 mRNA 3' end	-1.422
AF161371	LOC88745	HSPC253 mRNA partial cds	-1.416
NM_017828	FLJ20452	Hypothetical protein FLJ20452 (FLJ20452) mRNA	-1.409
AF085838		Full length insert cDNA clone YI44D08	-1.405
AK001296	SDCCAG3	cDNA FLJ10434 fis clone NT2RP1000481	-1.399
NM_016630	ACP33	Acid cluster protein 33 (ACP33) mRNA	-1.393
NM_002490	NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 6 14 kDa (NDUFA6) mRNA	-1.387
NM_001418	EIF4G2	Eukaryotic translation initiation factor 4 gamma 2 (EIF4G2) mRNA	-1.371
NM_004111	FEN1	Flap structure-specific endonuclease 1 (FEN1) mRNA	-1.359
AB037805	KIAA1384	mRNA for KIAA1384 protein partial cds	-1.358
AF149019	PLXNB3	Plexin-B3 (PLXNB3) mRNA complete cds	-1.358
AL050021		mRNA; cDNA DKFZp564D016 (from clone DKFZp564D016)	-1.337
NM_001379	DNMT1	DNA (cytosine-5-)-methyltransferase 1 (DNMT1) mRNA	-1.335
AK025495	KIAA0790	cDNA: FLJ21842 fis clone HEP01849	-1.333
AF119892		PRO2710 mRNA complete cds	-1.333
NM_000026	ADSL	Adenylosuccinate lyase (ADSL) mRNA	-1.333
AF090101		Clone IMAGE 30714	-1.331
AY007113		Clone TCCCTA00142 mRNA sequence	-1.33
NM_016144	PTD002	PTD002 protein (PTD002) mRNA	-1.326
NM_000903	NQO1	NAD(P)H dehydrogenase quinone 1 (NQO1) mRNA	-1.324
D28589	KIAA0114	Human mRNA (KIAA00167) partial sequence	-1.318
U20582	LOC81569	Human actin-like peptide mRNA partial cds	-1.31
AF143882		Clone IMAGE: 121214 mRNA sequence	-1.306
AF085971		Full length insert cDNA clone YT85E08	-1.304
U81002	FLJ14502	TRAF4 associated factor 1 mRNA partial cds	-1.283
NM_002101	GYPC	Glycophorin C (Gerbich blood group) (GYPC) transcript variant 1 mRNA	-1.282
AF085883		Full length insert cDNA clone YP41H12	-1.281
U95006	MGC14480	Human D9 splice variant A mRNA complete cds	-1.278
AJ251759	SANG	GNAS1 antisense transcript RNA	-1.276
NM_006467	RPC32	Polymerase (RNA) III (DNA directed) (32 kDa) (RPC32) mRNA	-1.272
AF132733	DKFZP564G2022	Unknown mRNA	-1.267
NM_014362	HIBCH	3-hydroxyisobutyryl-Coenzyme A hydrolase (HIBCH) mRNA	-1.267
NM_002860	PYCS	Pyrroline-5-carboxylate synthetase (glutamate gamma-semialdehyde synthetase) (PYCS) mRNA	-1.263

