Abstract. Cantharidin (CTD) induces cytotoxic effects in different types of human cancer cell; however, to date, there have been no studies on the effects of CTD on gene expression in human lung cancer cells and the potential associated signaling pathways. Therefore, the present study aimed to investigate how CTD affects the expression of key genes and functional pathways of human H460 lung cancer cells using complementary DNA microarray analysis. Human H460 lung cancer cells were cultured for 24 h in the presence or absence of 10 µM CTD; gene expression was then examined using microarray analysis. The results indicated that 8 genes were upregulated > 4 -fold, 29 genes were upregulated >3 -4-fold and 156 genes were upregulated >2-3-fold. In addition, 1 gene was downregulated >4 fold, 14 genes were downregulated >3-4-fold and 150 genes were downregulated >2-3 fold in H460 cells following exposure to CTD. It was found that CTD affected DNA damage genes, including DNIT3 and GADD45A, which were upregulated 2.26- and 2.60-fold, respectively, as well as DdiT4, which was downregulated 3.14-fold. In addition, the expression of genes associated with the cell cycle progression were altered, including CCND2, CDKL3 and RASA4, which were upregulated 2.72-, 2.19- and 2.72-fold, respectively; however, CDC42EP3 was downregulated 2.16-fold. Furthermore, apoptosis-associated genes were differentially expressed, including CARD6, which was upregulated 3.54-fold. In conclusion, the present study demonstrated that CTD affected the expression of genes associated with DNA damage, cell cycle progression and apoptotic cell death in human lung cancer H460 cells.

Introduction

Lung cancer accounts for ~28% of cancer-associated mortalities (1), the occurrence of which is increasing worldwide. There are ~1.2 million novel cases of lung cancer and ~1 million mortalities from lung cancer each year (2). Lung cancer may be subdivided into small cell lung carcinoma and non-small cell lung carcinoma (NSCLC). The majority of lung cancer diagnoses are NSCLC (3,4), which has a five-year survival rate of ~33% (5). At present, the standard treatment for patients with resectable stage I to IIIA NSCLC is surgical excision; however, the prognosis remains poor (6). In addition, chemotherapy with or without surgery is not effective in the majority of cases; therefore, it is essential to identify novel compounds, including natural products, which may be employed for the treatment of lung cancer.

Cantharidin (CTD) is a component of mylabris (blister beetle), which has previously been used as a Traditional Chinese Medicine (7). Previous studies have reported that CTD induced cytotoxic effects in leukemia stem cells (8) as well as U937 (9), pancreatic cancer (10), hepatocellular carcinoma (11,12), colon cancer (13) and human lung cancer A549 (14) cells. In addition, CTD was found to inhibit the activity of protein phosphatase 2A (PP2A) (9) and heat shock factor 1 (HSF1) (15). Furthermore, it was shown that CTD induced cell death in human colorectal cancer cells, which was suggested to proceed through inhibiting the binding of heat shock protein 70 (HSP70), B cell lymphoma 2-associated anathogene domain 3 (BAG3) and HSF1 to promoters (15).
Genetic mutations in oncogenes and tumor suppressor genes are present in cancer cells (16,17). The development of cancer cells is well-known to be dependent on oncogenes for tumor initiation and progression; this concept has therefore been named oncogene addiction (18). Oncogenes are commonly used as targets for drug-screening programs (19); however, other signaling pathways have also been examined, such as the molecular chaperone pathway (20). The present study aimed to investigate the effect of CTD on the expression of key genes and functional pathways of human H460 lung cancer cells using complementary DNA microarray analysis. The results of the present study showed that CTD affected DNA damage, the cell cycle and the expression of apoptosis-associated genes in vitro. Differentially expressed genes were then used to generate interaction maps of signaling pathways. The epidermal growth factor and vascular endothelial growth factor receptor pathways, provided by the present study may be useful for the development of novel molecular targeted therapies against lung cancer (21).

Materials and methods

Chemicals and reagents. Cantharidin (CTD), propidium iodide and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Minimum essential medium (MEM), fetal bovine serum (FBS), L-glutamine and penicillin-streptomycin were purchased from Gibco-BRL (Carlsbad, CA, USA). CTD was dissolved in DMSO and stored at 20˚C.

Lung cancer cell culture. The NCI-H460 human lung cancer cell line was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were grown in MEM containing 10% (v/v) FBS as well as 100 U/ml penicillin and 100 µg/ml streptomycin in a 37˚C humidified incubator with 5% CO₂. Cells were then subcultured once they reached 80-90% confluence, as previously described (22).

Complementary (c)DNA microarray assay. H460 cells were placed on 12-well plates at a density of 5x10⁴ cells/well in 2 ml MEM with 10% (v/v) FBS and 2 mM L-glutamine, as well as 100 U/ml penicillin and 100 µg/ml streptomycin for 24 h. Subsequently, cells were treated with or without 10 µM CTD for a further 24 h. Cells (3x10⁵) were then harvested and washed twice with phosphate-buffered saline (Gibco-BRL). Cells were lysed in TRIzol® (Invitrogen Life Technologies, Carlsbad, CA, USA) and total RNA was extracted using a Qiagen RNeasy Mini kit (Qiagen, Valencia, CA, USA). RNA concentrations were determined using a Qubit™ Fluorocytometer (Invitrogen Life Technologies).

Total RNA of CTD-treated and untreated H460 cells was used for cDNA synthesis. Samples were hybridized using an Affymetrix GeneChip Human Gene 1.0 ST array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. Sample fluorescence was quantified by Asia Biotech Corporation (Taipei, Taiwan) after the arrays were dried and baked, as previously described (22).

