Available online at www.tox.or.kr

# Genomewide Expression Profile of Forsythia Suspensa on Lipopolysaccaride-induced Activation in Microglial Cells

**Sung-Hwa Sohn[1,2], Eunjung Ko[1], Yangseok Kim[1], Minkyu Shin[1,2], Moochang Hong[1] & Hyunsu Bae[1,2]**

[1]Department of Physiology, College of Oriental Medicine, KyungHee University, Seoul 130-701, Korea
[2]BK21 Oriental Medical Science Center, KyungHee University, Seoul 130-701, Korea
Correspondence and request for materials should be addressed to H. S. Bae (hbae@khu.ac.kr)

Accepted 1 April 2008

## Abstract

**Microglia, which is the primary immune effector cells in the central nervous system, constitutes the first line of defense against infection and injury in the brain. The goal of this study was to determine the protective (anti-inflammation) mechanisms of forsythia suspense (FS) on LPS-induced activation of BV-2 microglial cells. The effects of FS on gene expression profiles in activated BV-2 microglial cells were evaluated using microarray analysis. BV-2 microglial cells were cultured in a 100 mm dish ($1 \times 10^7$/dish) for 24 hr and then pretreated with 1 μg/mL FS or left untreated for 30 min. Next, 1 μg/mL LPS was added to the samples and the cells were reincubated at 37 °C for 30 min, 1 hr, and 3 hr. The gene expression profiles of the BV-2 microglial cells varied depending on the FS. The oligonucleotide microarray analysis revealed that MAPK pathway-related genes such as Mitogen activated protein kinase 1 (*Mapk1*), RAS protein activator like 2 (*Rasal2*), and G-protein coupled receptor 12 (*Gpr12*) and nitric oxide biosynthesis-related genes such as nitric oxide synthase 1 (neuronal) adaptor protein (*Nos1ap*), and dimethylarginine dimethylaminohydrolase 1 (*Ddah1*) were downregulated in FS-treated BV-2 microglial cells. FS can affect the MAPK pathway and nitric oxide biosynthesis in BV-2 microglial cells.**

**Keywords:** LPS, Forsythia suspensa, Gene expression profile, Microarray

It has been proposed that microglia, which is the primary immune effector cells, play a role in host defense and tissue repair in the central nervous system (CNS). Microglia constitute the first line of defense against infection and injury in the brain[1,2], and activated microglia release neurotoxic factors such as nitric oxide (NO), as well as cytokines and chemokines, such as interleukin (IL)-1β, IL6, tumor necrosis factor (TNF)-α, and MIP-1[3,4]. Nitric oxide (NO) plays an important role in diverse physiological processes, including smooth muscle relaxation, platelet inhibition, neurotransmission, immune responses and inflammation. In addition, NO is known to be an important mediator of acute and chronic inflammation[5]. Inhibition of microglial activation, therefore, would be an effective therapeutic approach to alleviate the progression of inflammation disease, Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases[3,6-8].

During a search for new agents from medicinal plants for use in the treatment of neuroinflammation disease, the spray-dried extracts of 270 herbal medicines in a phytolibrary™ kit were tested for their ability to inhibit LPS-induced NO production in BV-2 microglial cells. Of these medicinal plants, forsythia suspense (FS) was selected for this study based on its higher inhibitory activity. FS is a well-known traditional Chinese medicine that is used in its crude form as an antipyretic, antidotal and anti-inflammatory agent for the treatment of infections, such as acute nephritis, erysipelas and ulcer[9]. Many factors contribute to the appeal of herbal medicine, and its supporters claim that herbs may both treat and prevent diseases. This adds to a deep belief that these treatments are safe because they are natural and fit into the image of a gentle and, therefore, harmless alternative to conventional medicine[10,11].

This study was conducted to determine the protective effects of FS on LPS-induced activation in BV-2 microglial cells. Specifically, FS was evaluated to determine if it could prevent LPS induced activation of microglial cells. The anti-neuroinflammation strategies and their possible mechanisms are also discussed herein.

