Differential Expression of Gene Profiles in MRGX-treated Lung Cancer

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Abstract

Objectives: Modified regular ginseng extract (MRGX) has stronger anti-cancer activity-possessing ginsenoside profiles.

Methods: To investigate changes in gene expression in the MRGX-treated lung cancer cells (A549), we examined genomic data with cDNA microarray results. After completing the gene-ontology-based analysis, we grouped the genes into up- and down-regulated profiles and into ontology-related regulated genes and proteins through their interaction network.

Results: One hundred nine proteins that were up- and down-regulated by MRGX were queried by using IPA. IL8, MMP7 and PLAUR and were found to play a major role in the anti-cancer activity in MRGX-treated lung cancer cells. These results were validated using a Western blot analysis and a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis.

Conclusions: Most MRGX-responsive genes are up-regulated transiently in A549 cells, but down-regulated in a sustained manner in lung cancer cells.

1. Introduction

Ginseng has been most widely used as herbal medicine in Eastern Asian countries, including Korea, China, and Japan. Ginseng is a species of a perennial plant belonging to the genus Panax in the family Araliaceae, and the ginseng species are only found in the northern hemisphere from Siberia to Vietnam. In general, Asian ginseng (Panax ginseng) and American ginseng (Panax quinquefolius) are most well-characterized, and these ginseng ginsenosides have been quantitatively and qualitatively characterized by ginsenoside profiling [1]. In particular, P. ginseng contains more than thirty pharmacologically-valuable ginsenosides [2]. Among them, ginsenosides such as Rg3, Rh2, Rb2, Compound K, and Rf2 are known to possess anti-cancer activities against colorectal cancer, colon cancer, hepatoma and breast cancer [3-6]. The ginsenosides exhibit anti-cancer activity by directly inhibiting cancer cell growth and metastasis in vitro and in vivo [7]. The major ginsenosides, such as Rb1, Rb2 and Rg1, induce apoptosis of...
lung cancer via an extrinsic apoptotic pathway [8]. These results suggest that specific ginsenosides play critical roles in the inhibition of cancer cell proliferation.

Lung cancer, comprising of a majority of non-small-cell lung carcinomas (NSCLCs) of up to 85%, is the second leading cause of cancer death worldwide [9]. Thus, lung cancer is still considered as an extremely lethal cancer [10]. Surgical and combined therapies have contributed to reducing the mortality and the morbidity of lung cancer over last several decades; however, these therapies have limitations. Thus, complementary and alternative therapies with herbal medicine have recently been introduced in the Western medical society. However, few studies have reported on the anti-cancer activity of regular ginseng extract on a molecular biological level.

In this study, we prepared a modified regular ginseng extract (MRGX) that reinforced some gensenosides from regular ginseng butanol-extract (GBX) by using treatments with enzymes such as laminarinase and pectinase for stronger anti-cancer effect. The assessments of the gene expression profiles in human lung cancer samples by using cDNA microarray technology showed that some genes were down-regulated while others were up-regulated during the MRGX-treatment process. Because gene expression might change during the lung cancer cell death process, such studies should use specific components and not serum that contains a mixture of gensenosides.

2. Materials and Methods

2.1. Preparation of MRGX extract

The root of regular ginseng (4 yr old) was purchased from the National Agricultural Cooperative Foundation in Chuncheongnam-do, Korea. A total of 20 g of pulverized ginseng root powder was suspended in 380 ml of distilled water and then sterilized at 121°C for 15 min. The ginseng butanol extract (GBX) contained compounds with specific anti-lung cancer activity. We used GBX as a control of regular ginseng. In addition, the suspension was treated with an aliquot of filter-sterilized commercial enzymes (laminarinase: 100 mg, pectinase: 100 mg) with equimolar ratio (1:1, specific activity unit); the mixture was then incubated at 40°C for 2 days and evaporated to dryness at 60°C. These enzymes-modified ginseng powders were suspended in 400 ml of 80% (v/v) methanol. The suspension was treated in an ultrasound bath for 5 min and filtered through Whatman No. 2 filter paper. The filtrates were combined and evaporated to dryness at 50°C. The extract was diluted to a concentration of 10% (w/v) in 70% ethanol. In the present study, the finally-obtained sample was called the modified regular ginseng extract (MRGX).

