

Bisdemethoxycurcumin (BDMC) Alters Gene Expression-associated Cell Cycle, Cell Migration and Invasion and Tumor Progression in Human Lung Cancer NCI-H460 Cells

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Abstract. *Background/Aim:* Lung cancer is one of the most common malignancies and a predominant cause of cancer-related death. It can metastasize in almost all organs, and currently, while new cases are increasing, treatment is still insufficient. **Bisdemethoxycurcumin (BDMC)**, one of the components of turmeric, has been known to possess biological activities. However, the effects of BDMC on the genetic level remain unclear. *Materials and Methods:* Human lung cancer NCI-H460 cells were treated with 55 μ M BDMC for 24 h and cells were harvested for total RNA extraction. The purified RNA was used for cDNA synthesis, labeling, microarray hybridization, and flour-labeled cDNA on-chip hybridization. The expression Console software (Affymetrix) with default RNA parameters was used to detect and

quantitate concentrations of fluorescent molecules. The key genes involved and their possible interaction pathways were analyzed by the GeneGo software. **Results:** Seven genes, such as CCNE2 (cyclin E), associated with cell cycle, were over 4-fold overexpressed, 22 genes, such as ERCC6L (excision repair cross-complementing rodent repair deficiency, complementation group 6-like) associated with DNA damage and repair, were from 3- to 4-fold overexpressed and 266, such as cell division cycle, S-phase associated kinase and associated with cell death, genes were from 2- to 3-fold overexpressed. **Conclusion:** **BDMC induced changes in gene expression that may reveal cytotoxic information on the genetic level while presenting novel biomarkers or targets for treatment of human lung cancer in the future.**

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Lung cancer is one of the major causes of death due to cancer worldwide and early metastatic dissemination and resistance to therapy are causal factors giving a median survival of less than 12 months (1). Recently, in lung cancer patients, new improvements have focused on chemotherapy and molecular-targeted therapy, however, the outcome of patients remains unsatisfactory (2). Non small-cell lung cancer (NSCLC) accounts for 85% of lung cancer cases (3, 4) and is also the main cause of cancer-related death worldwide (5) and the overall 5-year survival rate of NSCLC patients remains lower than 15% (6). Thus, new therapeutic strategies for NSCLC are urgently needed. Many current investigations are focusing on anticancer drugs

originating from natural products. Furthermore, in psychiatric medicine, herbals and phytochemicals have been recognized to be of great interest, as complementary and/or alternative therapies (7).

Turmeric, a ground rhizome of *Curcuma longa*, is widely used in Asian traditional medicine for wound healing, inflammatory conditions, and blood purification (8, 9). In traditional Chinese medicine, turmeric has a long history of treating diseases associated with abdominal pain (10). Bisdemethoxycurcumin (BDMC), one of the components of turmeric, has been shown to possess anti-inflammatory and anti-proliferative activities (11), anti-metastasis potency via the differentially down-regulation of ECM degradation enzymes (12) and to induce rapid DNA double-strand breaks in the human colon cancer HCT116 cells (13). BDMC inhibited cell invasion and motility and modulated MMP-3 expression in human invasive breast carcinoma cells (14). BDMC decreased inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production (15) and inhibited the Wnt/beta-catenin pathway (16). Furthermore, the dual activity of BDMC on topoisomerases-II α (TOP2A) has been used in novel therapeutic strategies in order to induce apoptosis in cancer cells (17).

Existing studies included patients treated with targeted-agents based on specific molecular alterations of neoplastic cells (18, 19). Thus, molecular alterations in cancer cells are called to attention for aiding the development of specific targeted drugs. Moreover, in clinical practice, predictive biomarkers have been assessed and increased the availability of anticancer drugs corresponding to specific molecular alterations (20). In the past decade, the identification of specific predictive and/or prognostic molecular alterations for NSCLC and colorectal carcinoma (CRC) have been the center of attention (21, 22). Currently, the development of targeted-therapeutics for NSCLC treatment aim at defining genetic abnormalities in NSCLC such as mutations in *EGFR* or a fusion of the *EML4*, while the *ALK* gene has been recognized as a target for first-line therapies in NSCLC (23-25).

Although BDMC has been shown to induce cell death in NSCLC cells, the exact genes affected by BDMC remain unknown. Thus, we investigated altered gene expression in NCI-H460 cells after exposure of cells to BDMC and results indicated that affected genes are involved in apoptosis pathways.

Materials and Methods

Chemicals and reagents. DMSO was 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). BDMC was dissolved in DMSO and stored at -20°C before use in experiments.

Human lung cancer cells. NCI-H460 human non-small cell lung cancer cells were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2 air in RPMI-1640 medium supplemented with 10% (FBS), 1% L-glutamine and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). Cultured NCI-H460 cells were split every 4 days for maintaining exponential growth and were harvested with 0.025% trypsin and 0.52 mM EDTA in phosphate buffer saline (PBS), plated at required cell numbers and allowed to adhere for 24 h before BDMC treatment.

cDNA Microarray assay for gene expression in NCI-H460 cells after exposure to BDMC. NCI-H460 cells at a density of 5×10^5 cells/ml were maintained on 12-well plates with RPMI-1640 medium for 24 h and then incubated with or without $35 \mu\text{M}$ of BDMC for 24 h. At the end of incubation, cells were collected from each treatment and total RNA was extracted by using the Qiagen RNeasy Mini Kit (Qiagen, Inc, Valencia, CA, USA), as described previously (26) and individually quantitated 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) (26). The resulting localized concentrations of fluorescent molecules on the chip were detected and quantified (Asia BioInnovations Corporation, Taipei, Taiwan) and data were further analyzed by the Expression Console software (Affymetrix) with default RMA parameters (26, 27). Data are representative of three separate assays.

Statistical analysis. All results were presented as mean \pm SD of three independent experiments. Significant differences between BDMC-treated and -untreated groups were considered if at least a 2-fold change was recorded. +, Up-regulation; -, down-regulation.

Results

BDMC induced both up-regulation and down-regulation of gene expression in NCI-H460 cells. After NCI-H460 cells were treated with or without $35 \mu\text{M}$ of BDMC for 48 h, they were extracted for total RNA from each treatment. Isolated total RNA was quantified, followed by cDNA microarray analysis and results are shown in Tables I and II. Table I shows that 7 genes were over 4-fold up-regulated, such as *CCNE2* (cyclin E), 22 genes were from over 3- to 4-fold such as *ERCC6L* (excision repair cross-complementing rodent repair deficiency, complementation group 6-like), and 266 genes were over from 2- to 3-fold up-regulated, such as cell division cycle 6 homolog (*CDC6*), cell division cycle associated 5 (*CDCA5*), cell division cycle 25 homolog A (*CDC25A*) and cell division cycle associated 7-like (*CDCA7L*) associated with cell division, S-phase kinase-associated protein 2, E3 ubiquitin protein ligase (*SKP2*), Cdk5 and Abl enzyme substrate 2 (*CABLES2*), Cdk5 and Abl enzyme substrate 1 (*CABLES1*) and cyclin E1 (*CCNE1*).

Table II indicates that 41 genes were over 4-fold down-regulated such as *DDIT3* associated with DNA damage; 57 genes were down-regulated from 3- to 4 -fold such as *DDIT4* associated with DNA damage, *CCPG1* associated with cell

Table I. *H460 BDMC-treated vs. H460-BDMC-control.*

Fold change	Gene symbol	mRNA Description
7.03	<i>CYP1A1</i>	Cytochrome P450, family 1, subfamily A, polypeptide 1
5.67	<i>FAM111B</i>	Family with sequence similarity 111, member B
5.53	<i>TIPARP</i>	TCDD-inducible poly(ADP-ribose) polymerase
4.83	<i>CCNE2</i>	Cyclin E2
4.35	<i>DSCC1</i>	Defective in sister chromatid cohesion 1 homolog (<i>S. cerevisiae</i>)
4.04	<i>NAP1L2</i>	Nucleosome assembly protein 1-like 2
4.02	<i>SNAI2</i>	Snail homolog 2 (<i>Drosophila</i>)
3.80	<i>CLSPN</i>	Claspin
3.77	<i>ZNF483</i>	Zinc finger protein 483
3.70	<i>ORC1</i>	Origin recognition complex, subunit 1
3.66	<i>FIGNL1</i>	Fidgetin-like 1
3.65	<i>LPCAT2</i>	Lysophosphatidylcholine acyltransferase 2
3.58	<i>ERCC6L</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 6-like
3.57	<i>VDR</i>	Vitamin D (1,25-dihydroxyvitamin D3) receptor
3.52	<i>ID3</i>	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
3.45	<i>IFIT2</i>	Interferon-induced protein with tetratricopeptide repeats 2
3.44	<i>CLSPN</i>	Claspin
3.42	<i>METTL7B</i>	Methyltransferase like 7B
3.30	<i>HECW1</i>	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
3.18	<i>ID1</i>	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
3.17	<i>SKA3</i>	Spindle and kinetochore associated complex subunit 3
3.11	<i>MBOAT2</i>	Membrane bound O-acyltransferase domain containing 2
3.06	<i>WNK3</i>	WNK lysine deficient protein kinase 3
3.05	<i>DLX2</i>	Distal-less homeobox 2
3.04	<i>NRG4</i>	Neuregulin 4
3.01	<i>TLR3</i>	Toll-like receptor 3
3.00	<i>FEN1</i>	Flap structure-specific endonuclease 1
3.00	<i>CRYZ</i>	Crystallin, zeta (quinone reductase)
3.00	<i>MCM10</i>	Minichromosome maintenance complex component 10
2.99	<i>DHFR</i>	Dihydrofolate reductase
2.99	<i>CROT</i>	Carnitine O-octanoyltransferase
2.99	<i>GINS3</i>	GINS complex subunit 3 (Psf3 homolog)
2.98	<i>CDC6</i>	Cell division cycle 6 homolog (<i>S. cerevisiae</i>)
2.95	<i>RALGPS2</i>	Ral GEF with PH domain and SH3 binding motif 2
2.95	<i>DHFR</i>	Dihydrofolate reductase
2.93	<i>KLHL13</i>	Kelch-like 13 (<i>Drosophila</i>)
2.93	<i>C10orf122</i>	Chromosome 10 open reading frame 122
2.91	<i>HSPA1A</i>	Heat shock 70kDa protein 1A; heat shock 70kDa protein 1B
2.91	<i>HSPA1B</i>	Heat shock 70kDa protein 1B; heat shock 70kDa protein 1A
2.90	<i>SLC1A1</i>	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1
2.89	<i>TDO2</i>	Tryptophan 2,3-dioxygenase
2.89	<i>TMEM14A</i>	Transmembrane protein 14A
2.89	<i>HSPA1A</i>	Heat shock 70kDa protein 1A; heat shock 70kDa protein 1B
2.87	<i>EML1</i>	Echinoderm microtubule associated protein like 1
2.86	<i>ASF1B</i>	ASF1 anti-silencing function 1 homolog B (<i>S. cerevisiae</i>)
2.85	<i>C3orf14</i>	Chromosome 3 open reading frame 14
2.85	<i>STOX1</i>	storkhead-box 1
2.84	<i>HSPA1A</i>	Heat shock 70kDa protein 1A; heat shock 70kDa protein 1B
2.83	<i>STS</i>	Steroid sulfatase (microsomal), isozyme S
2.83	<i>HSPA1A</i>	Heat shock 70kDa protein 1A; heat shock 70kDa protein 1B
2.82	<i>SNORA3</i>	Small nucleolar RNA, H/ACA box 3; ribosomal protein L27a
2.80	<i>SFN</i>	Stratifin
2.80	<i>GTF2I</i>	General transcription factor III
2.79	<i>DHFR</i>	Dihydrofolate reductase; dihydrofolate reductase pseudogene
2.78	<i>PLEK2</i>	Pleckstrin 2
2.78	<i>RN5S494</i>	RNA, 5S ribosomal 494
2.74	<i>CDCA5</i>	Cell division cycle associated 5
2.72	<i>PKI55</i>	DKFZp434H1419