## Gene Expression Profiles in BV-2 Microglial Cells

Gene expression profiles were significantly up- or down-regulated in the experimental groups (LPS or LPS plus FS-treated BV-2 microglial cells) when compared with the control (non-treated BV-2 microglial cells). When the microglial cells were treated with LPS and FS were evaluated, 497 up-regulated probe sets and 758 down-regulated probe sets were selected from the experimental group using approximately 45,100 oligonucloetide probes. These up- and down-regulated probes revealed that several genes involved in various functional categories were coordinately induced in the experimental groups when compared with those in the control (Figure 1). These genes were involved in processes including metabolism, signal transduction, transcription, cell cycle, transport, apoptosis, biological process, development, cell adhesion, translation and proliferation (Figure 1). Genes showing highly altered expression levels were aligned according to the magnitude of the altered expression. The most differentially expressed genes (113 up-regulated, 122 down-regulated) are listed in Table 1 and 2, which shows a comparison of the expression levels of a variety of genes between the experimental group and the control. All genes were grouped into functional categories and metabolic pathways based on the KEGG database. To obtain a molecular portrait of the metabolism associated with the experimental group, we used a hierarchical clustering algorithm to group genes on the basis of similar expression patterns and then presented the data in a matrix format (Figure 2). Each row in Figure 2 represents the hybridization results for single oligonucleotide probes of the array, and each column represents the expression levels for all genes in a single hybridization sample. The expression level of each gene was visualized in color relative to its median expression level across all samples. Red represents expression greater than the mean and green represents expression less than the mean, and the color intensity denotes the degree of deviation from the mean. Gray represents the median expression level. Distinct samples representing similar gene patterns from control cells are aligned in adjacent rows. The cells included in this map were obtained from both the experimental group and the control. Coordinately expressed genes are grouped into clusters, which are named according to the cellular process in which they participate.

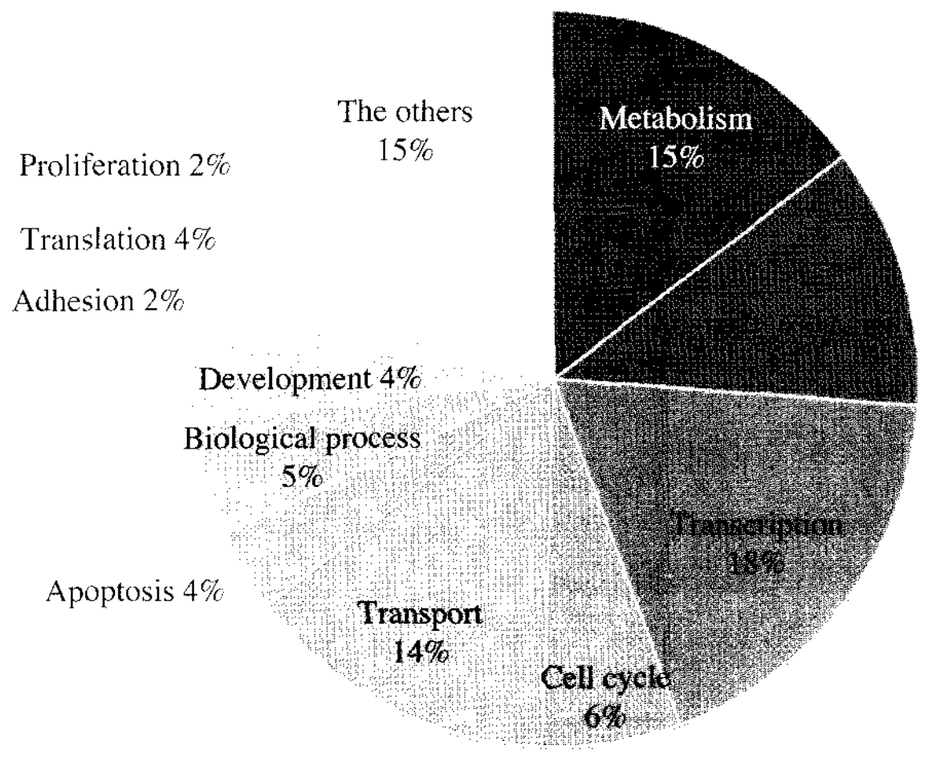


**Figure 1.** Gene Ontology classifications of genes based on comparison of gene expression between experimental (forsythia suspense (FS)-treated-treated) and control (non-treated or LPS-treated) BV-2 cells.