2.2. Cell culture

Human lung cancer (A549) cells were obtained from American Type Culture Collection (Rockville, MD). Cell lines were grown in DMEM (Dulbecco Modified Eagle’s Medium, Sigma, USA) supplemented with 10% (v/v) FBS (Fetal Bovine Serum, GIBCO, NY) and 1% (v/v) penicillin-streptomycin (GIBCO, NY). The cells were incubated at 37°C with 5% (v/v) CO₂ for 24 h. The density of A549 cells was adjusted to 5 × 10⁴ cells/well in a 96-well plate. After a 24-h incubation, the cells were treated with MRGX at 50 μg/ml concentrations. The appropriate dose was determined by evaluating the cytotoxicity of MRGX for 24 h. To the cell solution, 10 μl of cell-counting kit-8 solution (Dojindo, Japan) was added for 1 h. Cell viability was determined by using a microplate reader (Sunrise, Tecan, Switzerland) to measuring the absorbance at 450 nm.

2.3. Microarray analysis

For the microarray analysis of the MRGX-treated lung cancer cells, a human twin 44K cDNA chip was used for the transcription profiling analysis. Total RNA was extracted from vehicle- or MRGX-treated lung cancer cells, and cDNA probes were prepared by using reverse transcription of 50 μg of RNA in the presence of aminoallyl-dUTP, followed by coupling with Cy3 dye (vehicle) or Cy5 dye (MRGX-treated). The mixed Cy3- and Cy5-labeled RNA from lung cancer cells (A549) was hybridized with one side of the Twin Chip™ Human 44K (Genocheck, Seoul, Korea), and that from MRGX-treated cells was hybridized with the other side of the chip.

Fluorescent images were quantified and normalized, as described previously [11]. This experiment was repeated four times. Genes were considered to be differentially expressed when the global M value, log₂ (R/G), exceeded |1.0| (twofold) with a P-value < 0.05 after a significance analysis of the microarray (SAM). A student’s t-test was applied to assess the statistical significance of the differential expression of any gene between young and senescent HDFs (Human Dermal Fibroblasts) at each time point after MRGX treatment. To analyze the biological significance of the changes, we searched the gene ontology clone annotation (http://www.geneontology.org) and categorized the array data into specific gene groups. To verify the microarray data, we analyzed the total RNA extracted from cells by using a semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) and 2% agarose gel electrophoresis.

2.4. Ontology-related network analysis

To study the biological functions of ontology-relate regulated genes and proteins through their interaction network, we conducted a bioinformatic protein network anal-
ysis by using an ingenuity pathways analysis (IPA, http://www.ingenuity.com). The IPA identifies the protein interaction network on the basis of a regularly-updated “Ingenuity Pathways Knowledge-base.” The updatable database containing millions of individual protein-protein relationships was retrieved from the biological literature. Network generation was optimized to include as many proteins from the inputted expression profile as possible and aimed at the production of highly-connected networks.

2.5. Semi-quantitative RT-PCR confirmation
Total RNA was extracted from young and senescent cells by using an acid guanidinium thiocyanate phenol-chloroform extraction-based method. To compare the relative amounts of mRNA in young and senescent cells, we performed a semi-quantitative RT-PCR. A series of mixtures was prepared by mixing RNA from young and senescent cells as indicated, so that each mixture had the same total amount of RNA (2 μg) in a constant volume (12 μL). The RT reaction was carried out in a final volume of 20 μL by using Superscript II reverse transcriptase according to the manufacturer’s protocol, and 4 μL of the final RT product mix was then PCR amplified. The primer sets used were 5'-ATCTGGCAACCTAGCTGC-3' and 5'-GTGCTTCCA-CATGTCCTCAC-3' for IL8, 5'-GACATCATGGGTGGCT-TTGC-3' and 5'-TCCTACGAAGTGAGCATC-3' for MMP7 and 5'-GTGAGGAAGCCCAAGCTACT-3' and 5'-ATGTCCAGGTGGCTTCTTCC-3' for PLAUR.

