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# Toxicogenomics of A375 human malignant melanoma cells treated with arbutin

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**Summary**

Although arbutin is a natural product and widely used as an ingredient in skin care products, its effect on the gene expression level of human skin with malignant melanoma cells is rarely reported. We aim to investigate the genotoxic effect of arbutin on the differential gene expression profiling in A375 human malignant melanoma cells through its effect on tumorigenesis and related side-effect. The DNA microarray analysis provided the differential gene expression pattern of arbutin-treated A375 cells with the significant changes of 324 differentially expressed genes, containing 88 up-regulated genes and 236 down-regulated genes. The gene ontology of differentially expressed genes was classified as belonging to cellular component, molecular function and biological process. In addition, four down-regulated genes of AKT1, CLECSF7, FGFR3, and LRP6 served as candidate genes and correlated to suppress the biological processes in the cell cycle of cancer progression and in the downstream signaling pathways of malignancy of melanocytic tumorigenesis.

## Introduction

Arbutin, a natural compound of beta-D-glucopyranoside of hydroquinone, is widely used as an ingredient in skin care products [1]. It is effective in the treatment of various cutaneous hyperpigmentations and has the inhibitory effects on the melanogenesis in melanoma cells [2–4]. However, recent findings have raised serious questions of concern regarding both the safety and side-effect of arbutin. Although some inhibitory effects of arbutin on melanogenesis in melanoma cells and its mechanism have been elucidated, the intensive study of its biological effect on human genomics level in the regulation of malignant melanogenesis through the functional effect on carcinogenesis is not clear and rarely reported.

Recently, the high throughput technology of DNA microarray has become important in geneomic research and used to analyze gene expression profiling of human melanomas [5–7]. Since the study of gene expression profiling in human melanoma cells treated with arbutin has never been reported, it would be very challenging to use the DNA microarray in studying the biological effect of arbutin on malignant melanoma cells through its action on anti-cancer property. In this study, we aim to investigate the genotoxic effect of arbutin on the differential gene expression profiling

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in A375 human malignant melanoma cells through its effect on melanocytic tumorigenesis for cancer therapy and other related side-effects. We used the DNA microarray technology and bioinformatic tools to explore the differential gene expression profiling in arbutin-treated A375 cells and to classify the differentially expressed genes by Gene Oncology, which provided more understanding of the genetic basis of metabolic and cellular responses in tumergenesis of human skin cancer. In addition, we compared the biological effects of arbutin and kojic acid on differentially expressed genes in A375 cells and also proposed the hypothetical pathway of arbutin-responsive genes correlated in the suppression of tumorigenesis. These candidate genes may lead to consequently aid for early diagnostic and therapeutic applications.

## Materials and methods

### *Materials*

Human malignant melanoma cell line, A375 (CRL-1619), was obtained from the ATCC (Rockville, MD, USA). Dulbecco's Modified Eagle's Medium (DMEM), D-PBS and Trypsin-EDTA were purchased from Atlanta Biologicals (Norcross, GA, USA). Fetal bovine serum, sodium pyruvate, antibiotic-antimycotic and TRIzol reagent were purchased from Invitrogen (Carlsbad, CA, USA). Arbutin, MTT (methylthiazoletetrazolium) and sodium bicarbonate were purchased from Sigma (St. Louis, MO, USA). RNeasy Mini Kit was purchased from Qiagen (Valencia, CA, USA). Cyanine 3- and 5-labeled CTP were purchased from Perkin-Elemer/NEN Life Science (Boston, MS, USA). RNA 6000 Nano LabChip Kit, Low RNA Input Fluorescent Linear Amplification Kit, Human 1A Oligo Microarray Kit (V2), in situ Hybridization Kit Plus, and Stabilization and Drying Solution were purchased from Agilent Technologies (Palo Alto, CA, USA). All other chemicals were purchased from Sigma (St. Loius, MO, USA).

### *Cell culture*

A375 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1.5 g/l sodium bicarbonate, 1 mM sodium pyruvate and 1% antibiotic-antimycotic in a humidified incubator with 5% $CO_2$ and 95% air at 37 °C. Upon confluence, the cells were detached by treatment with 0.05% trypsin and 0.53 mM EDTA. During subculture, the medium was replaced every 2–3 days.

### *Treatment with arbutin and cell viability test*

To perform cell attachment, the cells were seeded at $5.0 \times 10^3$ cells/well in 96-well tissue culture-treated plates (NUNC$^{TM}$, Roskilde, Denmark) in 100 $\mu$l of culture media. They were then washed with PBS and were cultured either alone or in the presence of various concentrations of arbutin (0.32, 1.6, 8, 40, 200 and 1000 $\mu$g/ml) for 72 h. Arbutin was suspended in 0.01% DMSO, and the control was added with the same concentration of DMSO in order to reduce the variation of cell growth inhibition. Triplicate experiments were done for each pair group of arbutin concentration and control. The cell viability was determined by MTT assay [8]. The percentage of cell growth inhibition was calculated as follows: Inhibition (%) = [1-$A_{550}$ nm (arbutin)/$A_{550}$ nm (control)] $\times$ 100%.

### *RNA preparation and quantitative measurement*

After the cells were treated with 8 $\mu$g/ml arbutin for 24 h, RNA was extracted by a modified method using TRIzol combined with the RNeasy Mini Kit. The total RNA was quantified by a UV spectrophotometer and RNA quality was evaluated by capillary electrophoresis on an Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip Kit. For each paired sample, the number of independent paired samples of cultured cells that were treated with arbutin either present or absent was done in triplicate.

### *RNA amplification and labeling*

Targets of cRNA were amplified and fluorescently labeled from 0.5 $\mu$g total RNA in each reaction using the Agilent Low RNA Input Fluorescent Linear Amplification kit. For each sample pair, the control sample was labeled with Cy3 and the treated sample was labeled with Cy5. After purification using Qiagen's RNeasy mini-spin

columns, the quantification, quality and size distribution of the labeled cRNA targets were then determined by ultraviolet (UV) spectrophotometry and RNA 6000 Nano LabChip Assay.

*Microarray hybridization*

Hybridization was performed following the Agilent oligonucleotide microarray hybridization user's manual and Agilent in situ Hybridization Kit Plus. Briefly, 2 $\mu$g of the labeled cRNA per channel was mixed with 50 $\mu$l 10× control targets and nuclease-free water to a final volume of 240 $\mu$l. Each sample tube was added with 10 $\mu$l of 25× fragmentation buffer and was incubated at 60 °C in a water bath in the dark for 30 min. The reaction was then terminated by adding 250 $\mu$l of 2× hybridization buffer. A volume of 500 $\mu$l of hybridization mix was applied to Agilent's Human 1A Oligonucleotide Microarray, containing 20,173 (60 mer) oligonucleotide probes, and hybridized in a hybridization rotation oven at 60 °C for 17 h. The slides which disassembled in 6× SSPE and 0.005% N-Lauroylsarcosine were washed with 6× SSPE, 0.005% N-Lauroylsarcosine for 1 min at room temperature, then with 0.06× SSPE, 0.005% N-Lauroylsarcosine for 1 min and with Stabilization and Drying Solution for 30 s.

*Data analysis and bioinformatics*

The microarray chip was scanned using an Agilent G2565BA Microarray Scanner System, and the Agilent Feature Extraction software 7.5 used defaults for all parameters including a parameterized error model to compute the significance ($p$-values) of log ratios. The image quantities of interest produced by the image analysis methods were the (R, G) fluorescence intensity pairs for each gene on each array probe, where R = red for Cy5 and G = green for Cy3. An 'MA-plot' was used to represent the normalized (R, G) data, where $M = \log R/G$ and $A = \log \sqrt{(R \times G)}$ [9].

For the bioinformatics tools to search gene ontology, we used the combination of databases to gain information on gene name and symbol, subcellular location, family and superfamily classification, chromosome map location, similar gene, molecular function, biochemical function-related protein and references. The gene search programs were used the following sequential order of databases: NCBI (http://www.ncbi.nlm.nih.gov), Ensembl (http://www.ensembl.org), TIGR (http://www.tigr.org) and GeneCards (http://www.genecards.org). In addition, the category classification of gene expression was done by in-house Bulk Gene Search System for Java (BGSSJ) program that is a searching system accomplished by open database connectivity, UniGene database and Gene Ontology knowledgebase, and is available at http://servx8.sinica.edu.tw/bgss-cgi-bin/gene.pl. On the other hand, the protein search program used the Swiss-Prot/TrEMBL (http://www.expasy.ch/sprot), Proteome (http://www.proteome.com/databases/HumanPD/reports) and PubMed (http://www.ncbi.nl.nih.gov/PubMed). Moreover, the combining pathway databases of Biocarta (http://www.biocarta.com), KEGG (http://www.genome.ad.jp/kegg/pathway.html) and the PubMed literature were used to search the correlated signaling pathways and mechanisms in the malignant melanogenesis of A375 cells.

*Real-time quantitative PCR (RT-qPCR)*

Specific oligonucleotide primer pairs were designed using the analysis Beacon designer 4.00 (Premier Biosoft International) and were then used for real-time quantitative PCR. The sequences of the primers used are: (1) AKT1 (NM_005163, 150 bp): forward 5′-ACCATCACACCACCTGACCAAG-3′ and reverse 5′-CGCCTCTCCATCCCTCCAAG-3′; (2) CLECSF7 (NM_130441, 184 bp): forward 5′-ATCAACACCAGGGAAGAACAGG-3′ and reverse 5′-TCGCACAACGCTCAT CAAGG-3′; (3) FGFR3 (NM_000142, 102 bp): forward 5′-CTGCCAGCCGAGGAGGAG-3′ and reverse 5′-CACCACCAGGATGAACAGG AAG-3′; (4) LRP6 (NM_002336, 183 bp): forward 5′-AGAATGACCTCAGTGGCAACAG-3′ and reverse 5′-GGGTGGCGGTGGGTA GAG-3′. The specificity of each primer pair was tested by using a common reference RNA (Stratagene) to perform a RT-PCR reaction, followed by DNA 500 chip run on Bioanalyzer 2100 (Agilent Technologies) to check the size of the PCR product. Primer pairs of production predicted product size and no other side-product were chosen to conduct the following SYBR reaction.