## Discussion

The microarray technique is a molecular technology that enables the analysis of gene expression in conjunction with a very large number of genes that encompass a significant fraction of the human genome. This method is qualitative as well as quantitative because it possesses the sensitivity to detect changes in the levels of expression in the investigated cells when compared with the control samples[12,13]. An understanding of these molecular processes can then be used in the development of more advanced therapies for the herbal treatment of neuroinflammation disease.

During a search for new therapeutic agents for the treatment of neuroinflammation disease using medicinal herbs, 270 herbal medicines in the phytolibrary™ kit were tested for their ability to inhibit LPS -induced NO production in BV-2 microglial cells. This search revealed that FS showed considerable inhibition at a concentration of 1 μg/mL; therefore, it was selected for further analysis to determine the inhibition mechanisms of NS on LPS-induced NO production in BV-2 microglial cells. In addition, we found that the FS non-cytotoxicity affected the viability of BV-2 microglial cells (data not shown). The early signaling events involved in LPS-induced microglial activation are not completely understood; therefore the effects of FS on the gene expression profiles of BV-2 microglial cells that were treated for different lengths of time (30 min, 1 h, and 3 h) were evaluated.

Specific and significant alterations of the expression profile of FS-treated BV-2 microglial cells were observed (Table 1 and 2). The genes found to be differentially expressed were responsible for processes including cell cycle and proliferation, signal trans-

**Table 1.** Up-regulation of genes based on comparison of gene expression between experimental (forsythia suspense (FS)-treated) and control (non-treated or LPS-treated) BV-2 cells.