Table I. *Continued*

Table I. Continued

Fold change	Gene symbol	mRNA Description
2.70	<i>TMOD2</i>	Tropomodulin 2 (neuronal)
2.66	<i>PLCE1</i>	Phospholipase C, epsilon 1
2.66	<i>GMNN</i>	Geminin, DNA replication inhibitor
2.65	<i>CEP78</i>	Centrosomal protein 78kDa
2.65	<i>CXorf57</i>	Chromosome X open reading frame 57
2.65	<i>RNU5D-1</i>	RNA, U5D small nuclear 1
2.65	<i>FOXRED2</i>	FAD-dependent oxidoreductase domain containing 2
2.63	<i>POLR2A</i>	Polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
2.63	<i>IFIT1</i>	Interferon-induced protein with tetratricopeptide repeats 1
2.62	<i>PIK3R3</i>	Phosphoinositide-3-kinase, regulatory subunit 3 (gamma)
2.62	<i>CDC25A</i>	Cell division cycle 25 homolog A (S. pombe)
2.62	<i>ADI1</i>	Acireductone dioxygenase 1
2.59	<i>DTL</i>	Denticleless E3 ubiquitin protein ligase homolog (Drosophila)
2.57	<i>BLM</i>	Bloom syndrome, RecQ helicase-like
2.57	<i>FAM81A</i>	Family with sequence similarity 81, member A
2.55	<i>BBS5</i>	Bardet-Biedl syndrome 5
2.54	<i>DSN1</i>	DSN1, MIND kinetochore complex component, homolog (S. cerevisiae)
2.53	<i>NT5DC2</i>	5'-nucleotidase domain containing 2
2.53	<i>FRMD3</i>	FERM domain containing 3
2.52	<i>KIAA1009</i>	KIAA1009
2.51	<i>PRKAR2B</i>	Protein kinase, cAMP-dependent, regulatory, type II, beta
2.51	<i>POLE2</i>	Polymerase (DNA directed), epsilon 2, accessory subunit
2.51	<i>SKP2</i>	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase
2.51	<i>IQCG</i>	IQ motif containing G
2.49	<i>SMAD9</i>	SMAD family member 9
2.49	<i>SHCBP1</i>	SHC SH2-domain binding protein 1
2.49	<i>HMGCS1</i>	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
2.48	<i>NEFL</i>	Neurofilament, light polypeptide
2.48	<i>NAGK</i>	N-acetylglucosamine kinase
2.48	<i>BAMBI</i>	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)
2.47	<i>FAM40B</i>	Family with sequence similarity 40, member B
2.47	<i>ZNF136</i>	Zinc finger protein 136
2.46	<i>IFIT3</i>	Interferon-induced protein with tetratricopeptide repeats 3
2.45	<i>CCDC138</i>	Coiled-coil domain containing 138
2.45	<i>SEPW1</i>	Selenoprotein W, 1
2.45	<i>OR2AP1</i>	Olfactory receptor, family 2, subfamily AP, member 1
2.44	<i>HSPB11</i>	Heat shock protein family B (small), member 11
2.44	<i>LINC00324</i>	Long intergenic non-protein coding RNA 324
2.43	<i>ID2</i>	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
2.43	<i>MEPCE</i>	Methylphosphate capping enzyme
2.41	<i>EHHADH</i>	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase
2.41	<i>SNRNP25</i>	Small nuclear ribonucleoprotein 25kDa (U11/U12)
2.41	<i>SDAD1</i>	SDA1 domain containing 1
2.41	<i>ZNF701</i>	Zinc finger protein 701
2.40	<i>FGD6</i>	FYVE, RhoGEF and PH domain containing 6
2.40	<i>TRIP13</i>	Thyroid hormone receptor interactor 13
2.40	<i>BRMS1L</i>	Breast cancer metastasis-suppressor 1-like
2.40	<i>DPF1</i>	D4, zinc and double PHD fingers family 1
2.39	<i>MCM4</i>	Minichromosome maintenance complex component 4
2.39	<i>CABLES2</i>	Cdk5 and Abl enzyme substrate 2
2.39	<i>GPEX3</i>	Glutathione peroxidase 3 (plasma)
2.39	<i>NPTX2</i>	Neuronal pentraxin II
2.39	<i>TMEM171</i>	Transmembrane protein 171
2.37	<i>ESCO2</i>	Establishment of cohesion 1 homolog 2 (S. cerevisiae)
2.37	<i>ZNF280A</i>	Zinc finger protein 280A
2.37	<i>BFSPT</i>	Beaded filament structural protein 1, filensin
2.37	<i>GUCY1B3</i>	Guanylate cyclase 1, soluble, beta 3
2.36	<i>JAKMIP2</i>	Janus kinase and microtubule interacting protein 2
2.36	<i>C17orf53</i>	Chromosome 17 open reading frame 53

Table I. Continued

Table I. Continued

Fold change	Gene symbol	mRNA Description
2.35	<i>ZNF117</i>	Zinc finger protein 117
2.35	<i>APOBEC3B</i>	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
2.35	<i>GDAP1</i>	Ganglioside induced differentiation associated protein 1
2.35	<i>ZNF611</i>	Zinc finger protein 611
2.34	<i>HSPH1</i>	Heat shock 105kDa/110kDa protein 1
2.34	<i>MCM2</i>	Minichromosome maintenance complex component 2
2.34	<i>CCDC56</i>	Coiled-coil domain containing 56
2.33	<i>DHCR24</i>	24-dehydrocholesterol reductase
2.32	<i>CDCA2</i>	Cell division cycle associated 2
2.32	<i>TSPAN12</i>	Tetraspanin 12
2.32	<i>SSX1</i>	Synovial sarcoma, X breakpoint 1
2.32	<i>DYX1C1</i>	Dyslexia susceptibility 1 candidate 1; DYX1C1-CCPG1 readthrough (non-protein coding)
2.32	<i>WDR52</i>	WD repeat domain 52
2.31	<i>WDR51</i>	WD repeat and HMG-box DNA binding protein 1
2.31	<i>CERK</i>	Ceramide kinase
2.31	<i>ALDH3A1</i>	Aldehyde dehydrogenase 3 family, member A1
2.31	<i>THNSL1</i>	Threonine synthase-like 1 (<i>S. cerevisiae</i>)
2.31	<i>TMEM229B</i>	Transmembrane protein 229B
2.30	<i>PRKAR1B</i>	Protein kinase, cAMP-dependent, regulatory, type I, beta
2.30	<i>RPA2</i>	Replication protein A2, 32kDa
2.30	<i>CENPW</i>	Centromere protein W
2.30	<i>PCNA</i>	Proliferating cell nuclear antigen
2.30	<i>DDX46</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
2.30	<i>RNY4P10</i>	RNA, Ro-associated Y4 pseudogene 10
2.29	<i>EIF4EBP2</i>	Eukaryotic translation initiation factor 4E binding protein 2
2.29	<i>FOXM1</i>	Forkhead box M1
2.29	<i>FAR2</i>	Fatty acyl CoA reductase 2
2.28	<i>C15orf42</i>	Chromosome 15 open reading frame 42
2.28	<i>GINS1</i>	GINS complex subunit 1 (Psf1 homolog)
2.28	<i>C2orf44</i>	Chromosome 2 open reading frame 44
2.28	<i>CYP4Z2P</i>	Cytochrome P450, family 4, subfamily Z, polypeptide 2 pseudogene
2.28	<i>LYZL2</i>	Lysozyme-like 2
2.27	<i>SCRN1</i>	Secernin 1
2.26	<i>ACAT2</i>	Acetyl-CoA acetyltransferase 2
2.26	<i>NSF</i>	N-ethylmaleimide-sensitive factor; vesicle-fusing ATPase-like
2.25	<i>DENND2C</i>	DENN/MADD domain containing 2C
2.25	<i>ABRA1</i>	ABRA C-terminal like
2.25	<i>SYNJ2</i>	Synaptojanin 2
2.25	<i>POP4</i>	Processing of precursor 4, ribonuclease P/MRP subunit (<i>S. cerevisiae</i>)
2.25	<i>TUBA4A</i>	Tubulin, alpha 4a
2.25	<i>TCTN2</i>	Tectonic family member 2
2.24	<i>CKAP2</i>	Cytoskeleton associated protein 2
2.24	<i>ZWINT</i>	ZW10 interactor
2.24	<i>RN5S180</i>	RNA, 5S ribosomal 180
2.23	<i>CARD6</i>	Caspase recruitment domain family, member 6
2.23	<i>C11orf82</i>	Chromosome 11 open reading frame 82
2.23	<i>RAB27A</i>	RAB27A, member RAS oncogene family
2.23	<i>IDI1</i>	Isopentenyl-diphosphate delta isomerase 1
2.23	<i>ACACB</i>	Acetyl-CoA carboxylase beta
2.23	<i>SAMD9</i>	Sterile alpha motif domain containing 9
2.23	<i>DYDC2</i>	DPY30 domain containing 2
2.22	<i>IQGAP2</i>	IQ motif containing GTPase activating protein 2
2.22	<i>CDCA7L</i>	Cell division cycle associated 7-like
2.21	<i>EFCA10</i>	EF-hand calcium binding domain 10
2.21	<i>MYBL1</i>	V-myb myeloblastosis viral oncogene homolog (avian)-like 1
2.21	<i>UMPS</i>	Uridine monophosphate synthetase
2.21	<i>MYL6</i>	Myosin, light chain 6, alkali, smooth muscle and non-muscle
2.21	<i>COMMD4</i>	COMM domain containing 4
2.20	<i>SGK3</i>	Serum/glucocorticoid regulated kinase family, member 3; C8orf44-SGK3 readthrough