Results

Upregulated and downregulated gene expression in H460 cells exposed to CTD. H460 cells were incubated in the presence or absence of 10 µM CTD in a 12-well plate for 24 h. Cells were then harvested and the extraction of total RNA, RNA concentrations were determined and cDNA microarray analysis was performed in order to determine the expression of genes. The calculated upregulation and downregulation of gene expression, as determined by the microarray, are shown in Tables I and II, respectively. As shown in Table I, the results indicated that in CTD-treated H460 cells, 8 genes were upregulated >4-fold, 29 genes were upregulated >3-4-fold and 156 genes were upregulated >2-3-fold compared with expression levels in the untreated control cells. In addition, Table II indicated that one gene was downregulated >4 fold, 14 genes were downregulated >3-4 fold and 150 genes were downregulated >2-3 fold in H460 cells following exposure to CTD compared with those in the untreated control cells. The results presented in Table I demonstrated that genes associated with DNA damage, including DN1T3 and GADD45A were upregulated by 2.26- and 2.60-fold, respectively; in addition, the expression of genes associated with the cell cycle progression (checkpoint proteins) were upregulated, including CCND2, CDKL3 and RASA4, which were upregulated 2.72-, 2.19- and 2.72-fold, respectively. Furthermore, the
Table I. Upregulation of gene expression in catharidine-treated NCI-H460 cells.

| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|------------------|--------------------|
| 8108370      | 16.50       | EGR1        | Early growth response 1 | NM_001964          |
| 8012949      | 13.05       | CDRT1       | CMT1A duplicated region transcript 1 | NM_006382          |
| 8012951      | 10.85       | CDRT1       | CMT1A duplicated region transcript 1 | NM_006382          |
| 7916609      | 8.05        | JUN         | Jun oncogene       | NM_002228          |
| 7977075      | 6.45        | SNORA28     | Small nucleolar RNA, H/ACA box 28 | NR_002964          |
| 8041168      | 5.54        | SNORD53     | Small nucleolar RNA, C/D box 53 | NR_002741          |
| 7982084      | 5.06        | SNORD115-11 | Small nucleolar RNA, C/D box 115-11 | NR_003303          |
| 8097991      | 4.27        | TDO2        | Tryptophan 2,3-dioxygenase | NM_005651          |
| 8114468      | 3.99        | SNORD63     | Small nucleolar RNA, C/D box 63 | NR_002913          |
| 8158862      | 3.87        | SNORD62A    | Small nucleolar RNA, C/D box 62A | NR_002914          |
| 8158864      | 3.87        | SNORD62A    | Small nucleolar RNA, C/D box 62A | NR_002914          |
| 8007420      | 3.85        | AOC3        | Amine oxidase, copper-containing 3 (vascular adhesion protein 1) | NM_003734          |
| 7975779      | 3.81        | FOS         | FBJ murine osteosarcoma viral oncogene homolog | NM_005252          |
| 8001746      | 3.74        | SNORA46     | Small nucleolar RNA, H/ACA box 46 | NR_002978          |
| 7914322      | 3.70        | SNORD103A   | Small nucleolar RNA, C/D box 103A | NR_004054          |
| 7914324      | 3.70        | SNORD103A   | Small nucleolar RNA, C/D box 103A | NR_004054          |
| 793342       | 3.69        | PTEN20A     | Protein tyrosine phosphatase, non-receptor type 2 | NR_001042389       |
| 7918467      | 3.65        | Clorf103    | Chromosome 1 open reading frame 103 | NM_018372          |
| 8053797      | 3.60        | LOC400986   | Protein immuno-reactive with anti-parathyroid hormone polyclonla | ENST00000456556    |
| 8156848      | 3.59        | NR4A3       | Nuclear receptor subfamily 4, group A, member 3 | NM_006981          |
| 8005483      | 3.56        | FBXW10      | F-box and WD repeat domain-containing 10 | NM_031456          |
| 8105077      | 3.54        | CARD6       | Caspase recruitment domain family, member 6 | NM_032587          |
| 8139840      | 3.48        | ERV3        | Endogenous retroviral sequence 3 (includes zinc) | NM_001007253       |
| 8030831      | 3.45        | ZNF175      | Zinc finger protein 175 | NM_007147          |
| 7958200      | 3.45        | EID3        | EP300-interacting inhibitor of differentiation 3 | NM_001008394       |
| 7936637      | 3.44        | SNORA19     | Small nucleolar RNA, H/ACA box 19 | NR_002917          |
| 8107353      | 3.43        | ZRSR1       | Zinc finger (CCCH type), RNA-binding motif and serine/arginine rich 1 | BC104811           |
| 8173600      | 3.26        | NAP1L2      | Nucleosome assembly protein 1-like 2 | NM_021963          |
| 8126853      | 3.25        | C6orf138    | Chromosome 6 open reading frame 138 | NM_001013732       |
| 8025301      | 3.20        | CD209       | CD209 molecule | NM_021155          |
| 7923119      | 3.17        | ZBTB41      | Zinc finger and BTB domain-containing 41 | NM_194314          |
| 7985555      | 3.11        | EFTUD1      | Elongation factor Tu guanine triphosphate binding domain-containing | NM_024580          |
| 7952986      | 3.09        | HSN2        | Hereditary sensory neuropathy, type II | NM_213655          |
| 8114572      | 3.08        | HBEGF       | Heparin-binding epidermal growth factor-like growth factor | NM_001945          |
| 8049540      | 3.05        | LRRFIP1     | Leucine-rich repeat (in FLII) interacting protein 1 | NM_001137550       |
| 8047926      | 3.03        | MAP2        | Microtubule-associated protein 2 | NM_002374          |
| 7954382      | 3.02        | PYROXD1     | Pyridine nucleotide-disulphide oxidoreductase domain 1 | NM_024854          |
| 7957260      | 2.99        | GLIPR1      | GLI pathogenesis-related 1 | NM_006851          |
| 8054054      | 2.97        | ANKRD36B    | Ankryn repeat domain 36B | NM_025190          |
| 7907572      | 2.96        | PAPP2       | Pappalyrin 2 | NM_020318          |
| 8090688      | 2.93        | SNORA58     | Small nucleolar RNA, H/ACA box 58 | NR_002985          |
| 8139935      | 2.89        | TYW1B       | tRNA-yW synthesizing protein 1 homolog B | NM_001145440       |
| 7982028      | 2.87        | SNORD115-11 | Small nucleolar RNA, C/D box 115-11 | NR_003303          |
| 7982050      | 2.87        | SNORD115-11 | Small nucleolar RNA, C/D box 115-11 | NR_003303          |
| 7982064      | 2.87        | SNORD115-11 | Small nucleolar RNA, C/D box 115-11 | NR_003303          |
Table I. Continued.

| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|------------------|--------------------|
| 7982078      | 2.87        | SNORD115-11 | Small nucleolar RNA, C/D box 115-11 | NR_003303          |
| 7982092      | 2.87        | SNORD115-11 | Small nucleolar RNA, C/D box 115-11 | NR_003303          |
| 7905339      | 2.86        | GABPB2      | GA binding protein transcription factor, β subunit | NM_144618          |
| 8140782      | 2.84        | ABCB1       | ATP-binding cassette, sub-family B (multidrug resistance protein/transporter associated with antigen processing) | NM_000927          |
| 8139482      | 2.83        | SNORA5A     | Small nucleolar RNA, H/ACA box 5A | NR_002919          |
| 8124756      | 2.83        | PPP1R10     | Protein phosphatase 1, regulatory (inhibitor) subunit | NM_002714          |
| 8178358      | 2.83        | PPP1R10     | Protein phosphatase 1, regulatory (inhibitor) subunit | NM_002714          |
| 8179664      | 2.83        | PPP1R10     | Protein phosphatase 1, regulatory (inhibitor) subunit | NM_002714          |
| 8112731      | 2.77        | F2RL2       | Coagulation factor II (thrombin) receptor-like 2 | NM_004101          |
| 8043687      | 2.74        | ANKRD36     | Ankyrin repeat domain 36 | NM_001164315       |
| 7977273      | 2.74        | ADSL1       | Adenylosuccinate synthase like 1 | NM_152328          |
| 7953200      | 2.72        | CCND2       | Cyclin D2 | NM_001759          |
| 8057954      | 2.72        | C2prf66     | Chromosome 2 open reading frame 66 | AY358249           |
| 8049532      | 2.72        | LRRFIP1     | Leucine-rich repeat (in FLII) interacting protein 1 | NM_001137550       |
| 8141843      | 2.72        | RASA4       | RAS p21 protein activator 4 | NM_006989          |
| 8098752      | 2.72        | ABCA11P     | Adenosine triphosphate-binding cassette, sub-family A, member 11, pseudogene | NR_002451          |
| 7982046      | 2.70        | SNORD115-20 | Small nucleolar RNA, C/D box 115-20 | NR_003312          |
| 7982016      | 2.70        | SNORD115-12 | Small nucleolar RNA, C/D box 115-12 | NR_003304          |
| 7982024      | 2.70        | SNORD115-12 | Small nucleolar RNA, C/D box 115-12 | NR_003304          |
| 7982030      | 2.70        | SNORD115-12 | Small nucleolar RNA, C/D box 115-12 | NR_003304          |
| 8054064      | 2.69        | ANKRD36B    | Ankyrin repeat domain 36B | NM_025190          |
| 7910047      | 2.68        | DNAH14      | Dynein, axonemal, heavy chain 14 | NM_001373          |
| 8016239      | 2.68        | PLEKHM1     | Pleckstrin homology domain-containing, family M (with RUN domain) member 1 | NM_027774          |
| 8060949      | 2.67        | ANKRD5      | Ankyrin repeat domain 5 | NM_022096          |
| 8064375      | 2.62        | SRXN1       | Sulfiredoxin 1 homolog (S. Cerevisiae) | NM_080725          |
| 8077612      | 2.61        | TTLL3       | Tubulin tyrosine ligase-like family, member 3 | NM_001025930       |
| 8151559      | 2.60        | SLC10A5     | Solute carrier family 10 (sodium/bile acid cotransporter family), member 5 | NM_001010893       |
| 7902227      | 2.60        | GADD45A     | Growth arrest and DNA-damage-inducible, α | NM_001924          |
| 7982058      | 2.59        | SNORD115-26 | Small nucleolar RNA, C/D box 115-26 | NR_003343          |
| 8118023      | 2.55        | GTF2H4      | General transcription factor II human, polypeptide 4 | NM_001517          |
| 8006336      | 2.54        | LRRC37B     | Leucine-rich-repeat-containing 37B | NM_052888          |
| 8108006      | 2.51        | LEAP2       | Liver-expressed antimicrobial peptide 2 | NM_052971          |
| 7971388      | 2.50        | SLC25A30    | Solute carrier family 25, member 30 | NM_001010875       |
| 7987163      | 2.48        | FMN1        | Formin 1 | ENST00000414268    |
| 7938293      | 2.47        | SNORA45     | Small nucleolar RNA, H/ACA box 45 | NR_002977          |
| 8113651      | 2.45        | ATG12       | ATG12 autophagy-related 12 homolog (S. Cerevisiae) | NM_033362          |
| 8049542      | 2.45        | LRRFIP1     | Leucine-rich repeat (in FLII) interacting protein 1 | NM_001137550       |
| 8097435      | 2.45        | C4orf33     | Chromosome 4 open reading frame 33 | NM_173487          |
| 8168345      | 2.45        | ACRC        | Acidic repeat-containing | NM_052957          |
| 7980828      | 2.42        | CCDC88C     | Coiled-coil domain-containing 88C | NM_001080414       |
| 8045423      | 2.42        | SNORA40     | Small nucleolar RNA, H/ACA box 40 | NM_002973          |
| 8112331      | 2.42        | ISCA1       | Iron-sulfur cluster assembly 1 homolog | NM_030940          |
| 8043697      | 2.41        | ANKRD36B    | Ankyrin repeat domain 36B | NM_025190          |
| 8033667      | 2.40        | ZNF558      | Zinc finger protein 558 | NM_144693          |
| 8142232      | 2.39        | LAMB4       | Laminin, β4 | NM_007356          |
Table I. Continued.

| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|------------------|--------------------|
| 7960052      | 2.39        | SNORA49     | Small nuclear RNA, H/ACA box 49 | NR_002979          |
| 8031837      | 2.39        | ZNF587      | Zinc finger protein 587          | AF294842           |
| 8174715      | 2.38        | SNORA69     | Small nuclear RNA, H/ACA box 69 | NR_002584          |
| 8001748      | 2.38        | SNORA50     | Small nuclear RNA, H/ACA box 50 | NR_002980          |
| 8042503      | 2.38        | MXD1        | MAX dimerization protein 1       | NM_002357          |
| 8072678      | 2.36        | HMOX1       | Heme oxygenase (decycling) 1     | NM_002133          |
| 7979904      | 2.35        | ZNF778      | Zinc finger protein 778          | AK295122           |
| 8053648      | 2.35        | KRCC1       | Lysine-rich coiled-coil 1        | NM_016618          |
| 8035793      | 2.35        | ZNF737      | Zinc finger protein 737          | NM_001159293       |
| 7977732      | 2.34        | SNORD8      | Small nuclear RNA, C/D box 8     | NR_002916          |
| 8153457      | 2.31        | EEF1D       | Eukaryotic translation elongation factor 1δ | AY358690 |
| 8069574      | 2.31        | C21orf91    | Chromosome 21 open reading frame 91 | NM_001100420  |
| 8112841      | 2.30        | HOMER1      | Homer homolog 1 (Drosophila)     | NM_004272          |
| 8038919      | 2.29        | ZNF350      | Zinc finger protein 50           | NM_021632          |
| 9175288      | 2.29        | MOSPD1      | Motile sperm domain-containing 1 | NM_019556          |
| 8160912      | 2.28        | C9orf113    | Chromosome 9 open reading frame 131 | NM_203299 |
| 7934334      | 2.28        | TTC18       | Tetra(tripeptide) repeat domain 18 | NM_145170          |
| 8056572      | 2.27        | SPC25       | SPC25, NDC80 kinetochore complex component | NM_020675        |
| 8161919      | 2.27        | TLE1        | Transducin-like enhancer of split 1 (Drosophila) | NM_005077        |
| 7964460      | 2.26        | DDIT3       | DNA-damage-inducible transcript 3 | NM_004083          |
| 8019857      | 2.26        | NDC80       | NDC80 Homolog, kinetochore complex component (S. cerevisiae) | NM_006101        |
| 8045587      | 2.24        | ACVR2A      | Activin A receptor, type IIa     | NM_001616          |
| 8002660      | 2.24        | TXNL4B      | Thioredoxin-like 4B              | NM_017853          |
| 7911329      | 2.24        | 14-Sep      | Septin 14                       | NM_207366          |
| 8009890      | 2.24        | FLJ10213    | Endogenous Borna-like N element-1 | NM_018029        |
| 8014115      | 2.22        | MYOID       | Myosin ID                        | NM_015194          |
| 7949916      | 2.20        | CHKA        | Choline kinase α                 | NM_001277          |
| 7938295      | 2.20        | RPL27A      | Ribosomal protein L27a           | NM_000990          |
| 8168146      | 2.20        | KIF4A       | Kinesin family member 4A         | NM_012310          |
| 8114171      | 2.19        | CDKL3       | Cycline-dependent kinase-like 3  | NM_00113575        |
| 8008700      | 2.19        | FLJ11710    | Hypothetical protein FLJ11710    | AK021772           |
| 8108321      | 2.18        | FAM53C      | Family with sequence similarity 53, member C | NM_001135647       |
| 8047161      | 2.18        | OBFC2A      | Oligonucleotide/oligosaccharide-binding fold-containing 2a | NM_001031716       |
| 8053576      | 2.17        | RNF103      | Ring finger protein 103           | NM_005667          |
| 8006237      | 2.17        | LOC400590   | Hypothetical LOC400590            | ENST000000433145   |
| 8136341      | 2.17        | BPGM        | 2,3-bisphosphoglycerate mutase   | NM_199186          |
| 8146225      | 2.16        | C8orf40     | Chromosome 8 open reading frame 40 | NM_001135674       |
| 8147057      | 2.16        | CHMP4C      | Chromatin modifying protein 4C    | NM_152284          |
| 7921228      | 2.15        | ETV3        | E26 transforming-specific variant 3 | NM_001145312       |
| 8065607      | 2.15        | PLAGL2      | Pleiomorphic adenoma gene-like 2  | NM_002657          |
| 8096511      | 2.14        | BMPR1B      | Bone morphogenetic protein receptor, type 1B | NM_001203          |
| 7927389      | 2.14        | MAPK8       | Mitogen-activated protein kinase 8 | NM_002750          |
| 8099858      | 2.14        | POLN        | Polymerase (DNA directed) nu      | NM_181808          |
| 8038989      | 2.14        | ZNF600      | Zinc finger protein 600           | NM_198457          |
| 7951654      | 2.14        | FDXACB1     | Ferredoxin-fold anticyodon binding domain-containing 1 | NM_138378        |
| 8036341      | 2.13        | ZNF461      | Zinc finger protein 461           | NM_153257          |
| 7981998      | 2.13        | SNORD116-25 | Small nuclear RNA, C/D box 116-25 | NM_003339          |
| 8041179      | 2.13        | CLIP4       | Cytoskeleton-associated protein-glycine-rich domain-containing linker protein family member 4 | NM_024692        |
Table I. Continued.

| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|-----------------|-------------------|
| 7969096      | 2.13        | CDADC1      | Cytidine and deoxycytidine monophosphate deaminase domain-containing 1 | NM_030911         |
| 7969243      | 2.13        | CKAP2       | Cytoskeleton-associated protein 2 | NM_018204         |
| 7986350      | 2.12        | ARRDC4      | Arrestin domain-containing 4 | NM_183376         |
| 8063382      | 2.12        | SNAI1       | Snail homolog 1 (Drosophila) | NM_005985         |
| 8053801      | 2.12        | ANKRD36     | Ankyrin repeat domain 36 | NM_001164315      |
| 7995888      | 2.12        | PLA2G10     | Phospholipase A2, group X | NM_003561         |
| 8008795      | 2.11        | C17orf71    | Chromosome 17 open reading frame 71 | NM_018149         |
| 8029340      | 2.11        | ZNF155      | Zinc finger protein 155 | NM_003445         |
| 8166104      | 2.11        | OFD1        | Oral-facial-digital syndrome 1 | NM_003611         |
| 8123825      | 2.11        | SLC35B3     | Solute carrier family 35 member B3 | NM_015948         |
| 7901052      | 2.11        | SNORD38B    | Small nucleolar RNA, C/D box 38B | NM_001457         |
| 8084880      | 2.10        | HES1        | Hairy and enhancer of split 1 | NM_005524         |
| 7925672      | 2.10        | ZNF670      | Zinc finger protein 670 | NM_033213         |
| 7982294      | 2.10        | OTUD7A      | OTU domain-containing 7A | NM_130901         |
| 7962112      | 2.09        | CAPRIN2     | Caprin family member 2 | NM_001002259      |
| 7973948      | 2.09        | BRMSIL      | Breast cancer metastasis-suppressor 1-like | NM_032352         |
| 8117685      | 2.09        | ZKSCAN3     | Zinc finger with KRAB and SCAN domains 3 | NM_024493         |
| 8010082      | 2.08        | SNORD1A     | Small nucleolar RNA, C/D box 1A | NR_004395         |
| 8014982      | 2.08        | ACYP2       | Acylphosphatase 2, muscle type | NM_138448         |
| 8137693      | 2.08        | COX19       | Cytochrome c oxidase assembly homolog 19 | NM_001031617      |
| 7917779      | 2.08        | GCLM        | Glutamate-cysteine ligase, modifier subunit | NM_002061         |
| 7938364      | 2.08        | WEE1        | WEE1 homolog 1 (S. Pombe) | BX641032          |
| 8007414      | 2.08        | AOC2        | Amine oxidase, copper-containing 2 (retina-specific) | NM_009590         |
| 8139656      | 2.08        | GRB10       | Growth factor receptor-bond protein 10 | NM_001001555      |
| 8059852      | 2.08        | MSL3L2/MSL3-like 2 | Male-specific lethal 3-like 2 (Drosophila) | NM_001166217      |
| 8109484      | 2.07        | KIF4B       | Kinesin family member 4B | NM_001099293      |
| 8022559      | 2.07        | ANKRD29     | Ankyrin repeat domain 29 | NM_173505         |
| 7910030      | 2.07        | DNAH14      | Dynein, axonemal, heavy chain 14 | NM_00145154       |
| 8052143      | 2.07        | GPR75       | G protein-coupled receptor 75 | NM_006794         |
| 7931643      | 2.07        | CYP2E1      | Cytochrome P450, family 2, subfamily E, polypeptide 1 | NM_000773         |
| 8102789      | 2.06        | TERF1       | Telomeric repeat binding factor (NIMA-interacting) 1 | NM_003218         |
| 7953603      | 2.06        | C1S         | Complement component 1, s subcomponent | NM_201442         |
| 8104930      | 2.05        | SLC1A3      | Solute carrier family 1 (glial high affinity glutamate transporter), member 3 | NM_004172         |
| 7953211      | 2.05        | C12orf5     | Chromosome 12 open reading frame 5 | NM_020375         |
| 8114326      | 2.04        | FAM13B      | Family with sequence similarity 13, member B | NM_0166603        |
| 7936826      | 2.04        | IKZF5       | IKAROS family zinc finger (Pegasus) | NM_022466         |
| 8013567      | 2.04        | C17orf108   | Chromosome 17 open reading frame 108 | NM_001076680      |
| 7975066      | 2.04        | AKAP5       | A-kinase anchor protein 5 | NM_004857         |
| 8142524      | 2.04        | TSPAN12     | Tetraspanin 12 | NM_012338         |
| 7952673      | 2.04        | FLJ45950    | FLJ45950 protein | AKI27847          |
| 8081128      | 2.04        | NSUN3       | Nucleolar protein 2 homolog/Sun domain family, member 3 | NM_022072         |
| 7922846      | 2.04        | FAM129A     | Family with sequence similarity 129, member A | NM_052966         |
| 8013305      | 2.04        | ZNF286B     | Zinc finger protein 286B | NM_001145045      |
| 8153935      | 2.03        | ZNF252      | Zinc finger protein 252 | NM_023392         |
| 8162490      | 2.03        | HIATL1      | Hippocampus abundant transcript-like 1 | NM_032558         |
| 8128698      | 2.02        | SESN1       | Sestrin 1 | NM_014454         |
| 8010778      | 2.02        | CSNK1D      | Casein kinase 1, δ | NM_001893         |
### Table II. Downregulation of gene expressions in catharidine-treated NCI-H460 cells.

| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|------------------|--------------------|
| 8141311      | 2.02        | FAM200A     | Family with sequence similarity 200, member A | NM_145111          |
| 7944867      | 2.02        | SIAE        | Sialic acid acetylerase | NM_170601          |
| 7961829      | 2.02        | BCAT1       | Branched-chain amino-acid transaminase 1, cytosolic | NM_005504          |
| 7994161      | 2.02        | RBBP6       | Retinoblastoma binding protein 6 | NM_006910          |
| 7981273      | 2.02        | CCDC85C     | Coiled-coil domain-containing 85C | NM_001144995       |
| 8110649      | 2.02        | TRIM41      | Tripartite motif-containing 41 | NM_033549          |
| 8101839      | 2.01        | EIF4E       | Eukaryotic translation initiation factor 4E | NM_001968          |
| 8103226      | 2.01        | TMEM154     | Transmembrane protein 154 | NM_152680          |