Real-time quantitative PCR (RT-qPCR) was performed on the LightCycler instrument 1.5 using LightCycler® FastStart DNA Master$^{PLUS}$ SYBR

Green I kit (Roche Applied Science). Briefly, 10 $\mu$l reactions, containing 2 $\mu$l Master Mix, 2 $\mu$l of 0.25 $\mu$M gene specific primer and 6 $\mu$l of cDNA, were generated as described above. Each sample, which consisted of cDNA generated from all the samples as detailed above, was run in duplicate. The PCR parameters were 95 °C for 10 s, 45 cycles of 95 °C for 10 s, 60 °C for 3 s and 72 °C for 10 s. At the end of the program a melt curve analysis was made, the data were automatically analyzed by the system and an amplification plot was generated for each cDNA sample. From each of these plots, the LightCycler software calculated the threshold cycle (Cp), defined as the fractional cycle number at which the fluorescence reached the baseline. The fold expression or repression of the target gene relative to $\beta$-actin in each sample was then calculated by the formula:

$$2^{-\Delta\Delta Cp} \text{ where } \Delta\Delta Cp = \Delta Cp_{\text{arbutin sample}} - \Delta Cp_{\text{control sample}},$$
$$\Delta Cp_{\text{arbutin sample}} = Cp_{\text{arbutin-stimulated target gene}} - Cp_{\text{arbutin-stimulated } \beta\text{-actin}},$$
$$\Delta Cp_{\text{control sample}} = Cp_{\text{control target gene}} - Cp_{\text{control } \beta\text{-actin}}$$

## Results

### *Inhibition effect of arbutin on A375 cells*

The cell growth of A375 cells was directly inhibited by the increase of arbutin concentrations. After 72 h, the highest concentration of 1000 $\mu$g/ml arbutin allowed up to 40% inhibition of A375 cell growth, while the lower concentrations of 0.32, 1.6, 8 and 40 $\mu$g/ml arbutin allowed the inhibition of cell growth by only less than 20% (Figure 1). It is indicated that the A375 cell growth was not strongly affected by all arbutin concentrations. In addition, there were no morphological changes in the cells treated with 0.32–40 $\mu$g/ml arbutin. According to the safety recommendation of 1% prescription of drug in human skin care product [10], the concentration of 8 $\mu$g/ml arbutin was appropriated in the recommended concentration. Also, there was no morphological change in the cells within 24 h in the presence of 8 $\mu$g/ml arbutin and the inhibition of cell growth was less than 10%. However, it has never been reported the biological effect of arbutin on the gene expression profiling in A375 cells and other genotoxic side-effects. Thus, it is very interesting to study the genotoxic effect of arbutin on human skin and on the tumorigenesis that are become our purposes of this study.

### *Microarray analysis of differential gene expression in A375 cells after arbutin treatment*

The microarray results showed different fluorescence intensities between Cy3 (control) and Cy5 (arbutin-treated A375 cells), corresponding to the differential expression level of thousands of genes, and represented the different Cy3 and Cy5 signal intensities with a $p$-value of less than 0.01 ($p < 0.01$) that were considered to be significantly different (Figure 2). An analysis of gene expression changes with human oligonucleotide array revealed that the total number of 357 differentially expressed probes was matched with approximately 324 listed genes, containing 88 up-regulated genes (Table 1) and 236 down-regulated genes (Table 2). In addition, we used the in-house BGSSJ program to search the gene ontology that classified the gene category, according to cellular component, molecular function and biological process. The summary of the category classification of differentially expressed genes in arbutin-treated A375 cells is shown in Figure 3. The total numbers of 75 up-regulated genes and 123 down-regulated genes were classified into each category with different ratios. Some of genes could be classified into more than one category, according to their cellular component, molecular function and biological process. The expressed genes were found to be located in the subcellular region rather than organelle, extracellular region, protein complex and extracellular matrix, respectively. It may indicate the signal transduction pathway occurring from extracellular region to intracellular region because some of genes served as receptors and/or transporters and have mediated the signaling genes

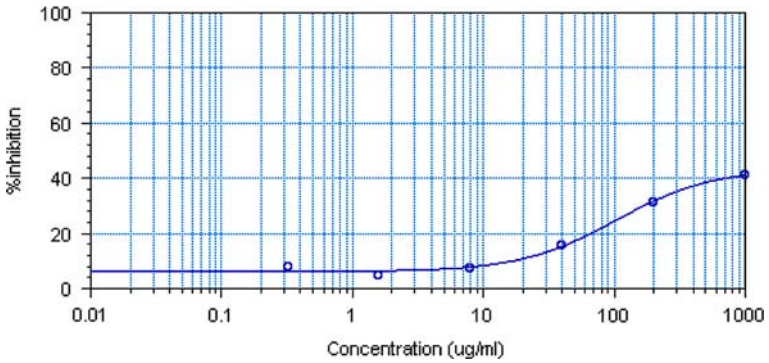


*Figure 1.* Effect of arbutin concentrations on the growth inhibition of A375 cells at 72 h. The concentrations of arbutin used were 0.32, 1.6, 8, 40, 200 and 1000 $\mu$g/ml.

to another region. So, it is possible to be found in both regions. Otherwise, they possessed the molecular functions in different ratios of binding, catalytic activity, signal transducer activity, transcription regulator activity, enzyme regulator activity, transporter activity, structural molecule activity and translation regulator activity. Moreover, the expressed genes involved some biological processes, such as cellular process, physiological process, development and regulation of biological process. Thus, this category classification provided more useful information of gene ontology in response to arbutin treatment. Furthermore, we suggest that all of the differentially expressed genes may be regulated by various gene networks and possess related molecular functions involving biological processes in the regulation of malignant tumorigenesis.

### *RT-qPCR validation of array analysis*

The arbutin-responsive genes in A375 cells chosen for RT-qPCR analysis were selected from the order of $p$ values that were determined by microarray analysis, and from the database search of biological functions correlated with carcinogenesis. The selected genes were validated by RT-qPCR analysis to confirm the result of gene expression level with microarray data and the result of gene expression level of four expressed

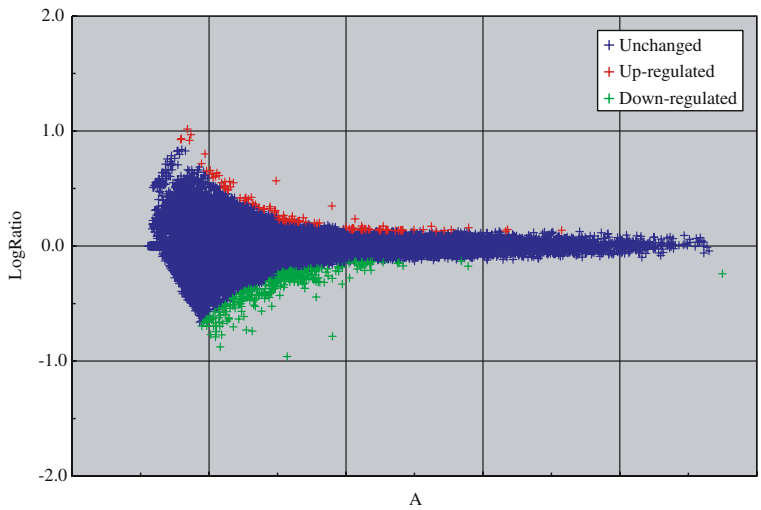


*Figure 2.* MA-plot of microarray data represents the differentially expressed probes of arbutin-treated A375 cells. A representative probe of comparative experiments (normal mRNA labeled with Cy3 and arbutin-treated mRNA labeled with Cy5) where R = log (Cy5/Cy3) represents the common log ratio of the two dyes and A = [log (Cy5) + log (Cy3)]/2 is the average logarithmic fluorescence intensities of both channels. The gene expression pattern shows approximately 357 significantly differentially expressed probes ($p < 0.01$). Blue + represents any data point whose log ratio is not significantly different from 0; red + and green + represent data points whose log ratios are greater or less than 0, respectively ($p < 0.01$).