Table I. Continued

Table I. Continued

Fold change	Gene symbol	mRNA Description
2.20	<i>IFI44</i>	Interferon-induced protein 44
2.20	<i>SLC10A5</i>	Solute carrier family 10 (sodium/bile acid cotransporter family), member 5
2.20	<i>DIO2</i>	Deiodinase, iodothyronine, type II
2.19	<i>DBR1</i>	Debranching enzyme homolog 1 (<i>S. cerevisiae</i>)
2.19	<i>ZNF680</i>	Zinc finger protein 680
2.19	<i>MFSD5</i>	Major facilitator superfamily domain containing 5
2.18	<i>ZNF382</i>	Zinc finger protein 382
2.18	<i>RCBTB2</i>	Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2
2.17	<i>PSG3</i>	Pregnancy specific beta-1-glycoprotein 3
2.17	<i>ZNF480</i>	Zinc finger protein 480
2.17	<i>RFWD3</i>	Ring finger and WD repeat domain 3
2.17	<i>CENPQ</i>	Centromere protein Q
2.17	<i>PPP2R3A</i>	Protein phosphatase 2, regulatory subunit B", alpha
2.17	<i>RPL41</i>	Ribosomal protein L41
2.17	<i>TUBB4B</i>	Tubulin, beta 4B class IVb
2.17	<i>CABLES1</i>	Cdk5 and Abl enzyme substrate 1
2.16	<i>BRCA1</i>	Breast cancer 1, early onset
2.16	<i>TRAF1</i>	TRAF interacting protein
2.16	<i>SLC25A21</i>	Solute carrier family 25 (mitochondrial oxoadipate carrier), member 21
2.16	<i>RPS6KA6</i>	Ribosomal protein S6 kinase, 90kDa, polypeptide 6
2.15	<i>SAMHD1</i>	SAM domain and HD domain 1
2.15	<i>SHMT1</i>	Serine hydroxymethyltransferase 1 (soluble)
2.15	<i>DNAJA1</i>	DnaJ (Hsp40) homolog, subfamily A, member 1
2.15	<i>HMOX1</i>	Heme oxygenase (decycling) 1
2.15	<i>REEP1</i>	Receptor accessory protein 1
2.14	<i>GIN5</i>	GIN5 complex subunit 4 (Sld5 homolog)
2.14	<i>SEPT3</i>	Septin 3
2.14	<i>KIAA0101</i>	KIAA0101
2.14	<i>TIMELESS</i>	Timeless homolog (<i>Drosophila</i>)
2.14	<i>MEIG1</i>	Meiosis expressed gene 1 homolog (mouse)
2.14	<i>CORO2A</i>	Coronin, actin binding protein, 2A
2.14	<i>C12orf26</i>	Chromosome 12 open reading frame 26
2.13	<i>FAM171B</i>	Family with sequence similarity 171, member B
2.13	<i>ATP6V0D1</i>	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1
2.13	<i>MSH6</i>	MutS homolog 6 (<i>E. coli</i>)
2.13	<i>FN3KRP</i>	Fructosamine 3-kinase related protein
2.13	<i>TUBB3</i>	Tubulin, beta 3 class III
2.12	<i>DNAJC6</i>	DnaJ (Hsp40) homolog, subfamily C, member 6
2.12	<i>HYAL1</i>	Hyaluronoglucosaminidase 1
2.12	<i>ZBTB9</i>	Zinc finger and BTB domain containing 9
2.11	<i>PCYT2</i>	Phosphate cytidyltransferase 2, ethanolamine
2.11	<i>BEND6</i>	BEN domain containing 6
2.11	<i>SRRT</i>	Serrate RNA effector molecule homolog (<i>Arabidopsis</i>)
2.11	<i>SHQ1</i>	SHQ1 homolog (<i>S. cerevisiae</i>)
2.11	<i>PHTF1</i>	Putative homeodomain transcription factor 1
2.10	<i>NAVI</i>	Neuron navigator 1
2.10	<i>MAP2</i>	Microtubule-associated protein 2
2.10	<i>TCF19</i>	Transcription factor 19
2.10	<i>C1orf112</i>	Chromosome 1 open reading frame 112
2.10	<i>TIMM21</i>	Translocase of inner mitochondrial membrane 21 homolog (yeast)
2.10	<i>TACC3</i>	Transforming, acidic coiled-coil containing protein 3
2.10	<i>ZNF468</i>	Zinc finger protein 468
2.10	<i>C2CD3</i>	C2 calcium-dependent domain containing 3
2.10	<i>GCA</i>	Grancalcin, EF-hand calcium binding protein
2.10	<i>MVK</i>	Mevalonate kinase
2.10	<i>DDX23</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 23
2.09	<i>COMMD9</i>	COMM domain containing 9
2.09	<i>RPGRIP1L</i>	RPGRIP1-like
2.09	<i>ZNF675</i>	Zinc finger protein 675

Table I. Continued

Table I. *Continued*

Fold change	Gene symbol	mRNA Description
2.09	<i>RFC5</i>	Replication factor C (activator 1) 5, 36.5kDa
2.09	<i>FTSJ3</i>	FtsJ homolog 3 (E. coli)
2.08	<i>PCYT2</i>	Phosphate cytidyltransferase 2, ethanolamine
2.08	<i>DHX9</i>	DEAH (Asp-Glu-Ala-His) box polypeptide 9
2.08	<i>GEMIN5</i>	Gem (nuclear organelle) associated protein 5
2.08	<i>C6orf225</i>	Chromosome 6 open reading frame 225
2.07	<i>TCF19</i>	Transcription factor 19
2.07	<i>SMG8</i>	Smg-8 homolog, nonsense mediated mRNA decay factor (C. elegans)
2.07	<i>GIMAP2</i>	GTPase, IMAP family member 2
2.07	<i>UST</i>	Uronyl-2-sulfotransferase
2.07	<i>KBTD8</i>	Kelch repeat and BTB (POZ) domain containing 8
2.07	<i>HRSP12</i>	Heat-responsive protein 12
2.07	<i>CASP8AP2</i>	Caspase 8 associated protein 2
2.07	<i>CENPP</i>	Centromere protein P
2.07	<i>ISG20L2</i>	Interferon stimulated exonuclease gene 20kDa-like 2
2.06	<i>TUBB4B</i>	Tubulin, beta 4B class IVb
2.06	<i>TUBB2A</i>	Tubulin, beta 2A class IIa
2.06	<i>DCLRE1B</i>	DNA cross-link repair 1B
2.06	<i>RAD51</i>	RAD51 homolog (S. cerevisiae)
2.06	<i>TSGA10</i>	Testis specific, 10
2.06	<i>VPS33A</i>	Vacuolar protein sorting 33 homolog A (S. cerevisiae)
2.06	<i>PRMT7</i>	Protein arginine methyltransferase 7
2.05	<i>MSX2</i>	Msh homeobox 2
2.05	<i>MCM3</i>	Minichromosome maintenance complex component 3
2.05	<i>POP1</i>	Processing of precursor 1, ribonuclease P/MRP subunit (S. cerevisiae)
2.05	<i>KIF3C</i>	Kinesin family member 3C
2.05	<i>MTBP</i>	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) binding protein, 104kDa
2.05	<i>SLFN13</i>	Schlafen family member 13
2.05	<i>ZNF695</i>	Zinc finger protein 695
2.05	<i>CTR9</i>	Ctr9, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)
2.05	<i>GCNT3</i>	Glucosaminyl (N-acetyl) transferase 3, mucin type
2.04	<i>FANCM</i>	Fanconi anemia, complementation group M
2.04	<i>TSPYL2</i>	TSPY-like 2
2.04	<i>OIP5</i>	Opa interacting protein 5
2.04	<i>MTMR4</i>	Myotubularin related protein 4
2.04	<i>PLCG1</i>	Phospholipase C, gamma 1
2.04	<i>MYBL2</i>	V-myb myeloblastosis viral oncogene homolog (avian)-like 2
2.04	<i>WDR52</i>	WD repeat domain 52
2.04	<i>TEX9</i>	Testis expressed 9
2.03	<i>CCNE1</i>	Cyclin E1
2.03	<i>EFNB2</i>	Ephrin-B2
2.03	<i>CBX5</i>	Chromobox homolog 5
2.03	<i>HMGN2</i>	High mobility group nucleosomal binding domain 2
2.03	<i>TM2D2</i>	TM2 domain containing 2
2.02	<i>TCEB3</i>	Transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A)
2.02	<i>CHAF1B</i>	Chromatin assembly factor 1, subunit B (p60)
2.02	<i>QDPR</i>	Quinoid dihydropteridine reductase
2.02	<i>PRAME</i>	Preferentially expressed antigen in melanoma
2.02	<i>TP53I3</i>	Tumor protein p53 inducible protein 3
2.01	<i>SGOL2</i>	Shugoshin-like 2 (S. pombe)
2.01	<i>PIM2</i>	Pim-2 oncogene
2.01	<i>CTSD1</i>	CDGSH iron sulfur domain 1
2.01	<i>TP53INP1</i>	Tumor protein p53 inducible nuclear protein 1
2.01	<i>CXCL14</i>	Chemokine (C-X-C motif) ligand 14
2.01	<i>AHSA1</i>	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)
2.01	<i>FLJ25715</i>	Uncharacterized protein FLJ25715
2.01	<i>ZNF780B</i>	Zinc finger protein 780B
2.01	<i>FAM98A</i>	Family with sequence similarity 98, member A
2.01	<i>ZNF138</i>	Zinc finger protein 138
2.01	<i>SQRDL</i>	Sulfide quinone reductase-like (yeast)