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AK026037		cDNA: FLJ22384 fis clone HRC07594	-1.26
AL133017	FLJ22865	mRNA; cDNA DKFZp434E0727 (from clone DKFZp434E0727)	-1.259
AK025996	MGC4415	cDNA: FLJ22343 fis clone HRC06043	-1.259
NM_006014	DXS9879E	DNA segment on chromosome X (unique) 9879 expressed sequence (DXS9879E) mRNA	-1.245
NM_004507	HUS1	HUS1 checkpoint homolog ( <i>S. pombe</i> ) (HUS1) mRNA	-1.244
AK021635		cDNA FLJ11573 fis clone HEMBA1003376	-1.242
NM_014907	KIAA0967	KIAA0967 protein (KIAA0967) mRNA	-1.24
NM_006276	SFRS7	Splicing factor arginine/serine-rich 7 35 kDa (SFRS7) mRNA	-1.24
NM_001916	CYC1	Cytochrome c-1 (CYC1) mRNA	-1.238
AF130083		Clone FLB6613 PRO1737 mRNA complete cds	-1.223
NM_006441	MTHFS	510-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase) (MTHFS) mRNA	-1.222
NM_002137	HNRPA2B1	Heterogeneous nuclear ribonucleoprotein A2/B1 (HNRPA2B1) transcript variant A2 mRNA	-1.212
NM_019605	DJ667H12.2	Hypothetical protein DJ667H12.2 (DJ667H12.2) mRNA	-1.21
NM_012343	NNT	Nicotinamide nucleotide transhydrogenase (NNT) mRNA	-1.209
NM_002494	NDUFC1	NADH dehydrogenase (ubiquinone) 1 subcomplex unknown1 6 kDa (NDUFC1) mRNA	-1.208
NM_004749	CPR2	Cell-cycle progression 2 protein (CPR2) mRNA	-1.2
NM_006330	LYPLA1	Lysophospholipase I (LYPLA1) mRNA	-1.199
NM_001890	CSN1	Casein alpha (CSN1) mRNA	-1.199
NM_004648		Protein tyrosine phosphatase non-receptor type substrate 1 (PTPNS1) mRNA	-1.191
NM_006278	SIAT4C	Sialyltransferase 4C (beta-galactoside alpha-23-sialyltransferase) (SIAT4C) mRNA	-1.189
AB033102	KIAA1276	mRNA for KIAA1276 protein partial cds	-1.189
AF123659	LZTS1	FEZ1 (FEZ1) mRNA complete cds	-1.182
NM_007059	KPTN	Kaptin (actin binding protein) (KPTN) mRNA	-1.17
AK022827	AGO3	cDNA FLJ12765 fis clone NT2RP2001511 moderately similar to putative RNA-binding protein Q99 mRNA	-1.15
NM_002953	RPS6KA1	Ribosomal protein S6 kinase 90 kDa polypeptide 1 (RPS6KA1) mRNA	-1.146
NM_001546	ID4	Inhibitor of DNA binding 4 dominant negative helix-loop-helix protein (ID4) mRNA	-1.141
D17188		Human HepG2 3' region MboI cDNA clone hmd3a05m3	-1.14
NM_002696	POLR2G	Polymerase (RNA) II (DNA directed) polypeptide G (POLR2G) mRNA	-1.131
NM_002097	GTF3A	General transcription factor IIIA (GTF3A) mRNA	-1.129
AJ227873		Partial mRNA; ID YG81-3A	-1.12
NM_000133	F9	Coagulation factor IX (plasma thromboplastic component Christmas disease hemophilia B) (F9) mRNA	-1.117
NM_016406	HSPC155	Hypothetical protein HSPC155 (HSPC155) mRNA	-1.116
NM_014597	HSU15552	Acidic 82 kDa protein mRNA (HSU15552) mRNA	-1.115
AK024011	FLJ13949	cDNA FLJ13949 fis clone Y79AA1001041	-1.115
NM_016467	LOC51240	Hypothetical protein LOC51240 (LOC51240) mRNA	-1.112
NM_016608	ALEX1	ALEX1 protein (ALEX1) mRNA	-1.089
NM_001665	ARHG	Ras homolog gene family member G (rho G) (ARHG) mRNA	-1.081
NM_014397	NEK6	NIMA (never in mitosis gene a)-related kinase 6 (NEK6) mRNA	-1.079
NM_001383	DPH2L1	Diphtheria toxin resistance protein required for diphthamide biosynthesis-like 1 ( <i>S. cerevisiae</i> ) (DPH2L1) mRNA	-1.079
NM_005729	PPIF	Peptidylprolyl isomerase F (cyclophilin F) (PPIF) mRNA	-1.078
NM_014473	HSA9761	Putative dimethyladenosine transferase (HSA9761) mRNA	-1.077

Table I. Continued.