*Table 1*. Up-regulated genes (88 genes) in arbutin-treated A375 cells.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| A_23_P170387 | A_23_P170387 | 10.41 | 3.28E−07 | Unknown |
| KLHL13 | NM_033495 | 9.31 | 4.34E−07 | Kelch-like 13 (Drosophila) (KLHL13) |
| EPX | NM_000502 | 8.42 | 3.14E−05 | Eosinophil peroxidase (EPX) |
| TSC1 | NM_000368 | 8.31 | 3.02E−06 | Tuberous sclerosis 1 (TSC1) |
| SCN1B | NM_001037 | 6.32 | 8.38E−07 | Sodium channel, voltage-gated, type I, beta (SCN1B), transcript variant a |
| A_23_P86598 | A_23_P86598 | 5.20 | 3.15E−05 | Unknown |
| GAGED3 | NM_130777 | 4.54 | 1.86E−05 | G antigen, family D, 3 (GAGED3) |
| IL3RA | NM_002183 | 4.50 | 5.11E−05 | Interleukin 3 receptor, alpha (low affinity) (IL3RA) |
| A_23_P32966 | A_23_P32966 | 4.31 | 6.51E−06 | Unknown |
| FBXO16 | NM_172366 | 4.22 | 2.94E−05 | F-box only protein 16 (FBXO16) |
| VPS39 | NM_015289 | 4.10 | 7.83E−06 | Vacuolar protein sorting 39 (yeast) (VPS39) |
| PARG | NM_003631 | 4.05 | 2.23E−05 | Poly (ADP-ribose) glycohydrolase (PARG) |
| NRP2 | NM_201266 | 3.94 | 5.99E−05 | Neuropilin 2 (NRP2), transcript variant 1, mRNA |
| ANKRD23 | NM_144994 | 3.65 | 2.80E−06 | Ankyrin repeat domain 23 (ANKRD23) |
| FLJ13611 | NM_024941 | 3.61 | 1.22E−05 | Hypothetical protein FLJ13611 (FLJ13611) |
| THC1991976 | THC1991976 | 3.55 | 3.44E−05 | AF244540 immunodominant membrane protein precursor {Aster yellows phytoplasma} |
| AEBP2 | NM_153207 | 3.54 | 1.63E−06 | AE binding protein 2 (AEBP2) |
| ZBTB20 | NM_015642 | 3.36 | 4.44E−05 | Zinc finger protein 288 (ZNF288) |
| NR2F1 | NM_005654 | 3.27 | 1.43E−05 | Nuclear receptor subfamily 2, group F, member 1 (NR2F1) |
| KLRC4 | NM_013431 | 3.24 | 6.16E−05 | Killer cell lectin-like receptor subfamily C, member 4 (KLRC4) |
| CAPNS2 | NM_032330 | 3.17 | 8.71E−05 | Calpain small subunit 2 (CAPNS2) |
| APOM | NM_019101 | 3.12 | 5.14E−05 | Apolipoprotein M (APOM) |
| SLC23A3 | NM_144712 | 2.65 | 5.34E−06 | Solute carrier family 23 (nucleobase transporters), member 3 (SLC23A3) |
| A_23_P10060 | A_23_P10060 | 2.62 | 2.04E−05 | Unknown |
| C18orf11 | NM_022751 | 2.62 | 7.04E−05 | Chromosome 18 open reading frame 11 (C18orf11) |
| DDEF1 | NM_018482 | 2.58 | 3.21E−05 | Development and differentiation enhancing factor 1 (DDEF1) |
| HIST1H2AC | NM_003512 | 2.55 | 2.23E−05 | Histone 1, H2ac (HIST1H2AC) |
| HH114 | NM_032499 | 2.49 | 6.79E−05 | Hypothetical protein HH114 (HH114) |
| CPE | NM_001873 | 2.35 | 4.34E−05 | Carboxypeptidase E (CPE) |
| TARDBP | NM_007375 | 2.28 | 4.80E−05 | TAR DNA binding protein (TARDBP) |
| ADAM7 | NM_003817 | 2.20 | 8.80E−06 | A disintegrin and metalloproteinase domain 7 (ADAM7) |
| KIAA1706 | NM_030636 | 2.16 | 7.74E−05 | KIAA1706 protein (KIAA1706) |
| CCAR1 | NM_018237 | 2.11 | 2.38E−05 | Cell division cycle and apoptosis regulator 1 (CCAR1) |
| CPNE5 | NM_020939 | 2.10 | 6.83E−05 | Copine V (CPNE5) |
| A_23_P251953 | A_23_P251953 | 2.09 | 7.22E−05 | Unknown |
| GPR32 | NM_001506 | 2.04 | 2.59E−05 | G protein-coupled receptor 32 (GPR32) |
| HAND1 | NM_004821 | 2.02 | 2.72E−05 | Heart and neural crest derivatives expressed 1 (HAND1) |
| TCF12 | NM_207038 | 1.97 | 6.27E−05 | Transcription factor 12 (HTF4, helix-loop-helix transcription factors 4) (TCF12), transcript variant 4 |
| GPD2 | NM_000408 | 1.85 | 9.10E−05 | Glycerol-3-phosphate dehydrogenase 2 (mitochondrial) (GPD2) |
| RAB3GAP | D31886 | 1.81 | 6.14E−05 | KIAA0066 |
| DNAJB9 | NM_012328 | 1.76 | 5.26E−05 | DnaJ (Hsp40) homolog, subfamily B, member 9 (DNAJB9) |
| MCM8 | NM_032485 | 1.73 | 0.000405 | MCM8 minichromosome maintenance deficient 8 (S. cerevisiae) (MCM8), transcript variant 1 |
| BC029424 | BC029424 | 1.71 | 0.000766 | cDNA clone MGC:32677 IMAGE:4285958 |
| FKSG43 | NM_032033 | 1.71 | 0.000588 | FKSG43 gene (FKSG43) |
| CYP2U1 | NM_183075 | 1.70 | 0.000978 | Cytochrome P450, family 2, subfamily U, polypeptide 1 (CYP2U1) |
| FLJ40432 | NM_152523 | 1.70 | 0.000645 | Hypothetical protein FLJ40432 (FLJ40432) |

*Table 1*. Continued.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| SPATA13 | NM_153023 | 1.69 | 0.000371 | Spermatogenesis associated 13 (SPATA13) |
| ECE1 | NM_001397 | 1.68 | 0.000863 | Endothelin converting enzyme 1 (ECE1) |
| MGC12466 | NM_033213 | 1.66 | 0.000982 | Hypothetical protein MGC12466 (MGC12466) |
| NEU4 | NM_080741 | 1.66 | 0.000833 | Sialidase 4 (NEU4) |
| TES | NM_152829 | 1.66 | 0.000333 | Testis derived transcript (3 LIM domains) (TES), transcript variant 2 |
| POLE2 | NM_002692 | 1.64 | 0.000625 | Polymerase (DNA directed), epsilon 2 (p59 subunit) (POLE2) |
| GALC | NM_000153 | 1.63 | 0.000618 | Galactosylceramidase (Krabbe disease) (GALC) |
| DLGAP4 | NM_014902 | 1.62 | 0.0007 | Discs, large (Drosophila) homolog-associated protein 4 (DLGAP4), transcript variant 1 |
| INPP4B | NM_003866 | 1.58 | 0.000504 | Inositol polyphosphate-4-phosphatase, type II, 105kDa (INPP4B) |
| ELKS | NM_178037 | 1.55 | 0.000781 | Rab6-interacting protein 2 (ELKS), transcript variant beta |
| FAM49B | NM_016623 | 1.49 | 0.00073 | Hypothetical protein BM-009 (BM-009) |
| MKI67 | NM_002417 | 1.49 | 0.00069 | Antigen identified by monoclonal antibody Ki-67 (MKI67) |
| RNF134 | NM_032154 | 1.49 | 0.000814 | Ring finger protein 134 (RNF134) |
| NMI | NM_004688 | 1.47 | 0.000352 | N-myc (and STAT) interactor (NMI) |
| EFTUD1 | NM_024580 | 1.43 | 0.00258 | Hypothetical protein FLJ13119 (FLJ13119) |
| IQCB1 | NM_014642 | 1.43 | 0.00317 | IQ calmodulin-binding motif containing 1 (IQCB1) |
| UBQLN1 | NM_013438 | 1.43 | 0.00305 | Ubiquilin 1 (UBQLN1), transcript variant 1 |
| ANKRD17 | NM_032217 | 1.41 | 0.00391 | Ankyrin repeat domain 17 (ANKRD17), transcript variant 1 |
| EPS8 | NM_004447 | 1.41 | 0.00383 | Epidermal growth factor receptor pathway substrate 8 (EPS8) |
| HIST1H1A | NM_005325 | 1.41 | 0.00382 | Histone 1, H1a (HIST1H1A) |
| FBXO11 | NM_025133 | 1.40 | 0.00418 | F-box only protein 11 (FBXO11), transcript variant 1 |
| HUMAUANTIG | NM_013285 | 1.40 | 0.0048 | Nucleolar GTPase (HUMAUANTIG) |
| RPS6KA3 | NM_004586 | 1.40 | 0.00514 | Ribosomal protein S6 kinase, 90kDa, polypeptide 3 (RPS6KA3) |
| A_23_P17152 | A_23_P17152 | 1.39 | 0.00504 | Unknown |
| CCT8 | D13627 | 1.39 | 0.00404 | KIAA0002 |
| DDX46 | NM_014829 | 1.39 | 0.00499 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 46 (DDX46) |
| HTATSF1 | NM_014500 | 1.39 | 0.00475 | HIV TAT specific factor 1 (HTATSF1) |
| BNIP2 | NM_004330 | 1.38 | 0.00782 | BCL2/adenovirus E1B 19kDa interacting protein 2 (BNIP2) |
| CGI-94 | NM_016037 | 1.38 | 0.0049 | Comparative gene identification transcript 94 (CGI-94) |
| OSBPL11 | NM_022776 | 1.38 | 0.00687 | Oxysterol binding protein-like 11 (OSBPL11) |
| PITPNB | NM_012399 | 1.38 | 0.00591 | Phosphotidylinositol transfer protein, beta (PITPNB) |
| PPHLN1 | NM_201515 | 1.38 | 0.00613 | Periphilin 1 (PPHLN1), transcript variant 2 |
| SPTLC1 | NM_006415 | 1.38 | 0.00681 | Serine palmitoyltransferase, long chain base subunit 1 (SPTLC1), transcript variant 1 |
| BCAS2 | NM_005872 | 1.37 | 0.0078 | Breast carcinoma amplified sequence 2 (BCAS2) |
| C20orf36 | NM_018257 | 1.37 | 0.00705 | Chromosome 20 open reading frame 36 (C20orf36) |
| KDELC1 | NM_024089 | 1.37 | 0.00851 | KDEL (Lys-Asp-Glu-Leu) containing 1 (KDELC1) |
| PGM2 | NM_018290 | 1.37 | 0.00679 | Phosphoglucomutase 2 (PGM2) |
| SF3B1 | NM_012433 | 1.37 | 0.0066 | Splicing factor 3b, subunit 1, 155kDa (SF3B1) |
| CRI1 | NM_014335 | 1.36 | 0.00788 | CREBBP/EP300 inhibitory protein 1 (CRI1) |
| TMEM32 | NM_173470 | 1.36 | 0.008 | Hypothetical protein LOC93380 (LOC93380) |
| ARL6IP | NM_015161 | 1.35 | 0.00971 | ADP-ribosylation factor-like 6 interacting protein (ARL6IP) |
| CGI-49 | NM_016002 | 1.35 | 0.00979 | CGI-49 protein (CGI-49) |

genes was agreed with the DNA microarray expression data (Table 3). There were v-akt murine thymoma viral oncogene homolog 1 (AKT1), c-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 7 (CLECSF7) or C-type lectin domain family 4, member C (CLEC4C), fibroblast growth factor receptor 3 (FGFR3) and low density