| Gene description | Gene symbol | Regulation profile and ratio | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPS | | | FS | | |
| | | 30 m | 1 hr | 3 hr | 30 m | 1 hr | 3 hr |
| <Transport-related genes> | | | | | | | |
| Similar to Potassium voltage-gated channel subfamily G member 2 | LOC240444 | −4.11 | −3.76 | −0.76 | 4.64 | 4.24 | 0.88 |
| solute carrier family 6 (neurotransmitter transporter), member 14 | Slc6a14 | −4.47 | −2.18 | −3.85 | 4.42 | 2.34 | 3.70 |
| transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) | Tap1 | −3.27 | −3.40 | −3.68 | 3.77 | 3.25 | 3.86 |
| cystic fibrosis transmembrane conductance regulator homolog | Cftr | −3.33 | −4.72 | −4.83 | 3.64 | 2.35 | 4.45 |
| cholinergic receptor, nicotinic, alpha polypeptide 5 | Chrna5 | −2.76 | −2.47 | −2.63 | 3.48 | 3.78 | 2.33 |
| glutamate receptor, ionotropic, kainate 1 | Grik1 | −2.17 | −3.00 | −4.46 | 2.95 | 3.15 | 4.49 |
| solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 | Slc1a1 | −0.81 | −0.54 | −1.21 | 2.93 | 3.00 | 2.49 |
| oxysterol binding protein 2 | Osbp2 | −2.01 | −3.05 | −1.87 | 2.85 | 2.23 | 2.50 |
| solute carrier family 25, member 37 | Slc25a37 | −4.17 | −4.50 | −2.98 | 2.45 | 1.06 | 3.60 |
| cytochrome P450, family 2, subfamily c, polypeptide 38 | Cyp2c38 | −1.94 | −0.61 | −2.17 | 2.14 | 0.78 | 2.77 |
| solute carrier family 25 (mitochondrial carrier, palmitoylcarnitine transporter), member 29 | Slc25a29 | −2.63 | −1.03 | −1.32 | 1.86 | 1.30 | 0.72 |
| lipocalin 9 | Lcn9 | −2.65 | −3.51 | −1.21 | 1.74 | 0.70 | 1.60 |
| expressed sequence AW146299 | AW146299 | −2.34 | −2.58 | −1.54 | 1.66 | 1.62 | 1.76 |
| gulonolactone (L-) oxidase | Gulo | −2.39 | −3.59 | −4.97 | 1.35 | 2.17 | 4.52 |
| orosomucoid 1 | Orm1 | −1.81 | −2.92 | −2.82 | 1.31 | 4.86 | 1.75 |
| potassium channel, subfamily K, member 5 | Kcnk5 | −2.99 | −1.96 | −1.57 | 0.64 | 2.81 | 1.66 |
| potassium inwardly-rectifying channel, subfamily J, member 2 | Kcnj2 | −1.70 | −1.27 | −2.08 | 0.57 | 3.00 | 4.62 |
| <Metabolism-related genes> | | | | | | | |
| phosphatidylinositol glycan anchor biosynthesis, class N | Pign | −4.07 | −4.05 | −2.15 | 4.49 | 3.96 | 3.04 |
| UDP-GalNAc:betaGlcNAc beta1,3-galactosaminyl transferase, polypeptide 2 | B3galnt2 | −3.36 | −3.70 | −2.85 | 4.31 | 3.36 | 3.12 |