2.6. Western blot analysis
Expression levels of signaling proteins were examined by using Western blot analyses. In brief, 30 μg of denatured protein were run using 12% polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose membrane. The transferred nitrocellulose membrane was stained with Ponceau S to position the proteins. The blotted membrane was blocked for 1 h with 5% (w/v) skimmed milk in TTBS (Tween-20 and Tris-buffered saline), followed by incubation with HRP-conjugated rabbit anti-goat IgG with a 1:2000 dilution in TBS-0.1% (v/v) Tween-20 for 5 min. The membrane was rinsed three times with TBS-0.1% (v/v) Tween-20 for 5 min. The Pierce® ECL system (Thermo Scientific, San Jose, CA) was used to develop the proteins on an X-ray film (Kodak, Seoul, Korea). The expression levels of proteins were quantitatively analyzed with GAPDH as an internal control.

3. Result

3.1. Analysis of MRGX related gene expression in lung cancer cells
To analyze the MRGX-related gene expression in lung cancer cells, we used a cDNA microarray analysis approach. Microarray data were filtered and combined using gene symbols; then, network analyses were performed. The clustered microarray data showed that groups of genes in lung cancer and MRGX-treated lung cancer cells were regulated differently by 50 mM of MRGX. Genes with at least one global M |1.0| are shown in Tables 1 and 2. A total of 109 were ultimately identified and categorized in terms of their cellular functions by using gene ontology and IPA annotation: The Venn-diagram for migration (45%), metabolism (45%), and cell death and survival (10%) are shown in Fig. 1. The relative abundances of the regulated genes between lung and MRGX-treated lung cancer cells determined by using a semi-quantitative analysis, as described previously [12], are listed in Table 1. Based on the criterion of a fold change > 1.0, 80 were up-regulated (Fig. 1A) whereas 29 were down-regulated in MRGX-treated lung cancer cells (Fig. 1B) compared to lung cancer.

3.2. Network analysis based on Gene Ontology analysis
To explore key MRGX-related proteins in the gene ontology analysis of gene functions, we used IPA to query 109 proteins belonging to the proteins up- and down-regulated by MRGX, resulting in a distinct interconnected network of 21 proteins (Fig. 2). There were quantitative and qualitative alterations in MRGX-treated lung cancer cells between the regulation groups. Among them, interleukin 8 (IL8) was identified as the center of the MRGX-related protein network in lung cancer cells. IL-8 is a lung giant-cell carcinoma-derived chemotactic protein that binds CXCR1 and CXCR2 [13]. In the present study, IL-8 is one of critical chemo-attractants responsible for leukocyte recruitment, cancer proliferation, and angiogenesis, and the potential mechanism of IL-8 production from human non-small-cell lung cancer was investigated [14]. IL-8 was decreased dramatically in the MRGX-treated lung cancer cells. The protein-protein network analysis suggests that IL-8 is a major protein that interacts with multiple proteins and that it is directly or indirectly down-regulated or up-regulated in MRGX-treat lung cancer cells. IL-8-linked proteins include chemokine (C-X-C motif) ligand 2 (CXCL2), MMP7 and PLAUR. PLAUR plays a key role in lung cancer [15]. However, the detailed mechanism has not been studied in lung cancer. Herein, the level of IL-8 in the MRGX-treat-
### Table 1: Up-regulated gene list