*Table 2*. Down-regulated genes (236 genes) in arbutin-treated A375 cells.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| 7h3 | NM_033025 | 0.74 | 0.00989 | Homo sapiens hypothetical protein FLJ13511 (7h3) |
| ATAD3B | NM_031921 | 0.74 | 0.00887 | ATPase family, AAA domain containing 3B (ATAD3B) |
| ARFIP2 | NM_012402 | 0.73 | 0.00693 | ADP-ribosylation factor interacting protein 2 (arfaptin 2) (ARFIP2) |
| ASNA1 | NM_004317 | 0.73 | 0.00759 | ArsA arsenite transporter, ATP-binding, homolog 1 (bacterial) (ASNA1) |
| MGC1203 | NM_024296 | 0.73 | 0.00731 | Hypothetical protein MGC1203 (MGC1203) |
| RGS19IP1 | NM_005716 | 0.73 | 0.0074 | Regulator of G-protein signaling 19 interacting protein 1 (RGS19IP1) |
| PIN1 | NM_006221 | 0.72 | 0.00526 | Protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1 (PIN1) |
| PSMD13 | NM_175932 | 0.72 | 0.00461 | Proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 (PSMD13) |
| SSP411 | NM_022827 | 0.72 | 0.00595 | Hypothetical protein FLJ21347 (FLJ21347) |
| CBX6 | NM_014292 | 0.71 | 0.00474 | Chromobox homolog 6 (CBX6) |
| CD151 | NM_004357 | 0.71 | 0.00415 | CD151 antigen (CD151) |
| JUN | NM_002228 | 0.71 | 0.00434 | v-Jun sarcoma virus 17 oncogene homolog (avian) (JUN) |
| PSMD3 | NM_002809 | 0.71 | 0.00298 | Proteasome (prosome, macropain) 26S subunit, non-ATPase, 3 (PSMD3) |
| ARHGEF2 | NM_004723 | 0.70 | 0.00218 | Rho/Rac guanine nucleotide exchange factor (GEF) 2 (ARHGEF2) |
| CD2BP2 | NM_006110 | 0.70 | 0.0022 | CD2 antigen (cytoplasmic tail) binding protein 2 (CD2BP2) |
| MGC5395 | NM_024060 | 0.69 | 0.00159 | Hypothetical protein MGC5395 (MGC5395) |
| PIGQ | NM_004204 | 0.69 | 0.00196 | Phosphatidylinositol glycan, class Q (PIGQ), transcript variant 2 |
| DPP7 | NM_013379 | 0.68 | 0.00152 | Dipeptidylpeptidase 7 (DPP7) |
| SDC4 | NM_002999 | 0.67 | 0.000717 | Syndecan 4 (amphiglycan, ryudocan) (SDC4) |
| UBE2M | NM_003969 | 0.67 | 0.000537 | Ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast) (UBE2M) |
| AP1S1 | NM_057089 | 0.66 | 0.000638 | Adaptor-related protein complex 1, sigma 1 subunit (AP1S1) |
| APTX | NM_175073 | 0.66 | 0.000804 | Aprataxin (APTX), transcript variant 1, mRNA |
| ARL2 | NM_001667 | 0.66 | 0.00061 | ADP-ribosylation factor-like 2 (ARL2 |
| Beta4GalNAc-T4 | NM_178537 | 0.64 | 0.000641 | Hypothetical protein FLJ25045 (FLJ25045) |
| BQ189193 | BQ189193 | 0.64 | 0.000536 | cDNA clone UI-E–EJ1-ajv-o-09–0-UI 5', mRNA |
| DPYSL2 | NM_001386 | 0.64 | 0.000382 | Dihydropyrimidinase-like 2 (DPYSL2) |
| FTHFSDC1 | NM_015440 | 0.64 | 0.000892 | Formyltetrahydrofolate synthetase domain containing 1 (FTHFSDC1) |
| ADRB3 | NM_000025 | 0.63 | 0.000605 | Adrenergic, beta-3-, receptor (ADRB3) |
| HOXB8 | NM_024016 | 0.63 | 0.000603 | Homeo box B8 (HOXB8) |
| ILT10 | NM_024317 | 0.63 | 0.000451 | Leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 5 (ILT10) |
| MAFK | NM_002360 | 0.63 | 0.000352 | v-Maf musculoaponeurotic fibrosarcoma oncogene homolog K (avian) (MAFK) |
| SYMPK | NM_004819 | 0.62 | 5.93E–05 | Symplekin (SYMPK) |
| A_23_P123389 | A_23_P123 389 | 0.61 | 0.000899 | Unknown |
| ENST00000329697 | ENST00000329697 | 0.61 | 0.000304 | Xenobiotic/medium-chain fatty acid:CoA ligase |
| FLJ12242 | NM_024681 | 0.61 | 0.00089 | Hypothetical protein FLJ12242 (FLJ12242) |
| GPRC5D | NM_018654 | 0.61 | 0.000349 | G protein-coupled receptor, family C, group 5, member D (GPRC5D) |
| INA | NM_032727 | 0.61 | 0.000181 | Internexin neuronal intermediate filament protein, alpha (INA) |
| MGC33867 | BC004501 | 0.61 | 5.93E–05 | Hypothetical protein MGC33867 (cDNA clone IMAGE:3835289) |
| PCTK1 | NM_033018 | 0.61 | 7.91E–05 | PCTAIRE protein kinase 1 (PCTK1) |
| PUNC | NM_004884 | 0.61 | 0.000473 | Putative neuronal cell adhesion molecule (PUNC) |
| CREBL1 | NM_004381 | 0.60 | 4.72E–05 | cAMP responsive element binding protein-like 1 (CREBL1) |
| KLPH | NM_207338 | 0.60 | 0.000992 | Likely ortholog of mouse klotho lactase-phlorizin hydrolase related protein (KLPH) |
| NYD-SP29 | NM_145172 | 0.60 | 0.000941 | Testis development protein NYD-SP29 (NYD-SP29) |

*Table 2*. Continued.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| OR8B8 | ENST00000 327930 | 0.60 | 0.000414 | Olfactory receptor 8B8 (Olfactory receptor TPCR85) (Olfactory-like receptor JCG8) [Source:SWISSPROT;Acc:Q15620] |
| TULP3 | AF045583 | 0.60 | 0.000618 | Tubby like protein 3 (TULP3) |
| MGC35308 | NM_175922 | 0.59 | 4.91E−05 | Hypothetical protein MGC35308 (MGC35308) |
| OTX1 | NM_014562 | 0.59 | 0.000342 | Orthodenticle homolog 1 (Drosophila) (OTX1) |
| SNIP1 | NM_024700 | 0.59 | 0.000202 | Smad nuclear interacting protein (SNIP1) |
| CEACAM1 | NM_001712 | 0.58 | 0.000249 | Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) (CEACAM1) |
| DLX1 | NM_178120 | 0.58 | 0.000192 | Distal-less homeo box 1 (DLX1) |
| FAM3D | NM_138805 | 0.58 | 0.000344 | Family with sequence similarity 3, member D (FAM3D) |
| KIAA1811 | NM_032430 | 0.58 | 0.000299 | KIAA1811 protein (KIAA1811) |
| MIDN | AK075506 | 0.58 | 7.37E−05 | cDNA PSEC0204 fis, clone HEMBA1002706. |
| MS4A6E | NM_139249 | 0.58 | 0.000482 | Membrane-spanning 4-domains, subfamily A, member 6E (MS4A6E) |
| SFXN2 | NM_178858 | 0.58 | 0.00037 | Sideroflexin 2 (SFXN2) |
| UPLC1 | NM_017707 | 0.58 | 0.000303 | Up-regulated in liver cancer 1 (UPLC1) |
| ZFP67 | NM_015872 | 0.58 | 0.000314 | Zinc finger protein 67 homolog (mouse) (ZFP67) |
| GPR26 | NM_153442 | 0.57 | 8.11E−05 | G protein-coupled receptor 26 (GPR26) |
| KIAA0669 | NM_014779 | 0.57 | 0.000263 | KIAA0669 gene product (KIAA0669) |
| LOC83468 | NM_031302 | 0.57 | 0.000162 | Gycosyltransferase (LOC83468) |
| MAPK8 | NM_002750 | 0.57 | 0.000385 | Mitogen-activated protein kinase 8 (MAPK8) |
| SERPINA5 | NM_000624 | 0.57 | 2.64E−05 | Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5 (SERPINA5) |
| TREH | NM_007180 | 0.57 | 0.000633 | Trehalase (brush-border membrane glycoprotein) (TREH) |
| AKT1[a] | NM_005163 | 0.56 | 2.14E−05 | v-Akt murine thymoma viral oncogene homolog 1 (AKT1) |
| MGC33407 | NM_178525 | 0.56 | 6.68E−05 | Hypothetical protein MGC33407 (MGC33407) |
| MVD | NM_002461 | 0.56 | 0.000205 | Mevalonate (diphospho) decarboxylase (MVD) |
| PRRX1 | NM_006902 | 0.56 | 1.66E−05 | Paired related homeobox 1 (PRRX1) |
| RAB6B | NM_016577 | 0.56 | 6.40E−05 | RAB6B, member RAS oncogene family (RAB6B) |
| SERPIND1 | NM_000185 | 0.56 | 9.94E−05 | Serine (or cysteine) proteinase inhibitor, clade D (heparin cofactor), member 1 (SERPIND1) |
| AFAP | NM_021638 | 0.55 | 0.000187 | Actin filament associated protein (AFAP) |
| AK026716 | AK026716 | 0.55 | 6.38E−05 | cDNA: FLJ23063 fis, clone LNG04745 |
| ENPP1 | NM_006208 | 0.55 | 1.98E−05 | Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) |
| FLJ11535 | NM_024888 | 0.55 | 4.58E−06 | Hypothetical protein FLJ11535 (FLJ11535) |
| PACSIN2 | NM_007229 | 0.55 | 4.91E−05 | Protein kinase C and casein kinase substrate in neurons 2 (PACSIN2) |
| SLC13A2 | NM_003984 | 0.55 | 0.000104 | Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2 (SLC13A2) |
| TEX13B | NM_031273 | 0.55 | 0.000121 | Testis expressed sequence 13B (TEX13B) |
| A_23_P86779 | A_23_P86779 | 0.54 | 4.10E−05 | Unknown |
| A_23_P9270 | A_23_P9270 | 0.54 | 1.36E−05 | Unknown |
| AY032661 | AY032661 | 0.54 | 0.000159 | Lung squamous cell carcinoma related protein-2 mRNA |
| IFIT3 | NM_001549 | 0.54 | 0.000147 | Interferon-induced protein with tetratricopeptide repeats 4 (IFIT4) |
| IL23A | NM_016584 | 0.54 | 2.18E−05 | Interleukin 23, alpha subunit p19 (IL23A) |
| NM_206995 | NM_206995 | 0.54 | 1.08E−05 | Chromosome 10 open reading frame 1 (C10orf1) |
| PRKX | NM_005044 | 0.54 | 0.000123 | Protein kinase, X-linked (PRKX) |
| ACVRL1 | NM_000020 | 0.53 | 4.56E−07 | Activin A receptor type II-like 1 (ACVRL1) |
| ARX | NM_139058 | 0.53 | 1.37E−05 | Aristaless related homeobox (ARX) |
| CLECSF7[a] | NM_130441 | 0.53 | 2.09E−05 | C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 7 (CLECSF7) |