Table II. *H460 BDMC-treated cells vs. H460-BDMC-control.*

Fold change	Gene symbol	mRNA Description
-2.01	<i>MIR32</i>	MicroRNA 32
-2.01	<i>TOM1L1</i>	Target of myb1 (chicken)-like 1
-2.01	<i>MAML3</i>	Mastermind-like 3 (Drosophila)
-2.02	<i>KCNN4</i>	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
-2.02	<i>NCAM2</i>	Neural cell adhesion molecule 2
-2.02	<i>CPS1</i>	Carbamoyl-phosphate synthase 1, mitochondrial
-2.02	<i>SPRY3</i>	Sprouty homolog 3 (Drosophila)
-2.02	<i>ICAM1</i>	Intercellular adhesion molecule 1
-2.02	<i>AXL</i>	AXL receptor tyrosine kinase
-2.03	<i>PRSS23</i>	Protease, serine, 23
-2.03	<i>NR2F1</i>	Nuclear receptor subfamily 2, group F, member 1
-2.03	<i>GADD45B</i>	Growth arrest and DNA-damage-inducible, beta
-2.03	<i>CDK17</i>	Cyclin-dependent kinase 17
-2.03	<i>PDE4DIP</i>	Phosphodiesterase 4D interacting protein
-2.03	<i>NEDD1</i>	Neural precursor cell expressed, developmentally down-regulated 1
-2.03	<i>SCCPDH</i>	Saccharopine dehydrogenase (putative)
-2.04	<i>FNI</i>	Fibronectin 1
-2.04	<i>PDE1A</i>	Phosphodiesterase 1A, calmodulin-dependent
-2.04	<i>GPR126</i>	G protein-coupled receptor 126
-2.04	<i>GADD45A</i>	Growth arrest and DNA-damage-inducible, alpha
-2.04	<i>ST6GAL1</i>	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1
-2.04	<i>SLPI</i>	Secretory leukocyte peptidase inhibitor
-2.05	<i>HIST1H2AC</i>	Histone cluster 1, H2ac
-2.05	<i>COL4A6</i>	Collagen, type IV, alpha 6
-2.05	<i>ACSS3</i>	Acyl-CoA synthetase short-chain family member 3
-2.05	<i>TRNAL45P</i>	Transfer RNA leucine 45 (anticodon UAA) pseudogene
-2.05	<i>RHBDD1</i>	Rhomboid domain containing 1
-2.05	<i>HIST1H2AL</i>	Histone cluster 1, H2al; histone cluster 1, H2ah; histone cluster 1, H2ag; histone cluster 1, H2am; histone cluster 1, H2ak; histone cluster 1, H2ai
-2.05	<i>SEPP1</i>	Selenoprotein P, plasma, 1
-2.05	<i>PTPN14</i>	Protein tyrosine phosphatase, non-receptor type 14
-2.06	<i>PTPRD</i>	Protein tyrosine phosphatase, receptor type, D
-2.06	<i>C6orf48</i>	Chromosome 6 open reading frame 48
-2.06	<i>PSAT1</i>	Phosphoserine aminotransferase 1
-2.07	<i>NT5C2</i>	5'-nucleotidase, cytosolic II
-2.07	<i>PTPRB</i>	Protein tyrosine phosphatase, receptor type, B
-2.07	<i>SLC2A14</i>	Solute carrier family 2 (facilitated glucose transporter), member 14
-2.07	<i>ANP32A-IT1</i>	ANP32A intronic transcript 1 (non-protein coding)
-2.07	<i>RASSF8</i>	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8
-2.08	<i>IFRD1</i>	Interferon-related developmental regulator 1
-2.08	<i>SESN2</i>	Sestrin 2
-2.08	<i>ITGB5</i>	Integrin, beta 5
-2.08	<i>RORA</i>	RAR-related orphan receptor A
-2.08	<i>LPAR6</i>	Lysophosphatidic acid receptor 6
-2.08	<i>KLRC4</i>	Killer cell lectin-like receptor subfamily C, member 4
-2.08	<i>CTAGE5</i>	CTAGE family, member 5
-2.09	<i>SLC39A8</i>	Solute carrier family 39 (zinc transporter), member 8
-2.09	<i>LAMB1</i>	Laminin, beta 1
-2.09	<i>TNFRSF19</i>	Tumor necrosis factor receptor superfamily, member 19
-2.10	<i>ITGA6</i>	Integrin, alpha 6
-2.10	<i>RCN3</i>	Reticulocalbin 3, EF-hand calcium binding domain
-2.10	<i>GREM2</i>	Gremlin 2
-2.10	<i>LOXL2</i>	Lysyl oxidase-like 2
-2.10	<i>NRN1</i>	Neuritin 1
-2.11	<i>PDCFA</i>	Platelet-derived growth factor alpha polypeptide
-2.12	<i>PMAIP1</i>	Phorbol-12-myristate-13-acetate-induced protein 1
-2.12	<i>ZC3H6</i>	Zinc finger CCCH-type containing 6
-2.12	<i>ALPK2</i>	Alpha-kinase 2
-2.13	<i>ST3GAL1</i>	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
-2.13	<i>CEBPG</i>	CCAAT/enhancer binding protein (C/EBP), gamma

Table II. *Continued*

Table II. Continued

Fold change	Gene symbol	mRNA Description
-2.13	<i>RAB31</i>	RAB31, member RAS oncogene family
-2.13	<i>UNC13A</i>	Unc-13 homolog A (C. elegans)
-2.13	<i>HIST1H2BF</i>	Histone cluster 1, H2bf; histone cluster 1, H2bc; histone cluster 1, H2bi; histone cluster 1, H2be; histone cluster 1, H2bg
-2.13	<i>MME</i>	Membrane metallo-endopeptidase
-2.13	<i>TERF1P2</i>	Telomeric repeat binding factor (NIMA-interacting) 1 pseudogene 2
-2.14	<i>ANTXR1</i>	Anthrax toxin receptor 1
-2.14	<i>EML2</i>	Echinoderm microtubule associated protein like 2
-2.14	<i>SREBF2</i>	Sterol regulatory element binding transcription factor 2
-2.14	<i>TF</i>	Transferrin
-2.15	<i>ETV4</i>	Ets variant 4
-2.15	<i>F2RL1</i>	Coagulation factor II (thrombin) receptor-like 1
-2.16	<i>SOCS3</i>	Suppressor of cytokine signaling 3
-2.16	<i>LAMA1</i>	Laminin, alpha 1
-2.16	<i>ARRDC4</i>	Arrestin domain containing 4
-2.17	<i>RAB3B</i>	RAB3B, member RAS oncogene family
-2.17	<i>AGA</i>	Aspartylglucosaminidase
-2.17	<i>COL5A2</i>	Collagen, type V, alpha 2
-2.17	<i>IFITM1</i>	Interferon induced transmembrane protein 1
-2.18	<i>DGKD</i>	Diacylglycerol kinase, delta 130kDa
-2.18	<i>NRP2</i>	Neuropilin 2
-2.18	<i>ALS2CR8</i>	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8
-2.19	<i>CCDC68</i>	Coiled-coil domain containing 68
-2.19	<i>PPFIBP1</i>	PTPRF interacting protein, binding protein 1 (liprin beta.1)
-2.19	<i>MARS</i>	Methionyl-tRNA synthetase
-2.19	<i>RPS6KA2</i>	Ribosomal protein S6 kinase, 90kDa, polypeptide 2
-2.19	<i>COL3A1</i>	Collagen, type III, alpha 1
-2.20	<i>DEPDC1</i>	DEP domain containing 1
-2.20	<i>AKR1B10</i>	Aldo-keto reductase family 1, member B10 (aldose reductase)
-2.20	<i>LONP1</i>	Lon peptidase 1, mitochondrial
-2.20	<i>ARHGEF10</i>	Rho guanine nucleotide exchange factor (GEF) 10
-2.20	<i>SCARNA5</i>	Small Cajal body-specific RNA 5
-2.21	<i>BACE2</i>	Beta-site APP-cleaving enzyme 2
-2.22	<i>RFK</i>	Riboflavin kinase
-2.22	<i>HIST1H3D</i>	Histone cluster 1, H3d; histone cluster 1, H2ad; histone cluster 1, H3f; histone cluster 1, H3b; histone cluster 1, H3h; histone cluster 1, H3j; histone cluster 1, H3g; histone cluster 1, H3i; histone cluster 1, H3e; histone cluster 1, H3c; histone cluster 1, H3a
-2.22	<i>DOCK4</i>	Dedicator of cytokinesis 4
-2.22	<i>RNY3P2</i>	RNA, Ro-associated Y3 pseudogene 2
-2.23	<i>CEBPB</i>	CCAAT/enhancer binding protein (C/EBP), beta
-2.23	<i>LIF</i>	Leukemia inhibitory factor
-2.23	<i>PDLIM5</i>	PDZ and LIM domain 5
-2.24	<i>LRRFIP1</i>	Leucine rich repeat (in FLII) interacting protein 1
-2.24	<i>PCSK1</i>	Proprotein convertase subtilisin/kexin type 1
-2.25	<i>PYCR1</i>	Pyroline-5-carboxylate reductase 1
-2.25	<i>CRY1</i>	Cryptochrome 1 (photolyase-like)
-2.26	<i>PDE1C</i>	Phosphodiesterase 1C, calmodulin-dependent 70kDa
-2.26	<i>SOX9</i>	SRY (sex determining region Y)-box 9
-2.26	<i>SPDYE8P</i>	Speedy homolog E8 (Xenopus laevis), pseudogene
-2.27	<i>HIST1H3F</i>	Histone cluster 1, H3f; histone cluster 1, H3b; histone cluster 1, H3h; histone cluster 1, H3j; histone cluster 1, H3g; histone cluster 1, H3i; histone cluster 1, H3e; histone cluster 1, H3c; histone cluster 1, H3d; histone cluster 1, H3a
-2.27	<i>DUSP5</i>	Dual specificity phosphatase 5
-2.27	<i>PDK1</i>	Pyruvate dehydrogenase kinase, isozyme 1
-2.27	<i>PP7080</i>	Uncharacterized LOC25845
-2.28	<i>GALNTL1</i>	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1
-2.28	<i>FAM129A</i>	Family with sequence similarity 129, member A
-2.28	<i>ZBTB38</i>	Zinc finger and BTB domain containing 38
-2.28	<i>EPST11</i>	Epithelial stromal interaction 1 (breast)
-2.28	<i>CARS</i>	CysteinyI-tRNA synthetase