GeneBank™ accession	Gene symbol	Gene name	Fold change
AL080063	DKFZP564I052	mRNA; cDNA DKFZp564I052 (from clone DKFZp564I052)	-1.069
NM_016068	CGI-135	CGI-135 protein (LOC51024) mRNA	-1.069
S67789		APC (human adenomatous polyposis coli kindreds 347 and 139 mRNA partial mutant 117 nt)	-1.049
AL137489	MGC4730	mRNA; cDNA DKFZp43401230 (from clone DKFZp43401230); partial cds	-1.046
NM_006867	RBPMS	RNA-binding protein gene with multiple splicing (RBPMS) mRNA	-1.045
NM_006810	PDIR	For protein disulfide isomerase-related (PDIR) mRNA	-1.044
NM_014764	DAZAP2	DAZ associated protein 2 (DAZAP2) mRNA	-1.042
NM_004315	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1 (ASAH1) mRNA	-1.034
AF286598	AMOT	Angiomotin mRNA complete cds	-1.034
NM_006634	VAMP5	Vesicle-associated membrane protein 5 (myobrevin) (VAMP5) mRNA	-1.032
NM_014239	EIF2B2	Eukaryotic translation initiation factor 2B subunit 2 beta 39 kDa (EIF2B2) mRNA	-1.03
NM_020300	MGST1	Microsomal glutathione S-transferase 1 (MGST1) transcript variant 1b mRNA	-1.028
NM_013442	STOML2	Stomatin (EPB72)-like 2 (STOML2) mRNA	-1.027
NM_004681	EIF1AY	Eukaryotic translation initiation factor 1A Y chromosome (EIF1AY) mRNA	-1.022
AF086140		Full length insert cDNA clone ZA92B11	-1.018
J03866	DLAT	M2 mitochondrial autoantigen dihydrolipoamide acetyltransferase mRNA complete cds	-1.005

but genes involved in G1/S cell-cycle progression and DNA replication (*CDC2*, *MCM5*, *MCM7*, *PCNA*, *E2F1* and *CDK4*) were suppressed (Fig. 2B and C).

*Molecular dissecting of cell-cycle regulation by Apicidin treatment through large-scale data analysis.* We asked whether this regulation of genes in the cell-cycle signaling pathway is specifically mediated by Apicidin or its cytotoxicity. Accordingly, we displayed the gene expression levels of all selected genes associated with the regulation of G1/S cell-cycle progression on a heatmap (Fig. 3A). For example, the SMADs are a class of proteins that modulate the activities of transforming growth factor ligands, and cytoplasmic SMADs form complexes and then translocate to the nucleus, where they serve as transcription factors. As shown in Fig. 3A, the expression level of *SMAD3* was immediately induced by Apicidin, whereas *SMAD4* was up-regulated after 24 h of treatment. Inhibitors of cyclin-dependent kinases (CDKs), such as p21<sup>Cip1</sup>, p27<sup>Kip1</sup> and p57<sup>Kip2</sup> are important molecules that negatively regulate cell-cycle progression through G1/S transition. It appeared that p21<sup>Cip1</sup> was specifically induced by Apicidin, and *CDK2* and *CDK4* (but not the other *CDKs*) were specifically down-regulated by Apicidin (Fig. 3A). These results were also confirmed at the protein level by Western blotting (Fig. 3B). Furthermore, *cyclin A2*, *B1*, *B2* and *cell division cycle 2 (CDC2)* were specifically down-regulated by Apicidin, but the other *cyclins* and *CDCs* were not. In fact, cyclin A, B and CDC2 are also major regulators in the G2/M rather than the G1/S transition, implying that Apicidin affects cell-cycle progression by modulating the expressions of core components of the cell-cycle clock.

In G1/S cell-cycle transition, activation of CDKs/cyclins complexes phosphorylates retinoblastoma (Rb) protein, which can no longer suppress E2F/DP-1, and leads to the transcriptional activation of downstream target molecules. As shown in Fig. 3A, among the E2F family members, only E2F1 was suppressed by Apicidin. It appears that some of the E2F/DP-1 downstream target genes, such as *MCMs*, *PCNA*, and *DHFR*, were down-regulated by Apicidin. Interestingly, some *HDAC* genes were specifically shown to be induced by Apicidin (Fig. 3A).