*Table 2*. Continued.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| MDFI | NM_005586 | 0.53 | 1.88E−06 | MyoD family inhibitor (MDFI) |
| BST2 | NM_004335 | 0.52 | 3.58E−06 | Bone marrow stromal cell antigen 2 (BST2) |
| FLJ14442 | NM_032785 | 0.52 | 1.43E−06 | Hypothetical protein FLJ14442 (FLJ14442) |
| FLJ20245 | NM_017723 | 0.52 | 6.35E−07 | Hypothetical protein FLJ20245 (FLJ20245) |
| GPR55 | NM_005683 | 0.52 | 1.23E−05 | G protein-coupled receptor 55 (GPR55) |
| HRMT1L1 | NM_001535 | 0.52 | 3.06E−05 | HMT1 hnRNP methyltransferase-like 1 (S. cerevisiae) (HRMT1L1) |
| LRP5 | NM_002335 | 0.52 | 1.73E−05 | Low density lipoprotein receptor-related protein 5 (LRP5) |
| LRP6[a] | NM_002336 | 0.52 | 3.36E−06 | LRP6 |
| M160 | NM_174941 | 0.52 | 5.81E−06 | Scavenger receptor cysteine-rich type 1 protein M160 (M160) |
| C19orf33 | NM_033520 | 0.51 | 7.70E−07 | Immortalization-upregulated protein (IMUP) |
| FRAS1 | NM_206841 | 0.51 | 2.96E−05 | Fraser syndrome 1 (FRAS1) |
| GMPR | NM_006877 | 0.51 | 2.09E−06 | Guanosine monophosphate reductase (GMPR) |
| MGC13275 | AK123855 | 0.51 | 8.94E−05 | cDNA FLJ41861 fis, clone NTONG2008672 |
| PRSS2 | NM_002770 | 0.51 | 3.41E−05 | Protease, serine, 2 (trypsin 2) (PRSS2) |
| SLCO5A1 | NM_030958 | 0.51 | 4.78E−05 | Solute carrier organic anion transporter family, member 5A1 (SLCO5A1) |
| A_23_P89499 | A_23_P89499 | 0.50 | 8.54E−06 | Unknown |
| COH1 | NM_015243 | 0.50 | 7.38E−05 | Cohen syndrome 1 (COH1), transcript variant 3, mRNA |
| CROC4 | AK124566 | 0.50 | 2.26E−05 | cDNA FLJ42575 fis, clone BRACE3008237. |
| NCALD | NM_032041 | 0.50 | 1.16E−06 | Neurocalcin delta (NCALD) |
| BAG5 | NM_004873 | 0.49 | 2.31E−07 | BCL2-associated athanogene 5 (BAG5) |
| BCKDK | NM_005881 | 0.49 | 1.99E−06 | Branched chain alpha-ketoacid dehydrogenase kinase (BCKDK) |
| BZRAP1 | NM_004758 | 0.49 | 1.16E−06 | Benzodiazapine receptor (peripheral) associated protein 1 (BZRAP1) |
| FGFR3[a] | NM_000142 | 0.49 | 8.82E−07 | FGFR3 (achondroplasia, thanatophoric dwarfism) (FGFR3) |
| KCNAB3 | NM_004732 | 0.49 | 1.78E−05 | Potassium voltage-gated channel, shaker-related subfamily, beta member 3 (KCNAB3) |
| NKD1 | NM_033119 | 0.49 | 6.73E−05 | Naked cuticle homolog 1 (Drosophila) (NKD1) |
| NM_017571 | NM_017571 | 0.49 | 6.95E−05 | Hypothetical protein LOC55580 (LOC55580) |
| OLFML1 | NM_198474 | 0.49 | 2.32E−06 | MVAL564 (UNQ564) |
| OSBP2 | AF288741 | 0.49 | 1.29E−07 | Oxysterol binding protein 2 (OSBP2) |
| RASGEF1B | NM_152545 | 0.49 | 3.17E−06 | RasGEF domain family, member 1B (RASGEF1B) |
| STARD8 | NM_014725 | 0.49 | 2.71E−05 | START domain containing 8 (STARD8) |
| BTN2A1 | NM_078476 | 0.48 | 1.59E−05 | Butyrophilin, subfamily 2, member A1 (BTN2A1) |
| TRAM2 | D31762 | 0.48 | 9.80E−08 | KIAA0057 |
| ZNF497 | NM_198458 | 0.48 | 5.89E−08 | Zinc finger protein 497 (ZNF497) |
| EFNA3 | NM_004952 | 0.47 | 1.93E−07 | Ephrin-A3 (EFNA3) |
| SFRP5 | NM_003015 | 0.47 | 1.28E−05 | Secreted frizzled-related protein 5 (SFRP5) |
| SLC25A2 | NM_031947 | 0.47 | 1.39E−07 | Solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 2 (SLC25A2) |
| WNT10A | NM_025216 | 0.47 | 6.74E−09 | Wingless-type MMTV integration site family, member 10A (WNT10A) |
| C19orf4 | NM_012109 | 0.46 | 1.27E−06 | Chromosome 19 open reading frame 4 (C19orf4) |
| FPGT | NM_003838 | 0.46 | 5.79E−06 | Fucose-1-phosphate guanylyltransferase (FPGT) |
| LRRTM4 | NM_024993 | 0.46 | 2.00E−05 | Leucine-rich repeat transmembrane neuronal 4 protein (LRRTM4) |
| ZNF483 | AB075842 | 0.46 | 1.37E−05 | mRNA for KIAA1962 protein |
| CD3G | NM_000073 | 0.45 | 8.24E−06 | CD3G antigen, gamma polypeptide (TiT3 complex) (CD3G) |
| MGC15716 | NM_032370 | 0.45 | 1.02E−06 | Hypothetical protein MGC15716 (MGC15716) |
| A_23_P122323 | A_23_P122323 | 0.44 | 1.85E−06 | Unknown |
| MGC33637 | NM_152596 | 0.44 | 3.00E−07 | Hypothetical protein MGC33637 (MGC33637) |
| A_23_P395326 | A_23_P395326 | 0.43 | 8.31E−07 | Unknown |
| COL17A1 | NM_000494 | 0.43 | 9.87E−05 | Collagen, type XVII, alpha 1 (COL17A1) |

*Table 2*. Continued.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| DNAH7 | NM_018897 | 0.43 | 7.67E−07 | Dynein, axonemal, heavy polypeptide 7 (DNAH7) |
| SLC27A4 | AF055899 | 0.43 | 6.94E−09 | Fatty acid transport protein (FATP) |
| THC1871477 | THC1871477 | 0.43 | 4.54E−06 | ALU7_HUMAN Alu subfamily SQ sequence contamination warning entry. |
| AK092138 | AK092138 | 0.42 | 1.54E−05 | cDNA FLJ34819 fis, clone NT2NE2008213 |
| CNKSR2 | AF418270 | 0.42 | 9.06E−07 | Connector enhancer of KSR2B mRNA |
| ZDHHC2 | NM_016353 | 0.42 | 2.83E−05 | Zinc finger, DHHC domain containing 2 (ZDHHC2) |
| ZP2 | NM_003460 | 0.42 | 5.84E−05 | Zona pellucida glycoprotein 2 (sperm receptor) (ZP2) |
| AY360464 | AY360464 | 0.41 | 8.63E−05 | Hypothetical protein LOC146177-like protein mRNA |
| TGFB3 | NM_003239 | 0.41 | 2.95E−05 | Transforming growth factor, beta 3 (TGFB3) |
| TLL1 | NM_012464 | 0.41 | 9.92E−05 | Tolloid-like 1 (TLL1) |
| C1orf1 | U88965 | 0.40 | 4.34E−07 | PO42 gene |
| FLJ11383 | AK021445 | 0.40 | 8.08E−05 | cDNA FLJ11383 fis, clone HEMBA1000518, weakly similar to PECANEX protein |
| HPS3 | NM_032383 | 0.40 | 3.81E−05 | Hermansky-Pudlak syndrome 3 (HPS3) |
| RSBN1 | NM_018364 | 0.40 | 2.94E−05 | Hypothetical protein FLJ11220 (FLJ11220) |
| CACNG7 | NM_031896 | 0.39 | 1.27E−07 | Calcium channel, voltage-dependent, gamma subunit 7 (CACNG7) |
| EFS | NM_005864 | 0.39 | 2.12E−07 | Embryonal Fyn-associated substrate (EFS) |
| ELA2 | NM_001972 | 0.39 | 6.18E−07 | Elastase 2, neutrophil (ELA2) |
| LRRC4 | NM_022143 | 0.39 | 1.43E−06 | Leucine rich repeat containing 4 (LRRC4) |
| MAP4K5 | NM_198794 | 0.39 | 8.47E−05 | Mitogen-activated protein kinase kinase kinase kinase 5 (MAP4K5) |
| A_23_P202572 | A_23_P202572 | 0.38 | 3.90E−06 | Unknown |
| EVI2B | NM_006495 | 0.38 | 6.35E−05 | Ecotropic viral integration site 2B (EVI2B) |
| FLJ20619 | NM_017904 | 0.38 | 5.58E−05 | Hypothetical protein FLJ20619 (FLJ20619) |
| LOC51619 | NM_015983 | 0.38 | 1.20E−06 | Ubiquitin-conjugating enzyme HBUCE1 (LOC51619) |
| UNQ689 | AY358528 | 0.38 | 1.64E−06 | Clone DNA66660 RSTI689 (UNQ689) |
| USP44 | NM_032147 | 0.38 | 5.64E−07 | Ubiquitin specific protease 44 (USP44) |
| ADAM19 | NM_023038 | 0.37 | 1.81E−07 | A disintegrin and metalloproteinase domain 19 (meltrin beta) (ADAM19) |
| BC039714 | BC039714 | 0.37 | 3.14E−06 | cDNA clone MGC:47794 IMAGE:5585073 |
| C1QB | NM_000491 | 0.37 | 5.73E−09 | Complement component 1, q subcomponent, beta polypeptide (C1QB) |
| CD160 | NM_007053 | 0.37 | 1.14E−07 | CD160 antigen (CD160) |
| MGC40179 | NM_173472 | 0.37 | 8.87E−07 | Hypothetical protein MGC40179 (MGC40179) |
| PDZK10 | AB002314 | 0.37 | 2.16E−05 | mRNA for KIAA0316 protein |
| A_23_P212149 | A_23_P212149 | 0.36 | 5.97E−05 | Unknown |
| A_23_P89841 | A_23_P89841 | 0.36 | 4.64E−06 | Unknown |
| DKFZP761H1710 | NM_031297 | 0.36 | 3.42E−12 | Hypothetical protein DKFZp761H1710 (DKFZP761H1710) |
| SLC18A2 | NM_003054 | 0.36 | 9.80E−05 | Solute carrier family 18 (vesicular monoamine), member 2 (SLC18A2) |
| TCN1 | NM_001062 | 0.36 | 1.98E−08 | Transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1) |
| ZNF354B | NM_058230 | 0.36 | 4.88E−06 | Zinc finger protein 354B (ZNF354B) |
| ABCA13 | NM_152701 | 0.35 | 1.12E−06 | ATP binding cassette gene, sub-family A (ABC1), member 13 (ABCA13) |
| AF090929 | AF090929 | 0.35 | 2.90E−07 | Clone HQ0477 PRO0477p mRNA |
| BC032472 | BC032472 | 0.35 | 9.92E−06 | Similar to caspase 4, apoptosis-related cysteine protease, clone IMAGE:5216666 |
| MGC34837 | NM_152377 | 0.35 | 5.69E−05 | Hypothetical protein MGC34837 (MGC34837) |
| TAL1 | NM_003189 | 0.35 | 3.85E−08 | T-cell acute lymphocytic leukemia 1 (TAL1) |
| TNFSF6 | NM_000639 | 0.35 | 1.29E−05 | Tumor necrosis factor (ligand) superfamily, member 6 (TNFSF6) |
| D82326 | D82326 | 0.34 | 7.13E−06 | mRNA for $Na^+$-independent neutral and basic amino acid transporter |
| TTC18 | NM_145170 | 0.34 | 8.71E−07 | Tetratricopeptide repeat-containing protein (LOC118491) |