### Table I. Continued.

| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|------------------|--------------------|
| 8150201      | -2.02       | FAM200A     | Family with sequence similarity 200, member A | NM_145111          |
| 8124413      | -2.06       | HIST1H4D    | Histone cluster 1, H4d | NM_003539          |
| 8111428      | -2.03       | FST         | Follicatin | NM_006350          |
| 7961365      | -2.04       | DPPA3       | Developmental pluripotency-associated 3 | NM_199286          |
| 8117426      | -2.99       | HIST1H2BH   | Histone cluster 1, H2bh | NM_003524          |
| 8117898      | -2.97       | HIST1H4J    | Histone cluster 1, H4j | NM_021968          |
| 8117337      | -2.95       | HIST1H1E    | Histone cluster 1, H1e | NM_005321          |
| 7911241      | -2.93       | OR2L8       | Olfactory receptor, family 2, subfamily L, member 8 | NM_001001963       |
| 8048749      | -2.88       | KCNE4       | Potassium voltage-gated channel, IsK-related family, member 4 | NM_080671          |
| 8124437      | -2.87       | HIST1H3F    | Histone cluster 1, H3f | NM_021018          |
| 8117395      | -2.83       | HIST1H2BF   | Histone cluster 1, H2bf | NM_003522          |
| 8015798      | -2.79       | LOC10013058 | Hypothetical LOC100130581 | NR_027413          |
| 7919642      | -2.78       | HIST2H2AB   | Histone cluster 2, H2ab | NM_175065          |
| 8059470      | -2.76       | IRS1        | Insulin receptor substrate 1 | NM_005544          |
| 8077270      | -2.75       | CHL1        | Cell adhesion molecule with homology to L1 cell adhesion molecule | NM_006614          |
| 7915592      | -2.74       | RNU5D       | RNA, U5D small nuclear | NR_002755          |
| 7906767      | -2.74       | FCGR2C      | Fc fragment of immunoglobulin G, low affinity IIc, receptor for (CD32) (gene/pseudogene) | NM_201563          |
| 8117594      | -2.74       | HIST1H2BM   | Histone cluster 1, H2bm | NM_003521          |
| 8117589      | -2.72       | HIST1H3H    | Histone cluster 1, H3h | NM_003536          |
| 7981728      | -2.72       | LOC10029321 | Similar to hCG2042717 | ENST00000390601    |
| 8124397      | -2.71       | HIST1H1C    | Histone cluster 1, H1c | NM_005319          |
| 8135734      | -2.71       | C7orf58     | Chromosome 7 open reading frame 58 | NM_024913          |
| 8138988      | -2.70       | DPY19L2P1   | Dpy-19-like 2 pseudogene 1 (C. elegans) | NR_002833          |
| 8117583      | -2.65       | HIST1H2AI   | Histone cluster 1, H2ai | NM_003509          |
| 8153258      | -2.65       | SLC45A4     | Solute carrier family 45, member 4 | BC033223           |
| 7960865      | -2.61       | SLC2A3      | Solute carrier family 2 (facilitated glucose transporter), member 3 | NM_006931          |
| 8116921      | -2.59       | EDN1        | Endothelin 1 | NM_001955          |
| 8101757      | -2.58       | GPRIN3      | G protein-regulated inducer of neurite outgrowth family member 3 | NM_198281          |
| 7971723      | -2.58       | FLJ37307    | Hypothetical LOC283521 | NR_027047          |
| Probe set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|--------------|-------------|------------------|--------------------|
| 8117580      | -2.56        | HIST1H2AI   | Histone cluster 1, H2ai | NM_003509          |
| 8167573      | -2.56        | GAGE1       | G antigen 1       | NM_001468          |
| 8165295      | -2.56        | LCN8        | Lipocalin 8       | ENST0000371686     |
| 7927876      | -2.53        | TET1        | Ten-eleven translocation oncogene 1 | NM_030625 |
| 7963054      | -2.52        | TUBA1A      | Tubulin, α1       | NM_006009          |
| 7957386      | -2.51        | ACS5S3      | Acyl-CoA synthetase short-chain family member 3 | NM_024560 |
| 7915919      | -2.49        | TAL1        | T cell acute lymphocytic leukemia 1 | NM_003189 |
| 8174985      | -2.48        | SMARCA1     | Switch/sucrose non-fermentable-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 1 | NM_003069 |
| 8105495      | -2.47        | PART1       | Prostate androgen-regulated transcript 1 (non-protein coding) | NR_028508 |
| 8146967      | -2.46        | CRISPLD1    | Cysteine-rich secretory protein LCCL domain-containing 1 | NM_031461 |
| 8144786      | -2.46        | SLC7A2      | Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 | NM_003046 |
| 8029280      | -2.45        | CD177       | CD177 molecule    | NM_020406          |
| 7984524      | -2.45        | PAQR5       | Progesterin and adipoQ receptor family member V | NM_001104554 |
| 7973182      | -2.44        | LOC554207   | Hypothetical LOC554207 | ENST0000320322 |
| 8145795      | -2.44        | LOC100293539 | Similar to ribosomal protein 10 | XM_002346094 |
| 8095697      | -2.44        | CXCL1       | Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α) | NM_001511 |
| 8124406      | -2.42        | HIST1H2BC   | Histone cluster 1, H2bc | NM_003526 |
| 7978260      | -2.42        | DHRS1       | Dehydrogenase/reductase family member 1 | NM_001136050 |
| 7920271      | -2.42        | S100A4      | S100 calcium binding protein A4 | NM_019544 |
| 8166469      | -2.42        | SAT1        | Spermidine/spermine N1-acetyltransferase 1 | NR_027783 |
| 8124527      | -2.40        | HIST1H1B    | Histone cluster 1, H1b | NM_005322 |
| 8008321      | -2.40        | ACSF2       | Acyl-CoA synthetase family member 2 | NM_025149 |
| 8124385      | -2.39        | HIST1H4B    | Histone cluster 1, H4b | NM_003544 |
| 800731       | -2.38        | TUBG2       | Tubulin, γ2     | NM_016437          |
| 8122365      | -2.36        | GPR126      | G protein-coupled receptor 126 | NM_020455 |
| 8015273      | -2.34        | KRT31       | Keratin 31       | NM_002277          |
| 7904465      | -2.34        | HIST2H2BA   | Histone cluster 2, H2ba | NR_027337         |
| 8124416      | -2.33        | HIST1H3D    | Histone cluster 1, H3d | NM_003530          |
| 8074458      | -2.33        | C22orf39    | Chromosome 22 open reading frame 39 | NM_173793 |
| 8046048      | -2.33        | CSRP3N      | Cysteine-serine-rich nuclear protein 3 | NM_001172173 |
| 8007493      | -2.32        | ARL4D       | Adenosine diphosphate-ribosylation factor-like 4D | NM_001661 |
| 8157804      | -2.31        | OLFML2A     | Olfactomedin-like 2A | NM_182487 |
| 7948836      | -2.31        | TMEM223     | Transmembrane protein 223 | NM_001080501 |
| 8033458      | -2.31        | LYPLA2      | Lyosphospholipase II | NM_007260 |
| 7919619      | -2.30        | HIST2H2AA3  | Histone cluster 2, H2aa3 | NM_003516 |
| 7905079      | -2.30        | HIST2H2AA3  | Histone cluster 2, H2aa3 | NM_003516 |
| 7927631      | -2.29        | DKK1        | Dickkopf homolog 1 (Xenopus laevis) | NM_012242 |
| 7975598      | -2.28        | ACOT1       | Acyl-CoA thioesterase 1 | NM_001037161 |
| 8071801      | -2.27        | GSTTP1      | Glutathione S-transferase 0 pseudogene 1 | NR_003081 |
| 7928429      | -2.27        | PLAU        | Plasminogen activator, urokinase | NM_002658 |
| 8068898      | -2.27        | HIST1H2BK   | Histone cluster 1, H2bk | NM_080593 |
| 8041467      | -2.26        | VIT         | Vitrin           | NM_053276          |
| 8077160      | -2.26        | ARSA        | Arylsulfatase A  | NM_000487          |
| 7991754      | -2.25        | HBZ         | Hemoglobin, ζ   | NM_005332          |
| 8049534      | -2.24        | LRRFIP1     | Leucine-rich repeat (in FLII) interacting protein 1 | NM_001137550 |
Table II. Continued.