| 1-acylglycerol-3-phosphate O-acyltransferase 6 | Agpat6 | −3.08 | −0.81 | −0.88 | 3.70 | 0.98 | 1.42 |
| phospholipase C, beta 4 | Plcb4 | −2.99 | −2.41 | −2.21 | 3.25 | 2.70 | 2.31 |
| phospholipase C, beta 1 | Plcb1 | −2.86 | −1.05 | −2.71 | 2.50 | 0.93 | 1.79 |
| glycine decarboxylase | Gldc | −2.48 | −1.93 | −0.96 | 2.49 | 2.16 | 0.87 |
| lipoic acid synthetase | Lias | −1.79 | −1.07 | −0.78 | 2.05 | 1.41 | 1.14 |
| glutamic-oxaloacetic transaminase 1-like 1 | Got1l1 | −3.66 | −3.74 | −2.51 | 1.76 | 0.86 | 1.51 |
| protein phosphatase 1, regulatory (inhibitor) subunit 3C | Ppp1r3c | −1.94 | −1.08 | −1.09 | 1.64 | 1.12 | 1.80 |
| Phosphatidylinositol-4-phosphate 5-kinase, type 1 beta | Pip5k1b | −1.47 | −1.06 | −0.98 | 1.59 | 1.17 | 1.92 |
| UDP glucuronosyltransferase 2 family, polypeptide B38 | Ugt2b38 | −4.18 | −3.38 | −2.32 | 1.11 | 0.62 | 1.47 |
| phospholipase A2, group VI | Pla2g6 | −0.64 | −4.11 | −1.59 | 1.10 | 4.74 | 1.82 |
| beta-1,3-glucuronyltransferase2 (glucuronosyltransferase S) | B3gat2 | −4.70 | −3.81 | −2.68 | 1.09 | 1.78 | 1.37 |
| phosphoglycerate mutase 2 | Pgam2 | −2.01 | −2.26 | −1.91 | 1.06 | 1.48 | 0.75 |
| ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2 | St8sia2 | −0.69 | −0.69 | −1.56 | 0.85 | 1.34 | 1.93 |
| <Transcription-related genes> | | | | | | | |
| RIKEN cDNA 4933403O03 gene | 4933403O03 | −2.84 | −2.81 | −1.15 | 4.10 | 3.20 | 2.04 |
| POU domain, class 6, transcription factor 2 | Pou6f2 | −3.27 | −3.28 | −0.99 | 3.75 | 4.33 | 1.13 |
| sine oculis-related homeobox 6 homolog (Drosophila) | Six6 | −1.80 | −1.21 | −1.01 | 3.27 | 1.50 | 2.34 |
| thymopoietin | Tmpo | −2.97 | −4.06 | −3.67 | 3.07 | 3.12 | 3.96 |
| centromere protein B | Cenpb | −2.43 | −1.45 | −1.16 | 2.87 | 2.26 | 1.54 |
| homeo box, msh-like 1 | Msx1 | −2.13 | −1.68 | −0.59 | 2.69 | 2.69 | 1.88 |
| paired-like homeobox 2a | Phox2a | −0.91 | −3.88 | −2.24 | 2.51 | 4.76 | 1.68 |
| RAR-related orphan receptor alpha | Rora | −2.78 | −1.81 | −2.80 | 1.87 | 1.84 | 0.65 |
| tripartite motif protein 27 | Trim27 | −1.00 | −2.58 | −2.24 | 1.45 | 1.37 | 2.24 |
| vestigial like 1 homolog (Drosophila) | Vgll1 | −0.84 | −2.78 | −2.72 | 1.32 | 2.35 | 2.84 |