*Table 2*. Continued.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| ADAMTS3 | NM_014243 | 0.33 | 4.34E−06 | A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 3 (ADAMTS3) |
| AGPAT3 | NM_020132 | 0.33 | 1.43E−05 | 1-Acylglycerol-3-phosphate O-acyltransferase 3 (AGPAT3) |
| BC063022 | BC063022 | 0.33 | 3.78E−06 | cDNA clone IMAGE:5246259 |
| MFAP4 | NM_002404 | 0.32 | 4.90E−06 | Microfibrillar-associated protein 4 (MFAP4) |
| DIAPH2 | NM_007309 | 0.31 | 1.82E−05 | Diaphanous homolog 2 (Drosophila) (DIAPH2) |
| GUCY1A2 | NM_000855 | 0.31 | 1.81E−06 | Guanylate cyclase 1, soluble, alpha 2 (GUCY1A2) |
| MGC35030 | BC042481 | 0.31 | 2.82E−08 | Hypothetical protein MGC35030 (cDNA clone MGC:35030 IMAGE:5164982) |
| SIN3B | AB014600 | 0.31 | 2.27E−06 | mRNA for KIAA0700 protein |
| SOCS4 | NM_080867 | 0.31 | 6.50E−05 | Suppressor of cytokine signaling 4 (SOCS4) |
| A_23_P253353 | A_23_P253353 | 0.30 | 6.70E−11 | Unknown |
| EMX2 | NM_004098 | 0.30 | 4.25E−05 | Empty spiracles homolog 2 (Drosophila) (EMX2) |
| FLJ22843 | AK026254 | 0.30 | 6.67E−06 | cDNA: FLJ22601 fis, clone HSI04471 |
| GSDM1 | NM_178171 | 0.30 | 8.77E−09 | Gasdermin (GSDM) |
| HLF | NM_002126 | 0.30 | 4.94E−07 | Hepatic leukemia factor (HLF) |
| SAA4 | NM_006512 | 0.30 | 3.45E−05 | Serum amyloid A4, constitutive (SAA4) |
| SH3GL2 | NM_003026 | 0.30 | 2.64E−05 | SH3-domain GRB2-like 2 (SH3GL2) |
| ACMSD | NM_138326 | 0.29 | 1.65E−06 | Aminocarboxymuconate semialdehyde decarboxylase (ACMSD) |
| ADAM20 | NM_003814 | 0.29 | 5.31E−06 | A disintegrin and metalloproteinase domain 20 (ADAM20) |
| C15orf26 | NM_173528 | 0.28 | 7.14E−05 | Hypothetical protein FLJ38615 (FLJ38615) |
| A_23_P29907 | A_23_P29907 | 0.27 | 6.46E−08 | Unknown |
| AX721299 | AX721299 | 0.27 | 7.60E−05 | Sequence 259 from Patent WO0220754 |
| C6orf97 | NM_025059 | 0.27 | 9.23E−06 | Chromosome 6 open reading frame 97 (C6orf97) |
| LGALS14 | NM_020129 | 0.27 | 5.56E−06 | Placental protein 13-like protein (PPL13) |
| SLC15A3 | NM_016582 | 0.27 | 1.03E−10 | Solute carrier family 15, member 3 (SLC15A3) |
| ABCC13 | NM_138726 | 0.25 | 7.39E−06 | ATP-binding cassette, sub-family C (CFTR/MRP), member 13 (ABCC13) |
| NM_152768 | NM_152768 | 0.25 | 2.33E−06 | Hypothetical protein FLJ25378 (FLJ25378) |
| PKHD1 | NM_138694 | 0.25 | 8.75E−05 | Polycystic kidney and hepatic disease 1 (autosomal recessive) (PKHD1) |
| ABCA10 | NM_080282 | 0.24 | 2.86E−05 | ATP-binding cassette, sub-family A (ABC1), member 10 (ABCA10) |
| BMPR1B | NM_001203 | 0.24 | 3.08E−07 | Bone morphogenetic protein receptor, type IB (BMPR1B) |
| C14orf148 | NM_138791 | 0.24 | 5.68E−06 | Hypothetical protein FLJ32809 (LOC122945) |
| FGF5 | NM_004464 | 0.24 | 2.93E−09 | Fibroblast growth factor 5 (FGF5) |
| ADAMTS9 | NM_020249 | 0.23 | 6.69E−05 | A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 9 (ADAMTS9) |
| CPB1 | NM_001871 | 0.23 | 5.82E−09 | Carboxypeptidase B1 (tissue) (CPB1) |
| FMNL3 | NM_175736 | 0.23 | 1.84E−07 | Formin-like 3 (FMNL3) |
| L17325 | L17325 | 0.23 | 7.47E−05 | Pre-T/NK cell associated protein (1D12A2) |
| SEMG1 | NM_003007 | 0.23 | 4.55E−05 | Semenogelin I (SEMG1) |
| SLC7A3 | NM_032803 | 0.23 | 2.09E−05 | Solute carrier family 7 (cationic amino acid transporter, y + system), member 3 (SLC7A3) |
| ZNF141 | NM_003441 | 0.23 | 2.11E−05 | Zinc finger protein 141 (clone pHZ-44) (ZNF141) |
| DMXL1 | NM_005509 | 0.22 | 1.45E−07 | Dmx-like 1 (DMXL1) |
| GPC3 | NM_004484 | 0.22 | 3.05E−06 | Glypican 3 (GPC3) |
| NEGR1 | NM_173808 | 0.22 | 5.06E−05 | Neuronal growth regulator 1 (NEGR1) |
| PDZK1 | NM_002614 | 0.22 | 2.16E−05 | PDZ domain containing 1 (PDZK1) |
| RFPL2 | NM_006605 | 0.22 | 8.26E−05 | Ret finger protein-like 2 (RFPL2) |
| ZNF167 | NM_018651 | 0.22 | 3.58E−05 | Zinc finger protein 167 (ZNF167) |
| MYO3B | NM_138995 | 0.21 | 3.06E−07 | Myosin IIIB (MYO3B) |

*Table 2*. Continued.

| Gene name | Accession no. | Ratio | *p* Value | Description |
|---|---|---|---|---|
| AK025116 | AK025116 | 0.20 | 9.77E−07 | cDNA: FLJ21463 fis, clone COL04765 |
| C20orf17 | NM_173485 | 0.20 | 4.87E−05 | Chromosome 20 open reading frame 17 (C20orf17) |
| CASP8 | NM_033357 | 0.20 | 1.57E−05 | Caspase 8, apoptosis-related cysteine protease (CASP8) |
| GPR155 | NM_152529 | 0.20 | 1.42E−09 | G protein-coupled receptor 155 (GPR155) |
| NTF5 | NM_006179 | 0.20 | 2.06E−06 | Neurotrophin 5 (neurotrophin 4/5) (NTF5) |
| RFPL3 | NM_006604 | 0.20 | 2.77E−07 | Ret finger protein-like 3 (RFPL3) |
| LOC162967 | NM_207333 | 0.19 | 1.57E−12 | Hypothetical protein LOC162967 (LOC162967) |
| IL6ST | NM_002184 | 0.18 | 1.50E−07 | Interleukin 6 signal transducer (gp130, oncostatin M receptor) (IL6ST) |
| PF4 | NM_002619 | 0.18 | 7.15E−14 | Platelet factor 4 (chemokine (C-X-C motif) ligand 4) (PF4) |
| HSD3B1 | NM_000862 | 0.17 | 3.81E−07 | Hydroxy-delta-5-steroid dehydrogenase, 3 beta-and steroid delta-isomerase 1 (HSD3B1) |
| THC1910111 | THC1910111 | 0.17 | 6.32E−09 | BC022679 D13Ertd275e protein {Mus musculus} |
| F13B | NM_001994 | 0.16 | 3.00E−08 | Coagulation factor XIII, B polypeptide (F13B) |
| ZNF41 | NM_153380 | 0.13 | 9.11E−11 | Zinc finger protein 41 (ZNF41) |

[a]The down-regulated genes were significantly expressed in agreement with RT-qPCR result.

lipoprotein receptor-related protein 6 (LRP6). They were down-regulated in arbutin-treated cells and have been found to be the tumor suppressor genes in the regulation of carcinogenesis mechanism. In addition, four differentially expressed genes were classified by BGSSJ program and showed the localization in subcellular region, except that CLECSF7 was not found in the classification. AKT1 and FGFR3 were found to be located in the organelle and extracellular regions, respectively. The molecular functions of those genes were involved in binding, catalytic activity and signal transducer activity, and their biological processes were involved in physiological process, cellular process, regulation of biological process and development.