| Genbank Accession ID | Gene Symbol | Protein Name                                                                 | Fold Change |
|----------------------|-------------|-------------------------------------------------------------------------------|-------------|
| NM_001159699         | FHL1        | four and a half LIM domains 1                                                 | 2.158241    |
| NM_001184717         | TIPARP      | TCDD-inducible poly (ADP-ribose) polymerase                                   | 1.982939    |
| NM_000612             | IGFL2       | insulin-like growth factor 2, somatotedin A                                   | 1.871624    |
| NM_002203             | ITGA2       | integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)                  | 1.83122     |
| NM_002526             | NTS8E       | 5′-nucleotidase, ecto (CD73)                                                  | 1.808669    |
| NM_032141             | NSRP1       | nuclear speckle splicing regulatory protein 1                                 | 1.734883    |
| NM_000104             | CYP1B1      | cytochrome P450, family 1, subfamily B, polypeptide 1                         | 1.694663    |
| NM_000104             | UBAH3B      | ubiquitin associated and SH3 domain containing B                              | 1.555049    |
| NM_003822             | NR5A2       | nuclear receptor subfamily 5, group A, member 2                               | 1.503484    |
| NM_000104             | CYP1B1      | cytochrome P450, family 1, subfamily B, polypeptide 1                         | 1.493132    |
| NM_005891             | ACAT2       | acetyl-CoA acetyltransferase 2                                                | 1.490562    |
| NM_004487             | GOLGB1      | golgin B1                                                                    | 1.477885    |
| NM_005633             | SOS1        | son of sevenless homolog 1                                                   | 1.463831    |
| NM_004454             | ETV5        | ets variant 5                                                                | 1.445291    |
| NM_001723             | DST         | dystonin                                                                     | 1.432908    |
| NM_00137550           | LRRFIP1     | leucine rich repeat (in FLII) interacting protein 1                          | 1.410612    |
| NM_004925             | AQP3        | aquaporin 3 (Gill blood group)                                                | 1.374904    |
| NM_00173463           | KIF21A      | kinesin family member 21A                                                     | 1.363929    |
| NM_001105244          | PTPRM       | protein tyrosine phosphatase, receptor type, M                                | 1.351404    |
| NM_002483             | CEACAM6     | carcinoembryonic antigen-related cell adhesion molecule 6                     | 1.34875     |
| NM_022118             | RBM26       | RNA binding motif protein 26                                                  | 1.333552    |
| NM_003861             | DCAF5       | DDB1 and CUL4 associated factor 5                                             | 1.315943    |
| NM_001023587          | ABC5C       | ATP-binding cassette, sub-family C (CFTR/MRP), member 5                       | 1.313605    |
| NM_003344             | UBE2H       | ubiquitin-conjugating enzyme E2H                                              | 1.287242    |
| NM_00174087           | NCOA3       | nuclear receptor coactivator 3                                                | 1.286065    |
| NM_001946             | DUSP6       | dual specificity phosphatase 6                                                | 1.253698    |
| NM_003870             | IQGAP1      | IQ motif containing GTPase activating protein 1                               | 1.250989    |
| NM_00193455           | NSUN2       | NOP2/Sun RNA methyltransferase family, member 2                               | 1.24729     |
| NM_001025356          | ANO6        | anoctamin 6                                                                  | 1.2465      |
| NM_021925             | C2orf43     | chromosome 2 open reading frame 43                                            | 1.242904    |
| NM_001098272          | HMGC51      | 3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)                           | 1.210537    |
| NM_005063             | SCD         | stearoyl-CoA desaturase (delta-9-desaturase)                                  | 1.201598    |
| NM_014331             | SLC7A11     | solute carrier family 7 (anionic amino acid transporter light chain) member 1 | 1.186217    |
| NM_001146276          | NCEH1       | neutral cholesterol ester hydrolase 1                                        | 1.185191    |
| NM_000693             | ALDH1A3     | aldehyde dehydrogenase 1 family, member A                                    | 1.183801    |
| NM_004100             | EYA4        | eyes absent homolog 4                                                         | 1.172664    |
| Accession      | Gene      | Description                                              | Score |
|---------------|-----------|----------------------------------------------------------|-------|
| NM_001032281  | TFPI      | tissue factor pathway inhibitor                           | 1.171704 |
| NM_001008938  | CKAP5     | cytoskeleton associated protein 5                         | 1.171153 |
| NM_001142568  | BBX       | bobby sox homolog                                        | 1.168988 |
| NM_001113239  | HIPK2     | homeodomain interacting protein kinase 2                 | 1.164367 |
| NM_016284     | CNOT1     | CCR4-NOT transcription complex, subunit 1                | 1.162079 |
| NM_004775     | B4GALT6   | UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6 | 1.160644 |
| NM_014646     | LPIN2     | lipin 2                                                  | 1.14518  |
| NM_172037     | RDH10     | retinol dehydrogenase 10 (all-trans)                     | 1.144987 |
| NM_001005376  | PLAUR     | plasminogen activator, urokinase receptor                | 1.13818  |
| NM_001077181  | CDC14B    | cell division cycle 14B                                  | 1.135517 |
| NM_001083592  | ROR1      | receptor tyrosine kinase-like orphan receptor 1          | 1.135517 |
| NM_007350     | PHLDA1    | plekstrin homology-like domain, family A, member 1       | 1.131633 |
| NM_020738     | KIDINS220 | kinase D-interacting substrate, 220kDa                  | 1.11258  |
| NM_017694     | MFSD6     | major facilitator superfamily domain containing 6        | 1.110798 |
| NM_031897     | CACNG6    | calcium channel, voltage-dependent, gamma subunit 6     | 1.094036 |
| NM_001130107  | KLC1      | kinesin light chain 1                                    | 1.093194 |
| NM_001174159  | SH2D4A    | SH2 domain containing 4A                                 | 1.081308 |
| NM_012090     | MACF1     | microtubule-actin crosslinking factor                     | 1.078294 |
| NM_001025081  | MBP       | myelin basic protein                                     | 1.077327 |
| NM_001001894  | TTC3      | tetratricopeptide repeat domain 3                        | 1.068548 |
| NM_001190438  | NCO1      | nuclear receptor corepressor 1                           | 1.068334 |
| NM_001077484  | SLC38A1   | solute carrier family 38, member 1                       | 1.066673 |
| NM_001962     | EFNA5     | ephrin-A5                                                | 1.064771 |
| NM_014827     | ZC3H11A   | zinc finger CCCH-type containing 11A                     | 1.058277 |
| NM_015554     | GLCE      | glucuronic acid epimerase                                | 1.051527 |
| NM_012197     | RABGAP1   | RAB GTPase activating protein 1                           | 1.04982 |
| NM_001184998  | KIAA0430  | KIAA0430                                                 | 1.049638 |
| NM_015635     | GAPVD1    | GTPase activating protein and VPS9 domains 1             | 1.048175 |
| NM_152641     | ARID2     | AT rich interactive domain 2                             | 1.044772 |
| NM_203365     | RAPH1     | Ras association (RalGDS/AF-6) and plekstrin homology domains 1 | 1.041108 |
| NM_145693     | LPIN1     | lipin 1                                                  | 1.039824 |
| NM_004462     | FDFT1     | farnesyl-diphosphate farnesyltransferase 1               | 1.03898 |
| NM_024817     | THSD4     | thrombospondin, type I, domain containing 4              | 1.038791 |
| NM_001002860  | BTBD7     | BTB (POZ) domain containing 7                            | 1.038677 |
| NM_001081550  | THOC2     | THO complex 2                                            | 1.035132 |
| NM_001128626  | SPIRE1    | spire homolog                                            | 1.026552 |
| NM_001197030  | ANKHD1    | ankyrin repeat and KH domain containing 1                | 1.023268 |
| NM_001142614  | EHBPI     | EH domain binding protein 1                              | 1.02271 |
| NM_014689     | DOCK10    | dedicator of cytokinesis 10                              | 1.013887 |