| Probe Set ID | Fold change | Gene symbol | Gene description | mRNA accession no. |
|--------------|-------------|-------------|------------------|-------------------|
| 7909789      | -2.23       | TGFβ2       | Transforming growth factor β2 | NM_001135599     |
| 7919612      | -2.23       | HIST2H3D    | Histone cluster 2, H3d | NM_001123375     |
| 8100578      | -2.22       | EPHA5       | Ephrin receptor A5 | NM_004439        |
| 8169541      | -2.22       | DOCK11      | Dedicator of cytokinesis 11 | NM_144658    |
| 8124430      | -2.21       | HIST1H1D    | Histone cluster 1, H1d | NM_005320        |
| 8124524      | -2.21       | HIST1H2AK   | Histone cluster 1, H2aK | NM_003510        |
| 8124524      | -2.21       | HIST1H2AK   | Histone cluster 1, H2aK | NM_003510        |
| 7906775      | -2.20       | HSPA7       | Heat shock 70kDa protein 7 (HSP70B) | NR_024151   |
| 7953291      | -2.20       | CD9         | CD9 molecule | NM_001769        |
| 8033319      | -2.19       | SH2D3A      | Src Homology 2 domain-containing 3A | NM_005490   |
| 7905088      | -2.19       | HIST2H2AC   | Histone cluster 2, H2ac | NM_003517        |
| 7975602      | -2.19       | ACOT2       | Acyl-CoA thioesterase 2 | NM_006821        |
| 7982854      | -2.19       | DLL4        | δ-like 4 (Drosophila) | NM_019074        |
| 8019778      | -2.19       | PCYT2       | Phosphate cytidylyltransferase 2, ethanolamine | NM_002861   |
| 8046048      | -2.19       | HIST1H4C    | Histone cluster 1, H4c | NM_003542        |
| 8007493      | -2.18       | VWASA       | Von Willebrand factor A domain-containing 5A | NM_001130142 |
| 8157804      | -2.18       | PLG         | Plasminogen | NM_000301        |
| 7948836      | -2.18       | CD24        | CD24 molecule | NM_013230        |
| 8033458      | -2.17       | FSTL4       | Follistatin-like 4 | NM_015082        |
| 7919619      | -2.16       | CA2         | Carbonic anhydrase II | NM_000067        |
| 7905079      | -2.16       | CDH19       | Cadherin 19, type 2 | NM_021153        |
| 7927631      | -2.16       | CDC42EP3    | Cell division cycle 42 effector protein (Rho guanosine triphosphatase binding) 3 | NM_006449   |
| 7975598      | -2.16       | ACCN2       | Amiloride-sensitive cation channel 2, neuronal | NM_020039        |
| 8071801      | -2.15       | HIST2H3D    | Histone cluster 2, H3d | NM_001123375   |
| 7928429      | -2.15       | RFX2        | Regulatory factor X, 2 (influences human leukocyte antigen class II expression) | NM_000635   |
| 8068898      | -2.15       | NES         | Nestin | NM_006617        |
| 8041467      | -2.15       | LOC25845    | Hypothetical LOC25845 | NR_024158 |
| 8077160      | -2.15       | THSD7A      | Thrombospondin, type I, domain-containing 7A | NM_015204   |
| 7991754      | -2.14       | LOC147727   | Hypothetical LOC147727 | NR_024333 |
| 8049534      | -2.14       | CALML6      | Calmodulin-like 6 | NM_138705        |
| 7909789      | -2.14       | DEFB109P1B  | Defensin, β 109, pseudogene 1B | NR_003668   |
| 7919612      | -2.13       | EPOR        | Erythropoietin receptor | NM_000121 |
| 8100578      | -2.13       | EEF2K       | Eukaryotic elongation factor-2 kinase | NM_013302   |
| 8169541      | -2.13       | EMP3        | Epithelial membrane protein 3 | NM_001425   |
| 8124430      | -2.13       | TMEM84      | Transmembrane protein 84 | NR_026949 |
| 8124524      | -2.13       | CXXC5       | CXXC finger 5 | NM_016463        |
| 7906775      | -2.12       | PCYT2       | Phosphate cytidylyltransferase 2, ethanolamine | NM_002861   |
| 7953291      | -2.12       | LYPD1       | Ly6/plasminogen activator, urokinase 1 receptor domain-containing | NM_144586 |
| 8033319      | -2.12       | PHLDB2      | Pleckstrin homology-like domain, family B, member 2 | NM_001134439 |
| 7905088      | -2.11       | LRFN2       | Leucine-rich repeat and fibronectin type III domain-containing 2 | NM_020737   |
| 7975602      | -2.11       | C9orf23     | Chromosome 9 open reading frame 23 | NM_148179   |
| 7982854      | -2.11       | FLJ13744    | Hypothetical FLJ13744 | BC070061 |
| 8018445      | -2.11       | UNK         | Unkempt homolog (Drosophila) | NM_001080419 |
| 8038407      | -2.10       | RRAS        | Related RAS viral (r-ras) oncogene homolog | NM_006270 |
| 7987230      | -2.10       | LPCAT4      | Lysophosphatidylcholine acyltransferase 4 | NM_153613 |
| 8031514      | -2.10       | LOC100133142| Hypothetical LOC100133142 | XM_001718400 |
expression of apoptosis-associated genes was upregulated, such as CARD6, which was upregulated 3.54-fold (Table I).

By contrast, the results presented in Table II demonstrated that genes associated with DNA damage, cell cycle progression and apoptosis were also downregulated, including DdiT4, CDC42EP3 and STAT2, respectively. These genes were found to be downregulated 3.14-, 2.16 and 2.04-fold, respectively (Table II). Overall, cDNA microarray analysis of H460 cells following treatment with CTD demonstrated that CTD induced the differential expression of numerous genes associated with DNA damage, cell cycle progression and apoptosis.
Figure 1. Top scored (by the number of pathways) AN network, as determined using GeneGo_cat_FC2 analysis. Thick cyan lines indicate the fragments of canonical pathways. Upregulated genes are marked with red circles and downregulated genes are indicated with blue circles. FC, Fold Change 2.0; AN, Analyze Networks algorithm.

Figure 2. Second scored (by the number of pathways) AN network, as determined using GeneGo_cat_FC2 analysis. Thick cyan lines indicate the fragments of canonical pathways. Upregulated genes are marked with red circles and downregulated genes are indicated with blue circles. FC, Fold Change 2.0; AN, Analyze Networks algorithm.
GeneGo analysis. A GeneGo analysis program was used to analyze the CTD-treated NCI-H460 cells in order to determine the top scoring genes which were differentially expressed, as determined by the number of pathway networks involved. The results of the GeneGo analyses are shown in Figs. 1-3, which reveal the top, second and third scored genes by the number of pathways, respectively. Experimental data were used to generate maps of the pathway interactions and genes which were upregulated (indicated by red circles) and downregulated (indicated by blue circles) in H460 cells following treatment with CTD. It was indicated that these genes may also be involved in DNA damage, cell cycle arrest and apoptosis-associated responses in CTD-treated H460 cells.