### *Correlation of differentially expressed genes with signaling pathways of malignant melanogenesis and tumerigenesis*

Combining the pathway databases of BioCarta, KEGG and the PubMed literatures, we built the hypothetical model of signaling pathway in the cell cycle and anti-apoptosis in tumorigenesis, in which the differentially expressed genes of AKT1, FGFR3 and LRP6 in arbutin-treated A375 cells are correlated in this pathway (Figure 4). Our hypothetical model suggests that the arbutin-responsive genes of AKT1, FGFR3 and LRP6 are correlated with the AKT signaling pathway, RAS, MAPK and WNT signaling pathways, respectively. FGFR3 and LRP6 normally activated in the membrane region and mediated the correlated genes to the downstream pathways of RAS, MAPK and AKT. The down-regulation of these genes may lead to the suppression of cell proliferation, survival, differentiation and apoptosis of the arbutin-treated A375 cells. Therefore, AKT1, FGFR3 and LRP6 play a key role in the regulation of both malignant melanognesis and tumorigenesis. They may become useful candidate markers for early diagnostic and therapeutic applications of melanoma carcinogenesis.

## Discussion

For the cell treatment, we examined the effect of arbutin concentrations on the growth inhibition of A375 cells and those concentrations did not strongly inhibit cell growth even though the incubation time was up to 72 h. We used the concentration of 8 $\mu$g/ml arbutin because (i) this was the same concentration as our previous study of kojic acid [11], in which this concentration (8 $\mu$g/ml or 0.8%w/v) was less than the saftety recommendation of 1% prescription in human skin care product [10], (ii) this concentration inhibited cell growth with an inhibition of less than 10% and no morpholical change of cells, and (iii) it is avoid the differential gene expression data resulting from cell death response of the

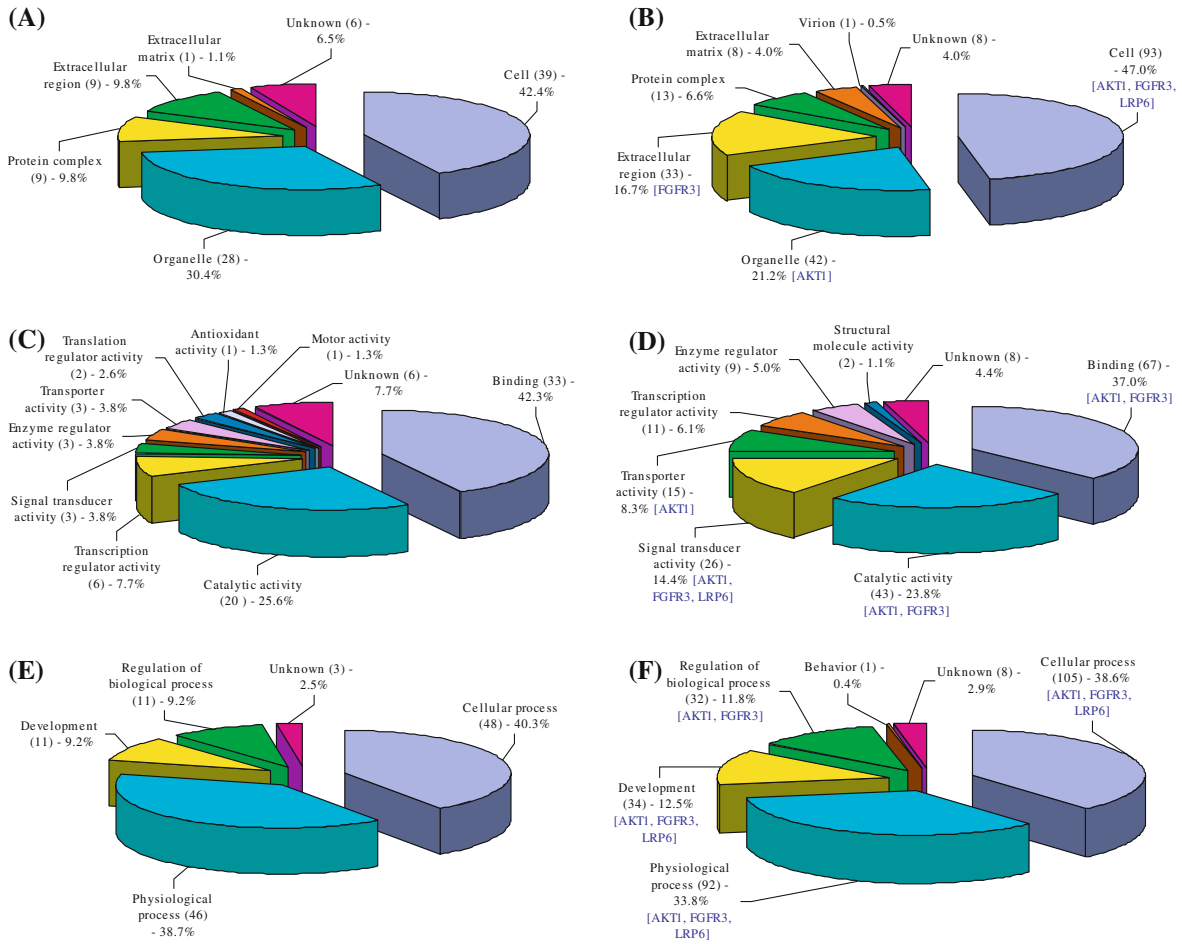


*Figure 3*. Category classification of up- and down-regulated genes in arbutin-treated A375 cells using the in-house BGSSJ program. The up- and down-regulated genes are classified according to cellular component (A, B), molecular function (C, D), and biological process (E, F), respectively. The following numbers are the classified genes and the percentage of genes in each category. Three down-regulated genes of AKT1, FGFR3 and LRP6 can be classified into each category.

cytotoxicity of a high concentration of arbutin. Under this consideration, the concentration of 8 $\mu$g/ml arbutin that is safe for use on human skin was used to study the genotoxic effect on gene expression profiling in A375 cells for examining the differential gene expression and another side-effect through the signaling pathways for cancer therapy. In order to study the effect of arbutin on the gene expression level of A375 cells, the time point of 24 h after arbutin treatment was

*Table 3*. Comparison of gene expression level of four differentially expressed genes in arbutin-treated A375 cells by microarray and RT-qPCR analysis.

| Gene name | Accessions | Description | *p* Value | Microarray | RT-qPCR |
|---|---|---|---|---|---|
| 1. AKT1 | NM_005163 | v-Akt murine thymoma viral oncogene homolog 1 | 2.14E−405 | 0.56 | 0.63 |
| 2. CLECSF7 | NM_130441 | C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 7, transcript variant 1 | 2.09E−05 | 0.53 | 0.52 |
| 3. FGFR3 | NM_000142 | FGFR3 (achondroplasia, thanatophoric dwarfism), transcript variant 1 | 8.82E−07 | 0.49 | 0.43 |
| 4. LRP6 | NM_002336 | LRP6 | 3.36E−06 | 0.52 | 0.60 |

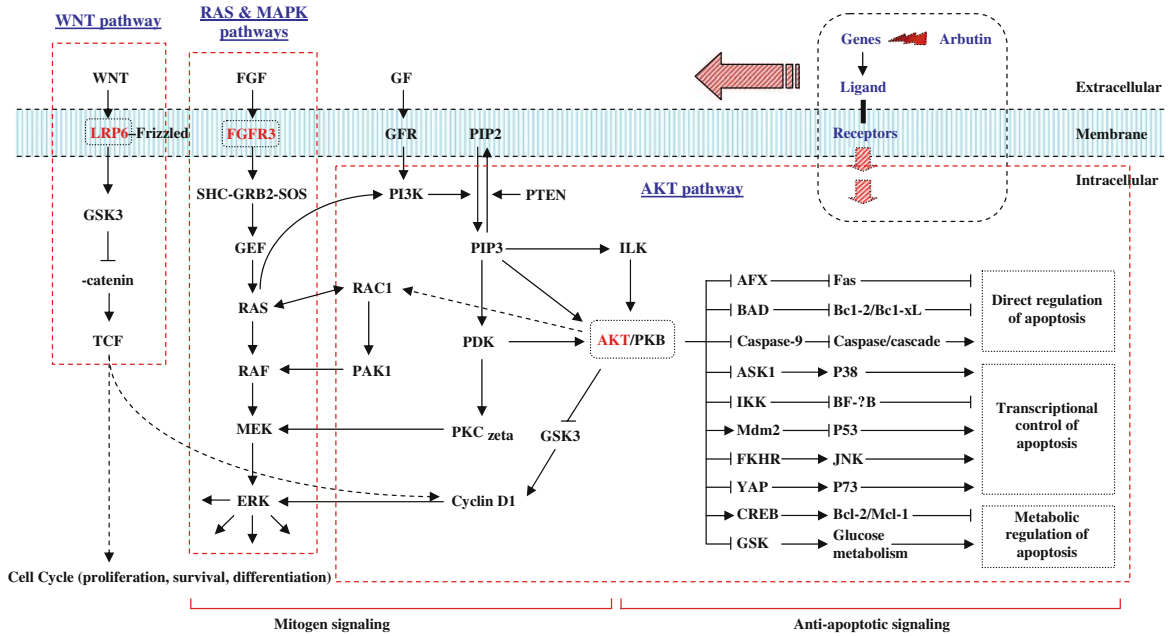


*Figure 4*. Hypothetical pathways of arbutin-responsive genes (AKT1, FGFR3 and LRP6) in A375 cells correlated with AKT, WNT, RAS and MAPK major signaling pathways in malignant melanogenesis and tumerigenesis. Abbreviations: FGF, fibroblast growth factor; GEF, guanine nucleotide exchange factor; GF, growth factor; GFR, growth factor receptor; GRB2, growth factor receptor-bound protein 2; GSK3, glycogen synthase kinase 3; ILK, integrin-linked kinase; MAPK, mitogen-activated protein kinase 3; PI3K, phosphoinoside-3 kinase; PIP2, phosphatidylinositol-4,5-biphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PDK-1, phosphainositide-dependent kinase-1; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; SON, son of sevenless homolog 1; TCF, transcription factor 7.

chosen for isolating the RNA from cells and studying the gene expression level using microarray analysis.