Table II. Continued

Table II. *Continued*

Fold change	Gene symbol	mRNA Description
-2.28	<i>WDR69</i>	WD repeat domain 69
-2.29	<i>GPR65</i>	G protein-coupled receptor 65
-2.30	<i>XYLT1</i>	Xylosyltransferase I
-2.30	<i>TCEA1</i>	Transcription elongation factor A (SII), 1
-2.30	<i>F2R</i>	Coagulation factor II (thrombin) receptor
-2.31	<i>CXCL5</i>	Chemokine (C-X-C motif) ligand 5
-2.31	<i>LOC100128252</i>	Uncharacterized LOC100128252
-2.31	<i>EGR1</i>	Early growth response 1
-2.32	<i>FRY</i>	Furry homolog (Drosophila)
-2.32	<i>CEP70</i>	Centrosomal protein 70kDa
-2.32	<i>SLC37A2</i>	Solute carrier family 37 (glycerol-3-phosphate transporter), member 2
-2.33	<i>TRMT2B</i>	TRNA methyltransferase 2 homolog B (<i>S. cerevisiae</i>)
-2.33	<i>CSGALNACT1</i>	Chondroitin sulfate N-acetylgalactosaminyltransferase 1
-2.33	<i>HIST3H2BB</i>	Histone cluster 3, H2bb
-2.34	<i>ZNF585B</i>	Zinc finger protein 585B
-2.34	<i>PCID2</i>	PCI domain containing 2
-2.34	<i>XBP1</i>	X-box binding protein 1
-2.34	<i>CDH13</i>	Cadherin 13, H-cadherin (heart)
-2.34	<i>GPR37</i>	G protein-coupled receptor 37 (endothelin receptor type B-like)
-2.35	<i>SERPINB9</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 9
-2.35	<i>GFPT1</i>	Glutamine--fructose-6-phosphate transaminase 1
-2.35	<i>SOCS2</i>	Suppressor of cytokine signaling 2
-2.35	<i>ZEB2</i>	Zinc finger E-box binding homeobox 2
-2.35	<i>IGFBP3</i>	Insulin-like growth factor binding protein 3
-2.35	<i>LCN1</i>	Lipocalin 1
-2.36	<i>TTC32</i>	Tetratricopeptide repeat domain 32
-2.36	<i>COL4A5</i>	Collagen, type IV, alpha 5
-2.36	<i>RN5S353</i>	RNA, 5S ribosomal 353
-2.37	<i>ZFP36</i>	Zinc finger protein 36, C3H type, homolog (mouse)
-2.37	<i>PLEKHG2</i>	Pleckstrin homology domain containing, family G (with RhoGef domain) member 2
-2.37	<i>DUSP5P</i>	Dual specificity phosphatase 5 pseudogene
-2.38	<i>CRLF2</i>	Cytokine receptor-like factor 2
-2.38	<i>LRRC63</i>	Leucine rich repeat containing 63
-2.38	<i>SLITRK4</i>	SLIT and NTRK-like family, member 4
-2.39	<i>ITPRIP</i>	Inositol 1,4,5-trisphosphate receptor interacting protein
-2.40	<i>OCN</i>	Occludin; occludin pseudogene
-2.40	<i>PLAU</i>	Plasminogen activator, urokinase
-2.40	<i>KIAA0825</i>	KIAA0825
-2.41	<i>OSMR</i>	Oncostatin M receptor
-2.41	<i>LAT2</i>	Linker for activation of T cells family, member 2
-2.41	<i>SLC25A37</i>	Solute carrier family 25 (mitochondrial iron transporter), member 37
-2.42	<i>EDN1</i>	Endothelin 1
-2.42	<i>THSD4</i>	Thrombospondin, type 1, domain containing 4
-2.43	<i>PLA2R1</i>	Phospholipase A2 receptor 1, 180kDa
-2.43	<i>CTIF</i>	CBP80/20-dependent translation initiation factor
-2.43	<i>CEP72</i>	Centrosomal protein 72kDa
-2.43	<i>BMP6</i>	Bone morphogenetic protein 6
-2.45	<i>PCK2</i>	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)
-2.45	<i>DTWD1</i>	DTW domain containing 1
-2.45	<i>THBS1</i>	Thrombospondin 1
-2.45	<i>PABPC1L</i>	Poly(A) binding protein, cytoplasmic 1-like
-2.45	<i>AGXT2L2</i>	Alanine-glyoxylate aminotransferase 2-like 2
-2.45	<i>CPED1</i>	Cadherin-like and PC-esterase domain containing 1
-2.45	<i>FCDHBI7</i>	Protocadherin beta 17 pseudogene
-2.46	<i>TCEA1</i>	Transcription elongation factor A (SII), 1
-2.46	<i>ASNS</i>	Asparagine synthetase (glutamine-hydrolyzing)
-2.47	<i>FLNB</i>	Filamin B, beta
-2.47	<i>MCL1</i>	Myeloid cell leukemia sequence 1 (BCL2-related)
-2.47	<i>SPDYE8P</i>	Speedy homolog E8 (<i>Xenopus laevis</i>), pseudogene
-2.47	<i>NCAM1</i>	Neural cell adhesion molecule 1

Table II. *Continued*

Table II. Continued

Fold change	Gene symbol	mRNA Description
-2.48	<i>RSPO3</i>	R-spondin 3
-2.48	<i>SLC2A13</i>	Solute carrier family 2 (facilitated glucose transporter), member 13
-2.48	<i>SLC7A1</i>	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1
-2.48	<i>DUSP4</i>	Dual specificity phosphatase 4
-2.48	<i>CLK1</i>	CDC-like kinase 1
-2.48	<i>CRELD1</i>	Cysteine-rich with EGF-like domains 1
-2.48	<i>TRNAQ52P</i>	Transfer RNA glutamine 52 (anticodon UUG) pseudogene
-2.49	<i>RN5S506</i>	RNA, 5S ribosomal 506
-2.49	<i>SEMA3C</i>	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C
-2.49	<i>SSFA2</i>	sperm specific antigen 2
-2.49	<i>LOC344887</i>	NmrA-like family domain containing 1 pseudogene
-2.50	<i>SLC4A7</i>	Solute carrier family 4, sodium bicarbonate cotransporter, member 7
-2.50	<i>PDCD1LG2</i>	Programmed cell death 1 ligand 2
-2.50	<i>P4HA1</i>	Prolyl 4-hydroxylase, alpha polypeptide I
-2.51	<i>FAM27E3</i>	Family with sequence similarity 27, member E3; family with sequence similarity 27, member E2
-2.53	<i>KIAA0825</i>	KIAA0825
-2.53	<i>EFEMP1</i>	EGF containing fibulin-like extracellular matrix protein 1
-2.53	<i>FAM27E3</i>	Family with sequence similarity 27, member E3; family with sequence similarity 27, member E2
-2.53	<i>MIR21</i>	MicroRNA 21
-2.53	<i>GLRXP3</i>	Glutaredoxin (thioltransferase) pseudogene 3
-2.54	<i>GDF15</i>	Growth differentiation factor 15
-2.54	<i>LURAP1L</i>	Leucine rich adaptor protein 1-like
-2.55	<i>PRDM1</i>	PR domain containing 1, with ZNF domain
-2.55	<i>RNF19A</i>	Ring finger protein 19A, E3 ubiquitin protein ligase
-2.55	<i>SLC4A11</i>	Solute carrier family 4, sodium borate transporter, member 11
-2.56	<i>NR4A1</i>	Nuclear receptor subfamily 4, group A, member 1
-2.56	<i>SEMA3A</i>	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A
-2.56	<i>MIF4GD</i>	MIF4G domain containing
-2.56	<i>ANGPTL4</i>	Angiopoietin-like 4
-2.57	<i>FGG</i>	Fibrinogen gamma chain
-2.57	<i>ADAM19</i>	ADAM metallopeptidase domain 19
-2.57	<i>ITGA3</i>	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
-2.59	<i>SNRPA1</i>	Small nuclear ribonucleoprotein polypeptide A'
-2.59	<i>FBXL13</i>	F-box and leucine-rich repeat protein 13
-2.59	<i>ADAMTS6</i>	ADAM metallopeptidase with thrombospondin type 1 motif, 6
-2.59	<i>GPRIN3</i>	GPRIN family member 3
-2.59	<i>STC2</i>	Stanniocalcin 2
-2.60	<i>FAM27E3</i>	Family with sequence similarity 27, member E3; family with sequence similarity 27, member E2
-2.60	<i>IL18R1</i>	Interleukin 18 receptor 1
-2.60	<i>SUCNR1</i>	Succinate receptor 1
-2.61	<i>BTG1</i>	B-cell translocation gene 1, anti-proliferative
-2.61	<i>PLAT</i>	Plasminogen activator, tissue
-2.62	<i>CCPG1</i>	Cell cycle progression 1; DYX1C1-CCPG1 readthrough (non-protein coding)
-2.62	<i>GDPD1</i>	Glycerophosphodiester phosphodiesterase domain containing 1
-2.63	<i>HOXB9</i>	Homeobox B9
-2.64	<i>CD55</i>	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
-2.64	<i>LAMB3</i>	Laminin, beta 3
-2.65	<i>PSD3</i>	Pleckstrin and Sec7 domain containing 3
-2.65	<i>SLC35E3</i>	Solute carrier family 35, member E3
-2.67	<i>ODZ2</i>	Odd Oz/ten-m homolog 2 (Drosophila)
-2.68	<i>FLJ44124</i>	Uncharacterized LOC641737
-2.71	<i>SLCO5A1</i>	Solute carrier organic anion transporter family, member 5A1
-2.72	<i>FOSL2</i>	FOS-like antigen 2
-2.72	<i>CTBS</i>	Chitinase, di-N-acetyl-
-2.73	<i>FBN2</i>	Fibrillin 2
-2.73	<i>SLC9A3</i>	Solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3
-2.74	<i>THSD7A</i>	Thrombospondin, type I, domain containing 7A
-2.76	<i>FSTL1</i>	Follistatin-like 1
-2.76	<i>PPAP2B</i>	Phosphatidic acid phosphatase type 2B
-2.77	<i>IL8</i>	Interleukin 8