http://www.journal.ac
| Genbank Accession ID | Gene Symbol | Gene Name | Protein Name | Fold Change |
|----------------------|-------------|-----------|--------------|-------------|
| NM_000585            | IL15        | interleukin 15 | interleukin 15 | -1.00511    |
| NM_006763            | BTG2        | BTG family, member 2 | BTG family, member 2 | -1.01812    |
| NM_005195            | CEBPD       | CCAAT/enhancer binding protein (C/EBP), delta | CCAAT/enhancer binding protein (C/EBP), delta | -1.02364    |
| NM_001166599         | FAM122B     | family with sequence similarity 122B | family with sequence similarity 122B | -1.02554    |
| NM_152603            | ZNF567      | zinc finger protein 567 | zinc finger protein 567 | -1.02634    |
| NM_000366            | TPM1        | tropomyosin 1 (alpha) | tropomyosin 1 (alpha) | -1.03063    |
| NM_001416            | EIF4A1      | eukaryotic translation initiation factor 4A1 | eukaryotic translation initiation factor 4A1 | -1.03982    |
| NM_001008405         | BCAP29      | B-cell receptor-associated protein 29 | B-cell receptor-associated protein 29 | -1.05437    |
| NM_001145275         | ZFY         | zinc finger protein, Y-linked | zinc finger protein, Y-linked | -1.06241    |
| NM_152487            | TMEM56      | transmembrane protein 56 | transmembrane protein 56 | -1.0712     |
| NM_002423            | MMP7        | matrix metallopeptidase 7 (matrilysin, uterine) | matrix metallopeptidase 7 (matrilysin, uterine) | -1.07991    |
| NM_000598            | IGFBP3      | insulin-like growth factor binding protein 3 | insulin-like growth factor binding protein 3 | -1.08553    |
| NM_004617            | TM4SF4      | transmembrane 4 L six family member 4 | transmembrane 4 L six family member 4 | -1.09854    |
| NM_006761            | YWHAE       | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide | -1.11487    |
| NM_014696            | GPRIN2      | G protein regulated inducer of neurite outgrowth 2 | G protein regulated inducer of neurite outgrowth 2 | -1.13021    |
| NM_004428            | EFNA1       | ephrin-A1 | ephrin-A1 | -1.18932    |
| NM_020374            | C12orf4     | chromosome 12 open reading frame 4 | chromosome 12 open reading frame 4 | -1.19108    |
| NM_001143668         | AMIGO2      | adhesion molecule with Ig-like domain 2 | adhesion molecule with Ig-like domain 2 | -1.20343    |
| NM_144643            | SCLT1       | sodium channel and clathrin linker 1 | sodium channel and clathrin linker 1 | -1.2175     |
| NM_003821            | RIPK2       | receptor-interacting serine-threonine kinase 2 | receptor-interacting serine-threonine kinase 2 | -1.3159     |
| NM_002522            | NPTX1       | neuronal pentraxin I | neuronal pentraxin I | -1.36692    |
| NM_006528            | TFPI2       | tissue factor pathway inhibitor 2 | tissue factor pathway inhibitor 2 | -1.45019    |
| NM_005949            | MT1F        | metallothionein 1F | metallothionein 1F | -1.45041    |
| NM_001511            | CXCL1       | chemokine (C-X-C motif) ligand 1 | chemokine (C-X-C motif) ligand 1 | -1.74981    |
| NM_000584            | IL8         | interleukin 8 | interleukin 8 | -1.94821    |
| NM_002090            | CXCL3       | chemokine (C-X-C motif) ligand 3 | chemokine (C-X-C motif) ligand 3 | -2.2981     |
| NM_002089            | CXCL2       | chemokine (C-X-C motif) ligand 2 | chemokine (C-X-C motif) ligand 2 | -2.38018    |
| AF432419             | C75F        | C75F | C75F | -5.11758    |
| AY227436             | DSP1        | drug-sensitive protein 1 | drug-sensitive protein 1 | -6.3106     |
ed lung-cancer cells was 1.9-fold down-regulated while PLAUR was up-regulated. The accumulation of PLAUR has been associated with lung cancer therapy.