**Table 1.** Continued.

| Gene description | Gene symbol | Regulation profile and ratio | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPS | | | FS | | |
| | | 30 m | 1 hr | 3 hr | 30 m | 1 hr | 3 hr |
| enhancer of polycomb homolog 1 (Drosophila) | Epc1 | −3.38 | −4.00 | −1.85 | 0.62 | 0.69 | 0.69 |
| POU domain, class 6, transcription factor 1 | Pou6f1 | −1.51 | −2.62 | −2.35 | 0.57 | 1.50 | 0.88 |
| <Development-related genes> | | | | | | | |
| LIM domain binding 2 | Ldb2 | −2.77 | −2.48 | −0.64 | 3.56 | 2.78 | 1.05 |
| exostoses (multiple) 2 | Ext2 | −2.12 | −1.72 | −0.89 | 3.13 | 2.62 | 1.78 |
| testis-specific serine kinase 2 | Tssk2 | −1.19 | −1.98 | −0.74 | 2.93 | 2.41 | 2.47 |
| outer dense fiber of sperm tails 1 | Odf1 | −3.71 | −3.47 | −3.42 | 2.37 | 2.38 | 2.59 |
| dentin sialophosphoprotein | Dspp | −0.80 | −0.93 | −1.09 | 1.83 | 2.00 | 1.59 |
| Norrie disease homolog | Ndph | −1.54 | −4.09 | −3.94 | 0.54 | 2.73 | 2.26 |
| <Cell adhesion/migration-related genes> | | | | | | | |
| cadherin 13 | Cdh13 | −3.12 | −1.44 | −1.25 | 3.56 | 0.95 | 2.41 |
| nuclear receptor subfamily 2, group F, member 2 | Nr2f2 | −3.46 | −1.13 | −5.14 | 3.15 | 0.73 | 4.21 |
| myosin binding protein H | Mybph | −1.34 | −1.43 | −2.39 | 2.55 | 0.58 | 3.29 |
| laminin B1 subunit 1 | Lamb1-1 | −2.02 | −2.90 | −1.70 | 2.46 | 4.38 | 1.64 |
| killer cell lectin-like receptor, subfamily A, member 1 | Klra1 | −0.75 | −3.39 | −2.12 | 2.41 | 0.86 | 2.80 |
| scavenger receptor class F, member 2 | Scarf2 | −3.67 | −3.12 | −1.52 | 1.49 | 0.54 | 1.31 |
| podocalyxin-like 2 | Podxl2 | −4.37 | −5.35 | −4.98 | 1.20 | 0.60 | 1.32 |
| procollagen, type X, alpha 1 | Col10a1 | −2.85 | −2.88 | −2.96 | 0.97 | 1.38 | 1.35 |
| Fez family zinc finger 1 | Fezf1 | −0.79 | −2.76 | −1.83 | 0.59 | 2.84 | 1.69 |
| <Biological process-related genes> | | | | | | | |
| calmodulin regulated spectrin-associated protein 1 | Camsap1 | −2.35 | −2.09 | −0.60 | 3.42 | 3.33 | 2.33 |
| prion protein interacting protein 1 | Prnpip1 | −2.62 | −3.12 | −2.70 | 2.99 | 1.87 | 3.36 |
| AT hook, DNA binding motif, containing 1 | Ahdc1 | −2.32 | −1.68 | −2.19 | 2.86 | 2.05 | 3.32 |
| activating transcription factor 7 interacting protein 2 | Atf7ip2 | −1.00 | −2.36 | −1.72 | 2.47 | 3.70 | 3.59 |
| Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 13 (human) | Als2cr13 | −1.24 | −2.22 | −3.95 | 2.06 | 1.41 | 4.59 |
| melanoma antigen, family B, 5 | Mageb5 | −2.11 | −1.41 | −2.22 | 1.67 | 1.41 | 0.96 |
| cadherin 4 | Cdh4 | −1.64 | −1.45 | −0.94 | 1.40 | 1.81 | 0.71 |
| <Translation-related genes> | | | | | | | |
| mitochondrial ribosomal protein L41 | Mrpl41 | −1.04 | −1.41 | −1.75 | 3.31 | 0.98 | 4.25 |
| <Proteolysis-related genes> | | | | | | | |
| elastase 2A | Ela2a | −2.85 | −1.52 | −1.79 | 2.83 | 1.23 | 1.34 |
| transducin (beta)-like 3 | Tbl3 | −4.12 | −3.43 | −3.72 | 2.56 | 0.66 | 1.04 |
| transmembrane protease, serine 2 | Tmprss2 | −1.37 | −1.21 | −4.06 | 1.08 | 1.54 | 3.83 |
| Protease, serine, 23 | Prss23 | −2.36 | −2.10 | −1.67a | 0.81 | 0.79 | 2.74 |
| a disintegrin-like and metallopetidase (reprolysin type) with thrombospondin type 1 motif, 16 | Adamts16 | −0.78 | −1.78 | −3.65 | 0.80 | 1.48 | 3.24 |
| <Apoptosis-related genes> | | | | | | | |
| coagulation factor II (thrombin) receptor | F2r | −2.65 | −4.65 | −3.87 | 1.68 | 0.50 | 2.96 |
| B-cell leukemia/lymphoma 2 related protein A1a | Bcl2a1a | −0.81 | −1.34 | −2.00 | 0.93 | 3.80 | 4.00 |
| <Cell cycle-related genes> | | | | | | | |
| NIMA (never in mitosis gene a)-related expressed kinase1 | Nek1 | −5.58 | −1.71 | −3.52 | 5.79 | 2.89 | 0.94 |
| Adenomatosis polyposis coli 2 | Apc2 | −4.41 | −1.89 | −4.63 | 4.13 | 2.26 | 4.52 |
| inner centromere protein | Incenp | −3.02 | −2.81 | −2.63 | 3.28 | 3.05 | 2.07 |
| protein kinase C, alpha | Prkca | −1.90 | −2.73 | −1.46 | 1.79 | 1.41 | 0.60 |
| growth arrest-specific 2 like 1 | Gas2l1 | −1.38 | −2.84 | −3.15 | 0.92 | 0.50 | 1.44 |
| c-fos induced growth factor | Figf | −1.54 | −2.50 | −1.30 | 0.79 | 1.50 | 1.93 |
| <Signal transduction-related genes> | | | | | | | |
| dickkopf homolog 4 (Xenopus laevis) | Dkk4 | −5.23 | −1.06 | −4.82 | 4.99 | 0.95 | 3.74 |
| prokineticin receptor 1 | Prokr1 | −2.22 | −2.41 | −1.76 | 4.56 | 2.84 | 1.37 |
| N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1 | Ndst1 | −3.64 | −1.19 | −2.30 | 4.40 | 0.91 | 0.96 |
| endothelin 3 | Edn3 | −4.19 | −3.09 | −2.41 | 4.23 | 0.72 | 2.11 |