Table II. Continued

Table II. *Continued*

Fold change	Gene symbol	mRNA Description
-2.77	<i>JHDM1D</i>	Jumonji C domain containing histone demethylase 1 homolog D (<i>S. cerevisiae</i>)
-2.77	<i>PSD3</i>	Pleckstrin and Sec7 domain containing 3
-2.78	<i>TFPI2</i>	Tissue factor pathway inhibitor 2
-2.79	<i>PTPRM</i>	Protein tyrosine phosphatase, receptor type, M
-2.80	<i>SERPINB1</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 1
-2.82	<i>PAPPA2</i>	Pappalysin 2
-2.85	<i>TUBE1</i>	Tubulin, epsilon 1
-2.86	<i>PHLDA1</i>	Pleckstrin homology-like domain, family A, member 1
-2.86	<i>RGS2</i>	Regulator of G-protein signaling 2, 24kDa
-2.86	<i>OLFML2A</i>	olfactomedin-like 2A
-2.87	<i>SLC7A2</i>	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2
-2.89	<i>LAMA5</i>	Laminin, alpha 5
-2.91	<i>EDIL3</i>	EGF-like repeats and discoidin I-like domains 3
-2.91	<i>TRPC6</i>	Transient receptor potential cation channel, subfamily C, member 6
-2.91	<i>ULBP1</i>	UL16 binding protein 1
-2.92	<i>VEGFA</i>	Vascular endothelial growth factor A
-2.92	<i>LOC284861</i>	Uncharacterized LOC284861
-2.94	<i>BDNF</i>	Brain-derived neurotrophic factor
-2.95	<i>ABCC3</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
-2.97	<i>FHAD1</i>	Forkhead-associated (FHA) phosphopeptide binding domain 1
-2.99	<i>DDIT4</i>	DNA-damage-inducible transcript 4
-3.02	<i>ANTXR2</i>	Anthrax toxin receptor 2
-3.03	<i>HERPUD1</i>	Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1
-3.04	<i>DPP10</i>	Dipeptidyl-peptidase 10 (non-functional)
-3.06	<i>BEST1</i>	Bestrophin 1
-3.06	<i>TMEM144</i>	Transmembrane protein 144
-3.06	<i>OGT</i>	O-linked N-acetylglucosamine (GlcNAc) transferase
-3.07	<i>UNC5B</i>	Unc-5 homolog B (<i>C. elegans</i>)
-3.07	<i>GLRX</i>	Glutaredoxin (thioltransferase)
-3.07	<i>SPOCK1</i>	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1
-3.08	<i>SLC6A9</i>	Solute carrier family 6 (neurotransmitter transporter, glycine), member 9
-3.10	<i>HSPA13</i>	Heat shock protein 70kDa family, member 13
-3.11	<i>BHLHE40</i>	Basic helix-loop-helix family, member e40
-3.13	<i>CYR61</i>	Cysteine-rich, angiogenic inducer, 61
-3.15	<i>DUSP1</i>	Dual specificity phosphatase 1
-3.16	<i>LSAMP</i>	Limbic system-associated membrane protein
-3.18	<i>CDH19</i>	Cadherin 19, type 2
-3.19	<i>GTPBP2</i>	GTP binding protein 2
-3.20	<i>CD36</i>	CD36 molecule (thrombospondin receptor)
-3.20	<i>HPGDS</i>	Hematopoietic prostaglandin G synthase
-3.20	<i>CLDN1</i>	Claudin 1
-3.20	<i>NRCAM</i>	Neuronal cell adhesion molecule
-3.21	<i>PITPNC1</i>	Phosphatidylinositol transfer protein, cytoplasmic 1
-3.27	<i>DLG2</i>	Discs, large homolog 2 (<i>Drosophila</i>)
-3.28	<i>PRKAA2</i>	Protein kinase, AMP-activated, alpha 2 catalytic subunit
-3.29	<i>ALDH1L2</i>	Aldehyde dehydrogenase 1 family, member L2
-3.30	<i>PDE3A</i>	Phosphodiesterase 3A, cGMP-inhibited
-3.30	<i>STARD4</i>	STAR-related lipid transfer (START) domain containing 4
-3.31	<i>NR1D1</i>	Nuclear receptor subfamily 1, group D, member 1
-3.33	<i>SLC25A37</i>	Solute carrier family 25 (mitochondrial iron transporter), member 37
-3.35	<i>H1FO</i>	H1 histone family, member 0
-3.37	<i>RAB39B</i>	RAB39B, member RAS oncogene family
-3.39	<i>CLGN</i>	Calmegin
-3.40	<i>IL6</i>	Interleukin 6 (interferon, beta 2)
-3.40	<i>VLDLR</i>	Very low density lipoprotein receptor
-3.43	<i>SMOX</i>	Spermine oxidase
-3.43	<i>SCARA5</i>	Scavenger receptor class A, member 5 (putative)
-3.45	<i>PLAUR</i>	Plasminogen activator, urokinase receptor
-3.47	<i>B4GALT1</i>	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1
-3.48	<i>TSPAN8</i>	Tetraspanin 8