## Discussion

Gene expression is in part regulated by the differential acetylation of nucleosomal histones, whereby hyperacetylation causes transcriptional activation and hypoacetylation causes repression (8,22,23). This phenomenon is regulated tightly by the balance between the activities of HAT and HDAC. In general, histone acetylation causes a more open chromatin structure, which facilitates transcriptional activation. HDACs can act as transcription repressors, due to histone deacetylation, and consequently promote chromatin condensation. Furthermore, HDAC inhibitors selectively alter gene transcription, in part, by causing chromatin remodeling and by changing the structures of proteins in transcription factor complexes (24). Eighteen HDAC enzymes have been identified and classified in human, based on homologies with yeast HDACs (25,26). The class I HDACs include HDAC1, 2, 3 and 8, which are related to yeast RPD3 deacetylase and have high homologies at their catalytic sites (27). Class II HDACs are related to yeast Hda 1 (histone deacetylase 1) and include

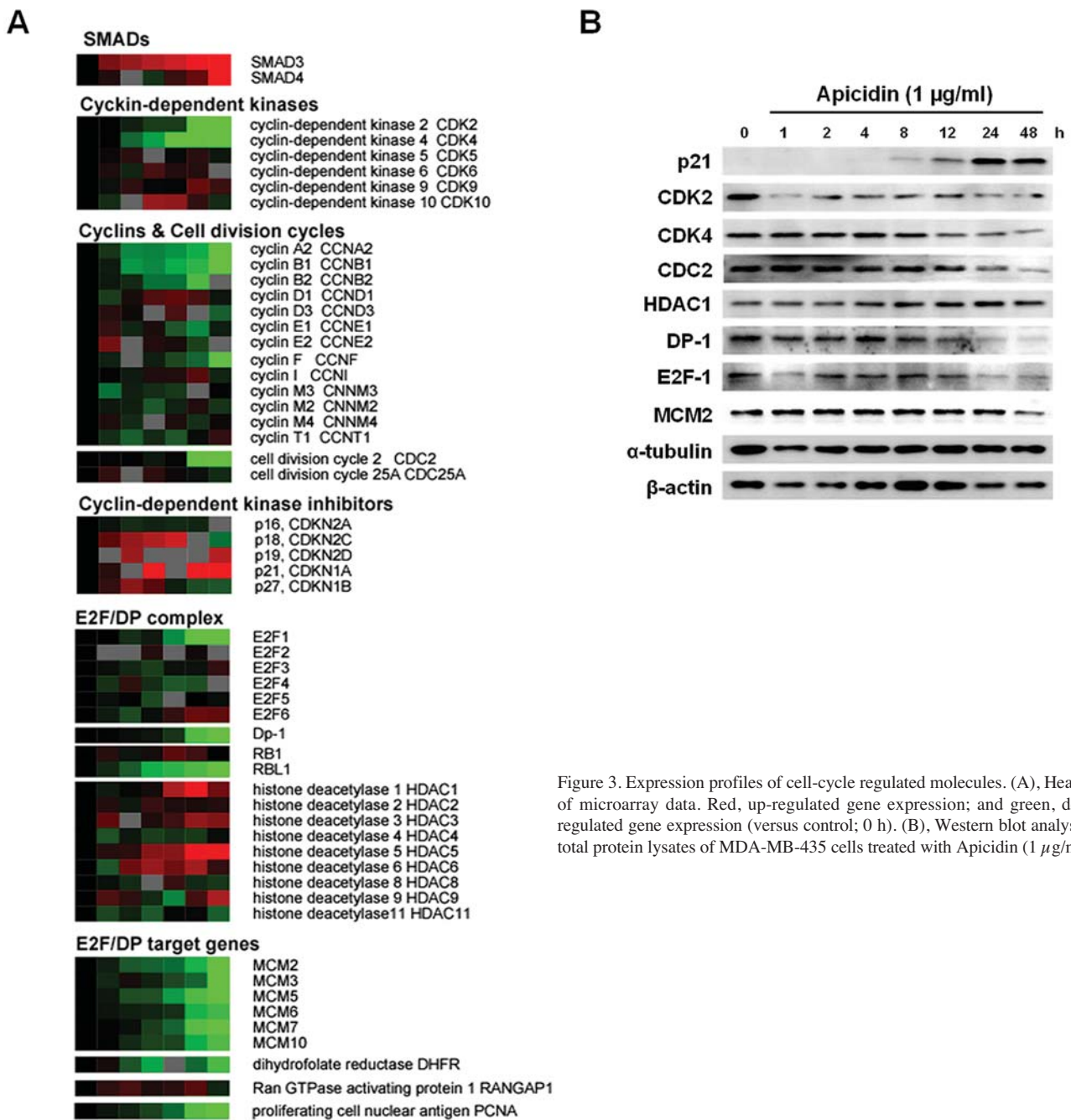


Figure 3. Expression profiles of cell-cycle regulated molecules. (A), Heatmap of microarray data. Red, up-regulated gene expression; and green, down-regulated gene expression (versus control; 0 h). (B), Western blot analysis of total protein lysates of MDA-MB-435 cells treated with Apicidin (1 µg/ml).

HDAC4, 5, 6, 9 and 10, which contain two catalytic sites (26,27). The human class III Sir2 or Hsts, SIRT1 (sirtuins) and Sir-related proteins, are related to HDACs in yeast (28), and class IV HDAC is represented by HDAC11, as conserved residues in the catalytic core region shared by both class I and II enzymes (29). A wide range of molecules, both natural and synthetic, have been identified that can inhibit the activities of class I, II and IV HDACs (1,30). With a few exceptions, these can be categorized as hydroxamic acid and its derivatives, carboxylates, benzamides, electrophilic ketones, and cyclic peptides (31,32). In particular, Apicidin is a fungal metabolite and an anti-protozoal agent that inhibits HDACs (14).

Although previous studies have shown that Apicidin has an anti-proliferative effect on various cancer cell lines (10,15,21), few studies have examined its effects in highly metastatic cancer cells, such as MDA-MB-435 cells. In