**Table 1.** Continued.

| Gene description | Gene symbol | Regulation profile and ratio | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPS | | | FS | | |
| | | 30 m | 1 hr | 3 hr | 30 m | 1 hr | 3 hr |
| Eph receptor A7 | Epha7 | −4.35 | −3.93 | −4.71 | 3.82 | 3.62 | 5.29 |
| insulin-like growth factor binding protein 4 | Igfbp4 | −3.86 | −1.43 | −1.24 | 3.67 | 1.47 | 0.56 |
| Centaurin, delta 1 | Centd1 | −1.92 | −3.16 | −1.98 | 3.58 | 5.61 | 2.80 |
| RAB36, member RAS oncogene family | Rab36 | −3.68 | −2.87 | −0.62 | 3.38 | 3.61 | 0.97 |
| phosphodiesterase 6H, cGMP-specific, cone, gamma | Pde6h | −2.77 | −1.06 | −0.60 | 2.87 | 3.20 | 2.17 |
| discs, large (Drosophila) homolog-associated protein 1 | Dlgap1 | −3.32 | −4.84 | −2.75 | 2.81 | 3.73 | 0.74 |
| plexin A4 | Plxna4 | −2.31 | −2.84 | −4.04 | 2.51 | 3.25 | 2.59 |
| succinate receptor 1 | Sucnr1 | −2.38 | −3.89 | −0.93 | 2.32 | 2.90 | 0.89 |
| olfactory receptor 1507 | Olfr1507 | −1.57 | −1.39 | −1.38 | 2.28 | 1.61 | 1.93 |
| connector enhancer of kinase suppressor of Ras 1 | Cnksr1 | −1.35 | −1.45 | −1.49 | 2.02 | 1.76 | 1.39 |
| taste receptor, type 2, member 119 | Tas2r119 | −2.17 | −2.89 | −0.87 | 1.66 | 3.02 | 0.65 |
| PDZ and LIM domain 3 | Pdlim3 | −1.24 | −1.74 | −1.70 | 1.37 | 1.59 | 1.40 |
| transforming growth factor, beta receptor I | Tgfbr1 | −0.52 | −2.19 | −0.92 | 1.12 | 2.06 | 1.53 |
| Centaurin, delta 1 | Centd1 | −3.65 | −2.40 | −4.14 | 1.03 | 1.14 | 2.30 |
| Eph receptor A5 | Epha5 | −2.80 | −2.01 | −2.36 | 0.99 | 2.53 | 1.95 |
| G protein-coupled receptor, family C, group 6, member A | Gprc6a | −1.66 | −2.50 | −2.16 | 0.83 | 2.56 | 3.20 |
| regulator of G-protein signaling 12 | Rgs12 | −1.14 | −2.48 | −2.33 | 0.74 | 2.55 | 0.63 |
| Nischarin | Nisch | −0.85 | −2.81 | −4.80 | 0.54 | 1.88 | 4.74 |
| <The others> | | | | | | | |
| U1 small nuclear ribonucleoprotein polypeptide A | Snrp70 | −4.11 | −4.74 | −1.92 | 4.93 | 4.88 | 2.33 |
| odd Oz/ten-m homolog 4 (Drosophila) | Odz4 | −4.88 | −1.69 | −1.98 | 4.45 | 0.77 | 2.51 |
| kinesin family member 21A | Kif21a | −3.63 | −2.92 | −4.69 | 2.75 | 0.63 | 4.72 |
| histone cluster 3, H2ba | Hist3h2ba | −0.93 | −2.04 | −1.58 | 1.32 | 2.66 | 1.58 |
| dihydropyrimidinase-like 5 | Dpysl5 | −2.31 | −2.07 | −1.84 | 1.15 | 2.74 | 0.07 |
| UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyl transferase 7 | B3gnt7 | −1.53 | −3.49 | −2.46 | 0.95 | 2.59 | 1.30 |
| C-type lectin domain family 2, member h | Clec2h | −1.76 | −2.14 | −4.52 | 0.58 | 1.21 | 3.83 |