Table II. *Continued*

Table II. *Continued*

Fold change	Gene symbol	mRNA Description
-3.49	<i>APLN</i>	Apelin
-3.51	<i>DUSP6</i>	Dual specificity phosphatase 6
-3.53	<i>PRICKLE1</i>	Prickle homolog 1 (Drosophila)
-3.53	<i>NR4A2</i>	Nuclear receptor subfamily 4, group A, member 2
-3.59	<i>CBS</i>	Cystathionine-beta-synthase
-3.59	<i>LINC00173</i>	Long intergenic non-protein coding RNA 173
-3.61	<i>TNFAIP3</i>	Tumor necrosis factor, alpha-induced protein 3
-3.62	<i>SHISA3</i>	Shisa homolog 3 (Xenopus laevis)
-3.62	<i>EPHA4</i>	EPH receptor A4
-3.70	<i>TSC22D3</i>	TSC22 domain family, member 3
-3.70	<i>KLRC3</i>	Killer cell lectin-like receptor subfamily C, member 3; killer cell lectin-like receptor subfamily C, member 2
-3.71	<i>TFPI</i>	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
-3.75	<i>CXCL2</i>	Chemokine (C-X-C motif) ligand 2
-3.82	<i>ANXA3</i>	Annexin A3
-3.84	<i>SLC16A4</i>	Solute carrier family 16, member 4 (monocarboxylic acid transporter 5)
-3.84	<i>KLRC4-KLRK1</i>	KLRC4-KLRK1 readthrough; killer cell lectin-like receptor subfamily K, member 1
-3.90	<i>PLAC4</i>	Placenta-specific 4
-3.97	<i>ABCC9</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
-4.03	<i>TMEM27</i>	Transmembrane protein 27
-4.03	<i>STC1</i>	Stanniocalcin 1
-4.05	<i>SEMA3D</i>	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D
-4.05	<i>TRIB3</i>	Tribbles homolog 3 (Drosophila)
-4.06	<i>CTGF</i>	Connective tissue growth factor
-4.09	<i>NUPR1</i>	Nuclear protein, transcriptional regulator, 1
-4.10	<i>ABI3BP</i>	ABI family, member 3 (NESH) binding protein
-4.21	<i>FBXO32</i>	F-box protein 32
-4.25	<i>RPE65</i>	Retinal pigment epithelium-specific protein 65kDa
-4.35	<i>PARK2</i>	Parkinson protein 2, E3 ubiquitin protein ligase (parkin)
-4.38	<i>C3orf49</i>	Chromosome 3 open reading frame 49
-4.48	<i>WARS</i>	Tryptophanyl-tRNA synthetase
-4.50	<i>SAT1</i>	Spermidine/spermine N1-acetyltransferase 1
-4.51	<i>DDIT3</i>	DNA-damage-inducible transcript 3
-4.52	<i>TCP11L2</i>	T-complex 11 (mouse)-like 2
-4.65	<i>KCNE4</i>	Potassium voltage-gated channel, Isk-related family, member 4
-4.65	<i>MIR29A</i>	MicroRNA 29a
-4.66	<i>ERRF1</i>	ERBB receptor feedback inhibitor 1
-4.67	<i>AREG</i>	Amphiregulin
-4.70	<i>AREG</i>	Amphiregulin
-4.80	<i>CTH</i>	Cystathionase (cystathionine gamma-lyase)
-4.89	<i>PAPPA</i>	Pregnancy-associated plasma protein A, pappalysin 1
-4.89	<i>ESM1</i>	Endothelial cell-specific molecule 1
-4.97	<i>C12orf39</i>	Chromosome 12 open reading frame 39
-5.03	<i>TXNIP</i>	Thioredoxin interacting protein
-5.06	<i>SMOC1</i>	SPARC related modular calcium binding 1
-5.10	<i>ASS1P11</i>	Argininosuccinate synthetase 1 pseudogene 11
-5.23	<i>LCN1</i>	Lipocalin 1
-5.39	<i>ASS1</i>	Argininosuccinate synthase 1
-5.44	<i>CHAC1</i>	ChaC, cation transport regulator homolog 1 (E. coli)
-5.47	<i>CHL1</i>	Cell adhesion molecule with homology to L1CAM (close homolog of L1)
-6.06	<i>GABRE</i>	Gamma-aminobutyric acid (GABA) A receptor, epsilon; microRNA 452; microRNA 224
-6.39	<i>FSTL4</i>	Follistatin-like 4
-6.44	<i>CD274</i>	CD274 molecule
-7.68	<i>TGFB2</i>	Transforming growth factor, beta 2
-7.75	<i>GABRE</i>	Gamma-aminobutyric acid (GABA) A receptor, epsilon; microRNA 452; microRNA 224
-8.71	<i>SERPINB11</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 11 (gene/pseudogene)
-9.43	<i>FST</i>	Follistatin
-9.94	<i>SLC2A3</i>	Solute carrier family 2 (facilitated glucose transporter), member 3
-10.77	<i>GPR133</i>	G protein-coupled receptor 133
-21.56	<i>IL1RL1</i>	Interleukin 1 receptor-like 1

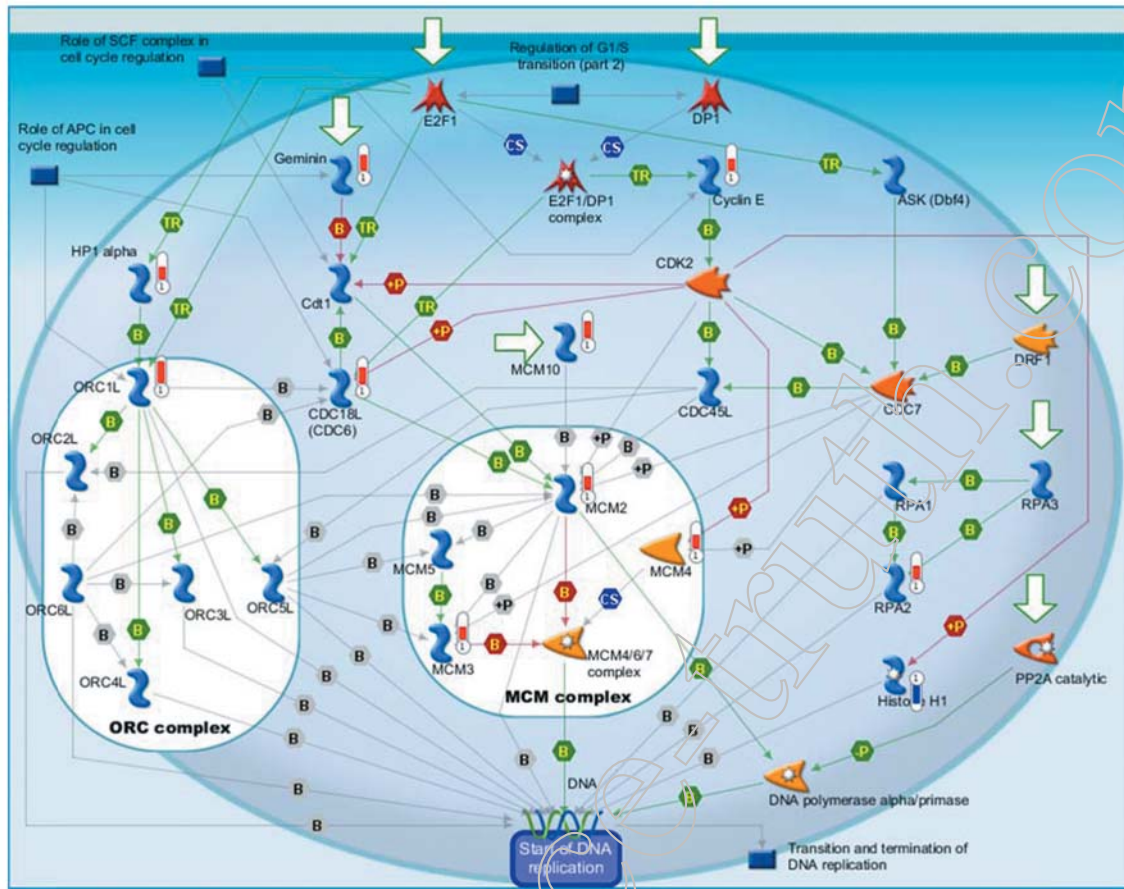


Figure 1. Cell cycle: Start of DNA replication. The top scored map (map-with the lowest p-value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files are linked to and visualized on the maps as thermometer like figures. Up ward thermometers have red color and indicate up regulated signals and down ward (blue) ones indicate down regulated expression levels of the genes.

cycle and 255 genes were down-regulated from 2 to 3 folds such as growth arrest and DNA-damage-inducible, alpha (*GADD45A*), DNA-damage-inducible transcript 4 (*DDIT4*), DNA-damage-inducible transcript 3 (*DDIT3*), cyclin-dependent kinase 17 (*CDK17*), CDC-like kinase 1 (*CLK1*), and cell cycle progression 1, *DYX1C1*-*CCPG1* read through (*CCPG1*) associated with cell cycle, tumor necrosis factor receptor superfamily, member 19 (*TNFRSF19*), early growth response 1 (*EGR1*), programmed cell death 1 ligand 2 (*PDCD1LG2*), and ATP-binding cassette, sub-family C (*CFTR/MRP*), member 3 (*ABCC3*) and member 9 (*ABCC9*), intercellular adhesion molecule 1 (*ICAM1*), Ras association (*RalGDS/AF-6*) domain family (N-terminal) member 8 (*RASSF8*), Rho guanine nucleotide exchange factor 10 (*ARHGEF10*) and cell adhesion molecule with homology to L1CAM (close homolog of L1) (*CHL1*) associated with cell migration.

BDMC affected the gene expression score measured by *GeneGo* analysis program in *NCI-H460* cells by the number of

pathway networks. After total mRNA was isolated from *BDMC*-treated or -untreated cells and used for cDNA microarrays, the analysis was followed with further processing by *GeneGo* and the results are shown in Figures 1, 2 and 3. Figure 1 indicates that *BDMC* affected associated gene expression with cell cycle that starts from DNA replication in the early S phase. For example, *BDMC* up-regulates *cyclin E* and *CDC6* but down-regulates *CDK2* and *CDC7*, leading to affect the start of DNA replication in *NCI-H460* cells. Figure 2 indicates that *BDMC* affected associated gene expression in development with TGF-beta-dependent induction of EMT *via* SMADs. For example, TGF-beta receptor type 1 and II were up-regulated by *BDMC* but *SNAIL*, *SIP1* and *MMP-2* were down-regulated affecting cell motility, adhesion and epithelial-to-mesenchymal transition in *NCI-H460* cells. Figure 3 indicates that *BDMC* affected gene expression, which associates with targets of tissue factor signaling in cancer. *BDMC* affected angiogenesis, inhibition of apoptosis, cell migration and invasion, leading to tumor progression.