Discussion

CTD has been reported to have cytotoxic effects in numerous different types of cancer cell (8-15). The results of previous studies have also demonstrated that CTD-induced cell death occurred due to the induction of apoptosis in human lung cancer cells (data not shown) (23). However, the effects of CTD on gene expression in cancer cells have remained to be elucidated. To the best of our knowledge, the present study was the first to report on the effects of CTD on gene expression in H460 cells. Therefore, the present study not only advanced the understanding of the differential gene expression following treatment with CTD in lung cancer cells, but may additionally provide several potential biomarkers for use as future therapeutic clinical targets for the treatment of lung cancer.

It has been well documented that the tumor microenvironment, which contains matrix proteins, stromal cells and associated secreted molecules, including cytokines and associated genes, which may be used as targets of cancer therapeutic drugs (24-26). Therefore, an increasing number of studies focus on elucidating the tumor microenvironment and associated gene expression in order to determine potential novel therapeutic agents for treating cancer patients (27). Over the past decade, there have been numerous clinical trials of treatments for lung cancer patients, including adjuvant chemotherapy trials and neo-adjuvant chemotherapy trials (28-30); however, the results of these trials have not yet provided a successful, effective treatment for lung cancer. Numerous studies have demonstrated that chemotherapeutics may result in cell death through DNA damage, cell cycle arrest and the induction of apoptosis (31,32). In the present study, H460 cells were treated with CTD and incubated in 12-well plates, and their RNA was then isolated in order to determine which genes exhibited altered expression following treatment with CTD. The results revealed that CTD effected the upregulation and downregulation, respectively, of the expression of certain genes which are known to be associated with DNA damage, cell cycle progression and apoptosis in H460 cells.

In order to further elucidate the molecular signaling pathways associated with altered gene expression in H460 cells following exposure to CTD, GeneGo Process Networks were
used in the present study in order to analyze the altered gene expression results of the microarray, in order to determine the possible signaling pathways involved. Based on GeneGo pathway and canonical pathway maps, which represent a set of ~650 signaling and metabolic maps covering human biology (signaling and metabolism) in a comprehensive way. A preset network of protein interaction characteristics for the process was used for each process, and the experimental data were mapped regarding the specific process. The obtained hypothetical molecular signaling pathways indicated that CTD affects numerous associated signaling pathways, indicated by the involvement of the differentially expressed genes in the network of the respective signaling pathways. The gene content of the uploaded files was used as the input list for the generation of biological networks using the Analyze Networks algorithm with default settings. This is a variant of the shortest paths algorithm, with main parameters of relative enrichment with the uploaded data, and relative saturation of the networks with canonical pathways. The network provides data listing interacting proteins. In this workflow the network is prioritized based on the number of fragments of canonical pathways on the network.

In conclusion, the results of the present study revealed that treatment with CTD induced the upregulation and downregulation of numerous genes in H460 cells. In addition, these differentially expressed genes were associated with DNA damage, cell cycle progression and apoptotic cell death in human lung cancer H460 cells. The present study also revealed possible signaling pathways, which may provide more information on the possible mechanism of CTD in H460 cells; however, further studies are required.

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References
1. Bilello KS, Murin S and Matthay RA: Epidemiology, etiology, and prevention of lung cancer. Clin Chest Med 23: 1-25, 2002.
2. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 55: 74-108, 2005.
3. Zhang P, Gao WY, Turner S and Ducatman BS: Gleevec (STI-571) inhibits lung cancer cell growth (A549) and potentiates the cisplatin effect in vitro. Mol Cancer 2: 1, 2003.
4. Midthun DE and Jett JR: Chemotherapy for advanced lung cancer. When to expect a response. Postgrad Med 101: 189-197, 1997.
5. Stewart DJ: Tumor and host factors that may limit efficacy of chemotherapy in non-small cell and small cell lung cancer. Crit Rev Oncol Hematol 75: 173-234, 2010.
6. Chansky K, Sculier JP, Crowley JJ, et al; National Cancer Institute of Canada and Intergroup Study FBR.10: Adjuvant vinorelbine and cisplatin in elderly patients: National Cancer Institute of Canada and Intergroup Study JBR.10. J Clin Oncol 25: 1553-1561, 2007.
7. Solimini NL, Luo J and Elledge SJ: Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 136: 823-837, 2009.
8. Zhang W, Han Y, Wang W, et al: Inhibitory effect of alpinate Oxyphylla fructus extracts on Ang II-induced cardiac pathological remodeling-related pathways in H9c2 cardiomyoblasts. BioMedicine 3: 148-152, 2013.
9. Hsia TC, Yu CC, Hsu SC, et al: Cantharidin induces apoptosis of H460 human lung cancer cells through mitochondria-dependent mitochondrial pathway in NCTD-induced cytotoxicity in human hepG2 cells. J Exp Clin Cancer Res 29: 145, 2010.
10. Li W, Xie L, Chen Z, et al: Cantharidin, a potent and selective PP2A inhibitor, induces an oxidative stress-independent growth inhibition of pancreatic cancer cells through G2/M cell-cycle arrest and apoptosis. Cancer Sci 101: 1226-1233, 2010.
11. Yeh CB, Su CJ, Hwang JM and Chou MC: Therapeutic effects of cantharidin analogues without bridging ether oxygen on human hepatocellular carcinoma cells. Eur J Med Chem 45: 3981-3985, 2010.
12. Huang WW, Ko SW, Tsai HY, et al: Cantharidin induces G2/M phase arrest and apoptosis in human colorectal cancer cell colo 205 cells through inhibition of CDK1 activity and caspase-dependent signaling pathways. Int J Oncol 38: 1067-1073, 2011.
13. Zhang WD, Zhao HR, Yan Y, Wang XH, Zong ZH and Liu Y: Apoptosis induced by cantharidin in human pulmonary carcinoma cells A549 and its molecular mechanisms. Zhonghua Zhong Liu Za Zhi 27: 330-334, 2005 (In Chinese).
14. Kim JA, Kim Y, Kwon BM and Han DC: The natural compound cantharidin induces cancer cell death through inhibition of heat shock protein 70 (HSP70) and Bcl-2-associated athanogene domain 3 (BAG3) expression by blocking heat shock factor 1 (HSF1) binding to promoters. J Biol Chem 288: 28713-28726, 2013.
15. Osborne C, Wilson P and Tripathy D: Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications. Oncologist 9: 361-377, 2004.
16. Heneghan HM, Miller N and Kerin MJ: MiRNAs as biomarkers and therapeutics targets in cancer. Curr Opin Pharmacol 10: 543-550, 2010.
17. Luftig MA, Okumura S, Kriwacki RW, et al: Apoptosis in prostate cancer: Mechanisms for activating cell death in cancer cells. Adv Exp Med Biol 615: 81-104, 2008.