4. Discussion

Microarray analyses may result in biased data, and inconsistent results are often obtained when different methods are used. Therefore, the data need to be validated with a specific method. Because we measured gene expression, we wanted to validate our screening results with conventional methods such as RT-PCR and Western blots. We selected genes from each of the two up- and down-regulated group of genes for validation by using semi-quantitative RT-PCR and Western blot analyses. Four genes were selected based on their high fold-change (a large number) in the analysis and the availability of commercial antibodies. IL-8, MMP7 and PLAUR are examples of up- and down-regulated genes. The mRNA levels of IL-8 and MMP7 were decreased in the MRGX-treated lung cancer cells, and the protein levels of IL-8 and MMP7 also decreased. The semi-quantitative PCR and Western blot results showed that PLAUR was increased (Fig. 3).

5. Conclusion

In conclusion, most MRGX-responsive genes are up-regulated transiently in A549 cells, but are down-regulated in a sustained manner in lung cancer cells. These genes might be involved in the altered MRGX responsiveness observed during the cancer migration and metabolism processes. The roles of these genes in MRGX responses, including the cell cycle and cell growth, should be evaluated in further studies by modulating their expressions.

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Figure 2 Network analysis based on a gene ontology analysis. Two-fold changed total proteins regulated by MRGX were queried by IPA, resulting in a distinct interconnected network of 21 proteins.