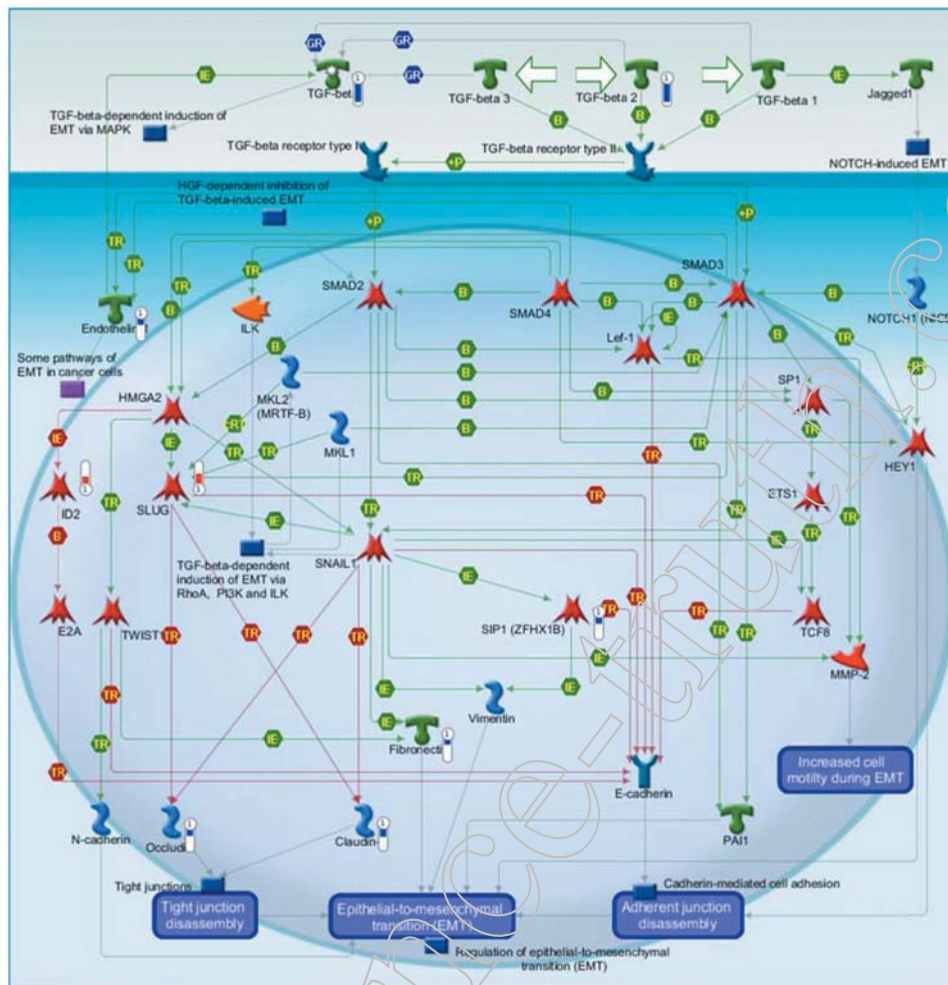


Figure 2. Development: TGF beta dependent induction of EMT via SMADs. The second top-scored map (map with the second lowest p-value) based on enrichment distribution, sorted by 'Statistically significant Maps' set. Experimental data from all files are linked to and visualized on the maps as thermometer like figures. Up ward thermometers have red color and indicate up regulated signals and down ward (blue) ones indicate down regulated expression levels of the genes.

Discussion

Several reports have shown that BDMC induces cytotoxic effects in human cancer cells *in vitro*, however, there is no available information on how BDMC affects gene expression and associated signaling pathways in human lung cancer cells. In the present study, we examined whether BDMC induced up- or down-regulation of genes associated with cell cycle, DNA replication, cell survival, cell migration and invasion and tumor progression in NCI H460 cells.

Table 1 indicates that *ERCC6L*, a gene associated with DNA damage and repair, was increased by 3.58-fold, four genes *CDC6*, *CDCA5*, *CDC25A* and *CDCA7L* associated with cell division, were increased by 2.98-, 2.74-, 2.62- and 2.22-fold, respectively, three genes *SKP2*, *CABLES2* and

CABLES1 associated with cell cycle, were increased by 2.51, 2.39, and 2.17 times respectively, three genes *CARD6*, *ATP6V0D1* and *CASP8AP2*, associated with cell death, were found increased by 2.23-, 2.13- and 2.07-times, respectively.

Table II demonstrates that BDMC suppressed expression of numerous genes associated with DNA damage, cell cycle, cell survival and cell migration and invasion. In particular, three genes *DDIT3*, *DDIT4* and *GADD45A* associated with DNA damage, were decreased by 4.51-, 2.99- and 2.04-times, respectively, three genes *CDK17*, *CLK1* and *CCPG1*, associated with cell cycle, were found decreased by 2.03-, 2.48- and 2.62-times, respectively, five genes *TNFRSF19*, *EGR1*, *PDCD1LG2*, *ABCC3* and *ABCC9* associated with cell death, were decreased 2.09-, 2.31-, 2.50-, 2.95- and 3.97-fold, respectively, four genes *ICAM1*, *RASSF8*, *ARHGEF10*

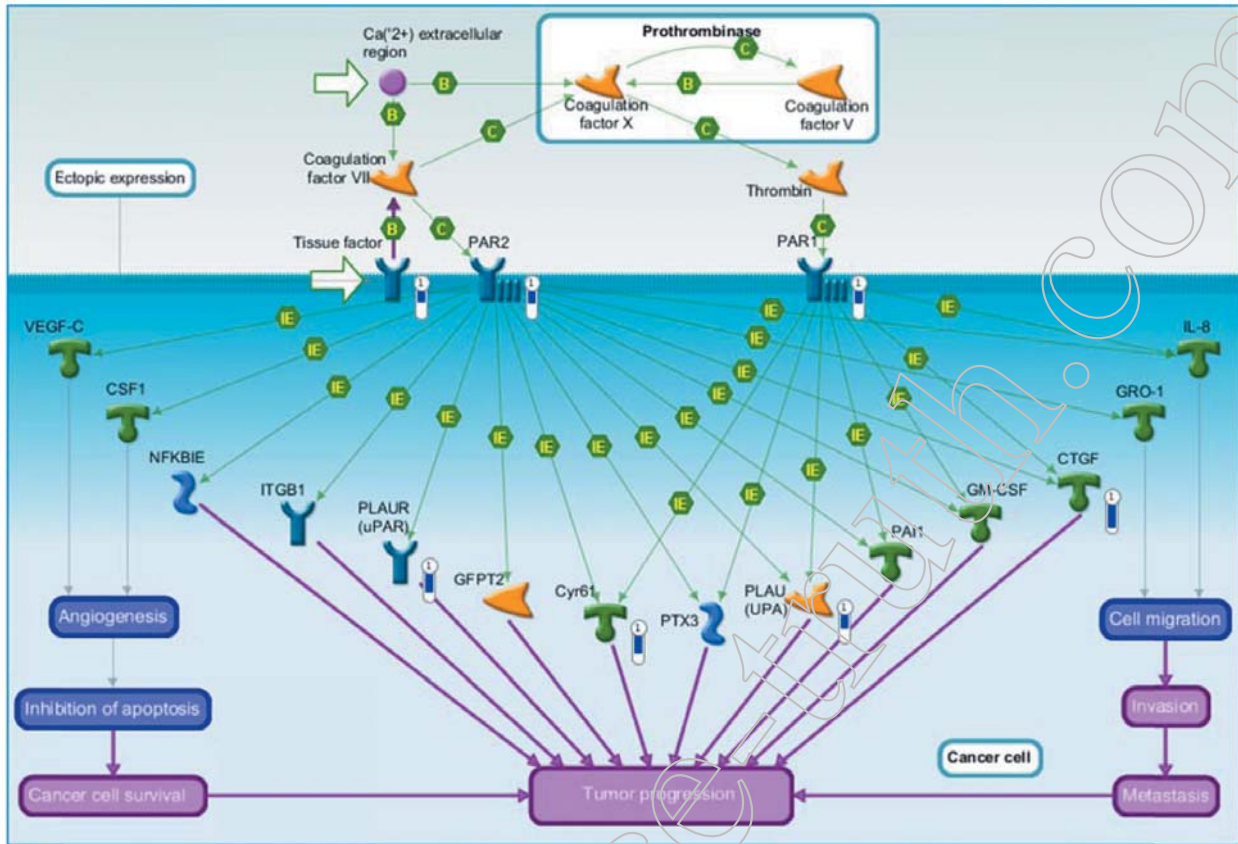


Figure 3. Expression targets of Tissue factor signaling in cancer. The third top-scored map (map with the third lowest *p* value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files are linked to and visualized on the maps as thermometer like figures. Up ward thermometers have red color and indicate up regulated signals and down ward (blue) ones indicate down regulated expression levels of the genes.

and *CHL1*, associated with cell migration, were decreased 2.02-, 2.07-, 2.20-, and 5.47-fold, respectively.

It is well-documented that cell-cycle regulation is the key mechanism of cancer cell growth (28, 29). Results from Figure 1 show that BDMC up-regulated cyclin E and CDC6, however, down-regulated CDK2 and CDC7 affecting the start of DNA replication in NCI-H460 cells, and in turn affecting S phase. It has been reported that cyclin E/Cdk2 complex is involved in G₁-S transition and is also associated with initiation of DNA synthesis. Furthermore, the cyclin A/Cdk2 complex is associated with the initiation of DNA synthesis and is also associated with the progression to S phase (30). It has also been reported that increase in Cdc7 and/or Dbf4 can arrest cells in G₁ phase, or slow down S-phase progression when cells are already in S phase (31). Furthermore, it has also been reported that Cdc6 and Mcm proteins are required to establish pre-replicative complex and the activities of Cdks and of Cdc7 kinase for triggering the G₁-S transition (32).

Results from Figure 2 demonstrate that BDMC up-regulated the transforming growth factor-beta (TGF-β) receptor type 1 and II, however down-regulated SNAIL, SIP1 and MMP-2 that were associated with cell motility, adhesion and epithelial-to-mesenchymal transition in NCI-H460 cells. It is well-known that TGF-β ligands play an important role in cell proliferation, extracellular matrix production, cell motility and apoptosis (33). It has also been reported that in the mammary gland of transgenic mice, if there was overexpression of active TGF-β1 or an activated type I TGF-β receptor (TβRI), it can lead to accelerated metastases derived from Neu-induced mammary tumors (34, 35).

The zinc-finger transcription factors Snail and SIP1 (Smad interacting protein 1) have been demonstrated to repress transcription of the E-cadherin (E-cad) gene by binding to E-boxes (CACCTG sequence) on the E-cad promoter (36, 37). E-cad plays a major role in the establishment and maintenance of intercellular adhesion, cell polarity, and tissue architecture (38, 39). Furthermore, matrix metalloproteinase

(MMP-1, MMP-2, MMP-7), and MT1-MMP expressions are strongly upregulated by Snail (40). Results from Figure 3 indicate that BDMC affects angiogenesis, inhibition of apoptosis, cell migration and invasion, leading to tumor progression. Thus, further studies are required to expand or append our current findings and possible understanding of them. In the present study, the genes affected by BDMC *in vitro* may offer certain insight on the cytotoxic mechanism of BDMC in the genetic level, which in turn may provide potentially useful biomarkers or targets for diagnosis and treatment of human lung cancer.

Acknowledgements

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