The changes of differentially expressed genes, which were validated by RT-qPCR analysis, gave the confirmatory quantitative results under stringent condition and revealed that the quantity or expression level of arbutin-responsive genes agreed with the DNA microarray data. It indicated that the validated genes in the robust biomarkers lists obtained precise data. Meanwhile, four significantly arbutin-responsive genes were found to be down-regulated in arbutin-treated A375 cells, and most of the genes are highly effective to the regulation of malignant melanogenesis and/or tumorigenesis process.

AKT1 (v-akt murine thymoma viral oncogene homolog 1 or serine/threonine protein kinase PKB) is frequently up-regulated in human tumors and has been shown to accelerate cell proliferation and to suppress programmed cell death. [12–14]. The gene ontology of its biological process involved in the protein amino acid phosphorylation, nitric oxide biosynthesis, anti-apoptosis, signal transduction and G-protein coupled receptor protein signaling pathway. Its activation via the AKT/PKB signaling pathway is triggered through the engagement of receptor tyrosine kinases by peptide growth factors and cytokines, and possesses a vital role in human malignancy tumorigenesis [15–17]. Consequently, the down-regulated AKT1 gene in arbutin-treated A375 cells may suppress the tumor progression of malignant transformation in melanoma cells [18–20]. Likewise, AKT1 has also been classified as a tumor suppressor gene in a wide variety of tumor cells, such as breast cancer cells, nonsmall cell lung cancer cells and pancreatic adenocarcinoma, and its down-regulation was shown to suppress the malignant progression of tumorigenesis [21–23].

C-type lectin, superfamily member 7 (CLECSF7) is a novel C-type lectin gene that maps close to the natural killer gene complex on human chromosome located at 12p13.2-p12.3 [24]. The protein encoding CLECSF7 gene, called C-type lectin domain family 4 member C (CLEC4C), is the membrane protein and involved in an oligosaccharide-recognition mechanism [25]. This

*Table 4*. Comparison of differentially expressed genes in arbutin- and kojic acid-treated A375 cells and category classification of matched genes.

| Total | Up-regulated genes | | Down-regulated genes | |
|---|---|---|---|---|
| | Arbutin | Kojic acid | Arbutin | Kojic acid |
| 1. Number | 88 | 136 | 236 | 225 |
| 2. Matched genes | 18 | | 98 | |
| 3. % Matching | 20.45 | 13.24 | 41.53 | 43.56 |
| 4. Category classification of matched genes | | | | |
| (a) Cellular component | 15 (100%) | | 70 (100%) | |
| Cell | 7 (46.67%) | | 39 (55.71%) | |
| Extracellular region | 3 (20.00%) | | 10 (14.29%) | |
| Organelle | 3 (20.00%) | | 11 (15.71%) | |
| Extracellular matrix | 1 (6.67%) | | 5 (7.14%) | |
| Protein complex | 1 (6.67%) | | 4 (5.71%) | |
| Virion | – | | 1 (1.43%) | |
| (b) Molecular function | 15 (100%) | | 60 (100%) | |
| Binding | 8 (53.33%) | | 23 (38.33%) | |
| Catalytic activity | 4 (26.67%) | | 13 (21.67%) | |
| Transcription regulator activity | 1 (6.67%) | | 4 (6.67%) | |
| Transporter activity | 1 (6.67%) | | 3 (5.00%) | |
| Signal transducer activity | – | | 13 (21.67%) | |
| Structural molecule activity | 1 (6.67%) | | 1 (1.67%) | |
| Enzyme regulator activity | – | | 3 (5.00%) | |
| (c) Biological function | 21 (100%) | | 101 (100%) | |
| Physiological process | 9 (42.86%) | | 33 (32.67%) | |
| Cellular process | 9 (42.86%) | | 43 (42.57%) | |
| Regulation of biological process | 1 (4.76%) | | 12 (11.88%) | |
| Development | 2 (9.52%) | | 13 (12.87%) | |

protein could interact with blood DC antigen type-2 (BDCA-2) receptor and involve in the target ligand into antigen processing and peptide loading compartments for presentation to T cells [26], in which its activation mediate induction of IFN-alpha/beta expression in plasmacytoid dendritic cells in the downstream pathway of apoptosis [27–29]. The down-regulated CLECSF7 gene in arbutin-treated A375 cells may be effective to the immune system and may inhibit the apoptosis of melanogenesis.

FGFR3 is one of the four high affinity tyrosine kinase receptors for the FGF family of ligands. On ligand stimulation, it undergoes dimerization and tyrosine autophosphorylation that result in cell proliferation or differentiation, depending on the cell context [30, 31]. The extracellular portion of the protein encoded by FGFR3 gene interacts with fibroblast growth factors, sets in motion an acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance [32, 33]. The inhibition of FGFR3 also induced differentiation and apoptosis in multiple myeloma and is treated as an oncogene that contributes to tumor progression in multiple myeloma including human breast cancer [34–36]. Therefore, the down-regulated FGFR3 gene may suppress the tumor progression of malignant transformation in melanoma cells.

LRP6 is a member of the expanding LDL receptor family and functions as an indispensable co-receptor for the Wnt/beta catenin signaling pathway [37–39]. It has been found to be a candidate oncogenes that promoted cancer cell proliferation and tumorigenesis by altering $\beta$-catenin subcellular distribution. In the absence or the deactivating of Wnts, $\beta$-catenin is phosphorylated by multiprotein complex, and leads to

suppress the tumorigenesis [40–43]. Thus, the down-regulated LRP6 gene in arbutin-treated A375 cells may be a key mediator in suppression of tumorigenesis of malignant transformation in melanoma cells.

According to our finding, we proposed the hypothetical model of signaling pathways of arbutin-responsive genes (AKT1, FGFR3 and LRP6) in A375 melanoma cells which were correlated with the major signaling pathways in cell cycle processes including proliferation, survival, differentiation, apoptosis and malignancy of melanogenesis (Figure 4). The AKT1 was normally activated via AKT signaling pathway that correlated with the upstream signaling pathways of RAS, MAPK, MEK and ERK [15–17]. The FGFR3 was correlated with several pathways, such as the MAPK, phospholipase C$\gamma$, RAC1 and AKT signaling pathways [30, 31]. The LRP6 is correlated with Wnt and MAPK signaling pathways [41–44]. Thus, this hypothetical model of signaling pathways is a simplified model which can represent the correlation of arbutin-responsive genes with other pathways and provides a clearer understanding of the arbutin-suppression effect on malignancy of melanocytic tumorigenesis. In addition, the CLECSF7 gene was not correlated with this hypothetical model but the deactivated CLECSF7 gene may correlate with another signaling pathway, occurring apoptosis and tumor suppression. Therefore, four down-regulated genes served as key mediators in the cell cycle's signaling pathways and possessed the biological function in the suppression of malignant transformation in arbutin-treated A375 cells.

Moreover, according to our previous study of A375 cells treated with kojic acid [11], we also compared the biological effects of arbutin and kojic acid on the gene expression in A375 cells, in which the same conditions of concentrations, cell treatment and microarray were used. It is the first study of comparative genotoxic effects between arbutin and kojic acid. The purpose of this comparison is to investigate the correlated genotoxic effects on gene expression profiling through the biological function of matched genes in the suppression of tumorigenesis. The comparison of differentially expressed genes between arbutin-and kojic acid-treated A375 cells showed the matched genes of 18 up-regulated genes and 98 down-regulated genes, including four significantly arbutin-responsive genes (AKT1, CLECSF7, FGFR3 and LRP6) (Table 4). The matched number of down-regulated genes was 5.4-fold of up-regulated genes and the matching percentages of down-regulated genes in arbutin and kojic acid were up to 40%, which may indicate the same direction of suppression of tumorigenesis in A375 cells. Although the comparison of differentially expressed genes according to category classification may not give any significant information, the category classification of matched genes can explain the similar molecular functions and biological functions of differentially expressed genes in both of arbutin-and kojic acid-treated A375 cells. Interesting, FGFR3 gene was found in both of treatments, in which FGFR3 gene may be an important key in the regulation of tumor progression of malignant transformation in melanoma cells. Therefore, we suggest that arbutin and kojic acid gave some biological effect on A375 cells with the similar differential gene expression profiling but the regulation of tumor progression may be regulated by different genes or signaling pathways.

In conclusion, we used the high throughput analysis of DNA microarray and bioinformatic tools for a global analysis of differentially expressed genes in arbutin treated A375 malignant melanoma cells. These genes were classified the gene oncology and led to an exploration of more valuable data in the regulation of melanoma carcinogenesis. In addition, four down-regulated genes served as candidate tumor suppressor genes in A375 cells after arbutin treatment. They may deactivate the regulation of malignant tumorigenesis in human malignant melanoma cells and may become useful markers for further diagnostic and therapeutic applications. Moreover, the genotoxic effect of arbutin on gene expression profiling of A375 cells was similar to kojic acid effect, which may indicate the similar regulation of malignant tumorigenesis. However, we will conduct further studies on the effects of arbutin on the biological and molecular mechanisms of human skin cells, and also examine other biological characterizations of arbutin for therapeutic applications.

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