Figure 3 Validation of microarray analysis results by using Western blot and RT-PCR analyses. Expressions of IL8 and MMP were examined by using a Western blot analysis with an anti-IL8 and MMP7 antibody (left panel) and by using a RT-PCR analysis (right panel). Expression of PLAUR was examined as described above. Beta-actin protein was used as a loading control for Western blot, and GAPDH was used as a normalization control for semi-quantitative PCR.

| Protein | mRNA |
|---------|------|
| IL8     |      |
| MMP7    |      |
| PLAUR   |      |

Figure 3 Validation of microarray analysis results by using Western blot and RT-PCR analyses. Expressions of IL8 and MMP were examined by using a Western blot analysis with an anti-IL8 and MMP7 antibody (left panel) and by using a RT-PCR analysis (right panel). Expression of PLAUR was examined as described above. Beta-actin protein was used as a loading control for Western blot, and GAPDH was used as a normalization control for semi-quantitative PCR.

References

1. Yun TK, Lee YS, Lee YH, Kim SI, Yun HY. Anticarcinogenic effect of Panax ginseng C.A. Meyer and identification of active compounds. J Kor Med Sci. 2001;16 Suppl:S6-18.

2. He BC, Gao JL, Luo J, Shen J, Wang L, Zhou Q, et al. Ginsenoside Rg3 inhibits colorectal tumor growth through the down-regulation of Wnt/β-catenin signaling. Int J Oncol. 2011;38(2):437-45.

3. Kim DY, Park MW, Yuan HD, Lee HJ, Kim SH, Chung SH. Compound K induces apoptosis via CAMK-IV/AMPK pathway in HT-29 colon cancer cells. J Agric Food Chem. 2009;57(22):10573-8.
4. Park HM, Kim SJ, Kim JS, Kang HS. Reactive oxygen mediated ginsenoside Rg3- and Rh2-induced apoptosis in hepatoma cells through mitochondrial signaling pathways. Food Chem Toxicol. 2012;50(3):2736-41.
5. Mai TT, Moon J, Song Y, Viet PQ, Phuc PV, Lee JM, et al. Ginsenoside F2 induces apoptosis accompanied by protective autophagy in breast cancer stem cells. Cancer Lett. 2012;321(2):144-53.
6. Shibata S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. J Kor Med Sci. 2001;16 Suppl:S28-37.
7. Hwang JW, Oh JH, Yoo HS, Lee YW, Cho CK, Kwon KR, et al. Mountain ginseng extract exhibits anti-lung cancer activity by inhibiting the nuclear translocation of NF-xB. Am J Chin Med. 2012;40(1):187-202.
8. Lee DG, Jang SI, Hwang JW, Yang KE, Yoon SJ, Lee ZW, et al. Anti-proliferative effects of ginsenosides extracted from mountain ginseng on lung cancer. Chin J Integ Med. Forthcoming 2013.
9. Ai Z, Yin L, Zhou X, Zhu Y, Zhu D, Yu Y, Feng Y. Inhibition of survivin reduces cell proliferation and induces apoptosis in human endometrial cancer. Cancer. 2006;107(4):746-56.
10. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009;59(4):225-49.
11. Kim JY, Lee JH, Park GW, Cho K, Kwon KH, Park YM. Utility of electrophoretically derived protein mass estimates as additional constraints in proteome analysis of human serum based on MS/MS analysis. Proteomics. 2005;5(13):3376-85.
12. Ishihama Y, Oda Y, Tabata T, Sato T, Nagasu T, Rappsilber J. Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. Mol Cell Proteomics. 2005;4(9):1265-72.
13. González-Quintela A, Domínguez-Santalla MJ, Loidi L, Quinteiro C, Perez LF. Relation of tumor necrosis factor (TNF) gene polymorphisms with serum concentrations and in vitro production of TNF-alpha and interleukin-8 in heavy drinkers. Alcohol. 2004;34(2-3):273-7.
14. Zhang Y, Wang L, Zhang M, Jin M, Bai C, Wang X. Potential mechanism of interleukin-8 production from lung cancer cells: an involvement of EGF-EGFR-PI3K-Akt-Erk pathway. J Cell Physiol. 2012;227(1):35-43.
15. Wang IM, Stepaniants S, Boie Y, Mortimer JR, Kennedy B, Elliott M. Gene expression profiling in patients with chronic obstructive pulmonary disease and lung cancer. Am J Respir Crit Care Med. 2008;177(4):402-11.