addition, because HDAC inhibitors are known to alter global gene expression, it was of interest to determine its effects on gene expression in carcinoma cells. Therefore, to understand the genomic effects of Apicidin on MDA-MB-435 cells, we investigated the anti-proliferative activity of Apicidin and its effects on global gene expression profiles. Our results show that Apicidin has potent anti-tumor effects on MDA-MB-435 cells (Fig. 1) and we identified 631 genes showing altered expressions by at least 2-fold through performing a large-scale serial data analysis (Fig. 2). In addition, the pathway mining analysis of these 631 genes suggested that Apicidin causes cell-cycle arrest by activating negative cell-cycle regulators and/or inactivating cell-cycle accelerating molecules at the transcriptional level (Figs. 2 and 3).

There have been some reports that the time of culture and/or concentration of HDAC inhibitor used affect the number

of genes detected with altered transcription. That is, short time points and low concentration cause fewer changes in gene transcription, while the magnitude of change and the number of genes altered increase to certain extent with the increase of time of culture and concentration of HDAC inhibitor (33-35). Thus, to identify the characteristic global gene expression changes induced by Apicidin, we performed serial gene expression analysis. Using this approach, we were able to identify 631 genes as Apicidin specific molecular signature that were continuously up- or down-regulated during 48 h of Apicidin treatment (Fig. 2).

In the present study, we utilized ArrayXPath, a web-interfaced pathway mining tool designed for microarray data (36,37), to map and visualize gene expression and to identify the biological pathways affected. This process identified cell-cycle regulation as one of the most significant biological processes affected; 11 genes were found to be obviously affected (Fig. 2B and C).

Many HDACs have non-histone proteins as their substrates, such as, hormone receptors, chaperone proteins, or cytoskeletal proteins, which are known to regulate cell proliferation and cell death (23). Thus, HDAC inhibitor-induced cell death involves both transcription-dependent and -independent mechanisms (25,38,39). CDK inhibitor *p21<sup>Cip1</sup>* is one of the most common genes induced by HDAC inhibitors (40,41), and although it was not included among the cell-cycle related 11 genes identified (probably because of missing data), our data indicated that *p21<sup>Cip1</sup>* was induced by Apicidin, and expression of protein level was confirmed by Western blot analysis. It has been well established that the activation of TGF- $\beta$  signaling stimulates the SMAD complex and causes the transcriptional activation of *p21<sup>Cip1</sup>* and *p15<sup>INK4</sup>*, which in turn leads to cell-cycle arrest. Thus, the transcriptional activation of the *p21<sup>Cip1</sup>* might be a consequence of the up-regulation of *SMAD3* by Apicidin.

Accumulating evidence suggests that HDAC inhibitors induce cell-cycle arrest in normal and transformed cells (25,42). Both G1 and G2 arrest are often associated with induction of *p21<sup>Cip1</sup>*, which inhibits the regulation of G1 progression (CDK4/6) and G1/S transition (CDK2) by inhibiting CDKs. Our array data also showed that *CDK4* and *CDK6* were specifically down-regulated by Apicidin, and this was supported by Western blot analysis (Fig. 3). During the G1/S cell-cycle transition, increased CDK inhibitor activity and decreased cyclin may act together to reduce CDK activity and then dephosphorylate Rb, which inactivates E2F and the transcriptions of genes required for G1 progression and G1/S transition are blocked (39). Interestingly, our data indicate that the *E2F1* and *DP-1* genes were down-regulated in parallel with the suppressions of *CDK4*, *CDK2* and induction of *CDKN1A* (*p21<sup>Cip1</sup>*) by Apicidin, which implies that Apicidin suppresses cell-cycle progression via the simultaneous transcriptional activation and inactivation of component molecules in the cell-cycle circuit. Indeed, many of E2F/DP-1 downstream target genes, such as, *MCMs*, *DHFR*, and *PCNA*, appeared to be underexpressed (Fig. 4). In addition, the observed down-regulations of *cyclin A2*, *cyclin B1*, *B2* and *CDC2*, which are major regulatory components of the G2/M phase, implies that Apicidin also exerts its anti-proliferative effect by influencing the G2/M transition. In conclusion, the

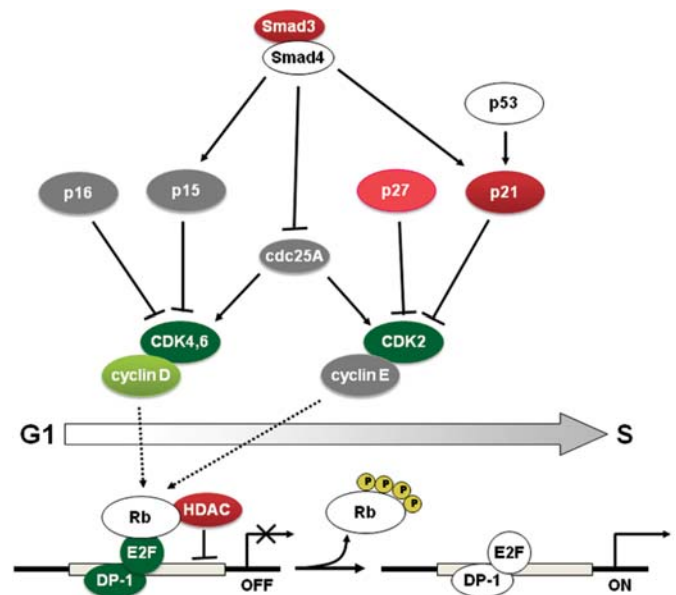


Figure 4. A schematic illustration of the cell-cycle regulation by Apicidin.

potent anti-mitogenic activity of Apicidin is due to the systemic transcriptional modulation of regulatory components of the cell-cycle circuit.

In summary, this study shows that the anti-tumor activity of Apicidin extends to highly metastatic cancer cells. Furthermore, large-scale DNA microarray data analysis findings showed that 631 genes are substantially modulated by Apicidin suggesting a characteristic molecular signature of Apicidin on MDA-MB-435 cells. Data mining of this molecular signature provides clues concerning the intracellular mechanism underlying the anti-tumor effect of Apicidin. Moreover, comparing these results to the known signaling pathway allows us to explain a systemic regulatory mechanism concerning the G1/S transition (Fig. 4).

Herein, we suggest that the potent anti-tumor activity of Apicidin is attributable to the modulation of the transcriptomic expressions of molecules in cell-cycle circuit. We hope that further comprehensive analyses of the identified 631 genes and intensive biological validations will provide useful information concerning the mechanisms underlying resistance and sensitivity to HDAC inhibitors.

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