duction, transport, metabolism, transcription, development, adhesion and migration, translation, inflammatory and immune response, proteolysis and nitric oxide biosynthesis, as well as other functions. The MAPK pathways-related genes (Mitogen activated protein kinase 1 (*Mapk1*), RAS protein activator like 2 (*Rasal2*), G-protein coupled receptor 12 (*Gpr12*) and nitric oxide biosynthesis-related genes (nitric oxide synthase 1 (neuronal) adaptor protein (*Nos1ap*) and dimethylarginine dimethylaminohydrolase 1 (*Ddah1*) were down-regulated in the FS-treated BV-2 microglial cells (Table 2). The MAPK pathways are deeply involved in signaling for various immune responses such as apoptosis. MAP kinases are serine/threonine kinases, which include the extracellular signal-related kinases (ERKs), p38 kinases, and c-Jun N-terminal kinases (JNKs). Activation of the MAPK kinase pathway often occurs in response to growth factor stimulation of receptor tyrosine kinases, which are coupled to the activation of Ras G-proteins through Src homology 2 domain-containing proteins, such as Shc and Grb2, and quinine nucleotide exchange factors such as SOS[14,15]. The results of this study revealed the down-regulation of Mapk1, Rasal2, and Gpr12 in FS-treated BV-2 microglial cells (Table 2). Each of the MAPKs has been implicated in neuroinflammatory events and the MAPK pathways have been linked to mediation of many of the physiological responses to NO. For example, NO regulation of matrix metalloproteinases proteins, including MMP1, during inflammatory and angiogenic responses may require MAP kinase proteins. NO is a signaling molecule, neurotransmitter, and immune effector[14,16,17] that is produced by the activity of the family of enzymes known as the nitrix-oxide synthases (NOSs). The *Nos1ap* gene encodes a cytosolic protein that binds to the signaling molecule, neuronal nitric oxide synthase (nNOS). This protein has a C-terminal PDZ-binding domain that mediates interactions with nNOS and an N-terminal phosphotyrosine binding (PTB) domain that binds to the small monomeric G protein, Dexras1. Studies of related mouse and rat proteins have shown that this protein functions as an adapter protein linking nNOS to specific targets, such as Dexras1 and the synapsins[18]. The results of this study indicated that *Nos1ap* was down-regulated in FS-treated BV-2 microglial

**Table 2.** Down-regulation of genes based on comparison of gene expression between experimental (forsythia suspense (FS)-treated) and control (non-treated or LPS-treated) BV-2 cells.

| Gene description | Gene symbol | Regulation profile and ratio | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPS | | | FS | | |
| | | 30 m | 1 hr | 3 hr | 30 m | 1 hr | 3 hr |
| <Nitric oxide biosynthesis-related genes> | | | | | | | |
| Dimethylarginine dimethylaminohydrolase 1 | Ddah1 | 3.94 | 3.29 | 3.88 | −5.39 | −0.54 | −3.50 |
| nitric oxide synthase 1 (neuronal) adaptor protein | Nos1ap | 2.75 | 3.21 | 3.58 | −1.09 | −1.52 | −3.22 |
| <Response to oxidative stress-related genes> | | | | | | | |
| zinc finger protein 292 | Zfp292 | 0.91 | 0.74 | 1.17 | −2.08 | −2.21 | −2.07 |
| <Cell cycle & proliferation-related genes> | | | | | | | |
| vascular endothelial growth factor C | Vegfc | 1.19 | 2.29 | 2.86 | −3.31 | −4.40 | −2.20 |
| transforming growth factor, beta 2 | Tgfb2 | 2.67 | 1.67 | 2.22 | −3.31 | −2.02 | −1.60 |
| Nipped-B homolog (Drosophila) | Nipbl | 0.53 | 1.05 | 0.58 | −2.71 | −3.31 | −1.43 |
| Non-SMC condensin II complex, subunit D3 | Ncapd3 | 2.29 | 1.16 | 1.80 | −2.05 | −0.90 | −1.57 |
| interleukin 11 | Il11 | 2.28 | 2.39 | 2.39 | −1.34 | −1.93 | −1.60 |
| <Signal transduction-related genes> | | | | | | | |
| sortilin-related VPS10 domain containing receptor 3 | Sorcs3 | 2.42 | 1.21 | 1.04 | −4.35 | −2.02 | −1.20 |
| Dystrobrevin alpha | Dtna | 2.94 | 3.03 | 2.85 | −3.98 | −1.61 | −1.73 |
| Mitogen activated protein kinase 1 | Mapk1 | 1.35 | 0.68 | 1.89 | −3.47 | −0.96 | −2.36 |
| Rho/rac guanine nucleotide exchange factor (GEF) 18 | Arhgef18 | 1.28 | 1.78 | 0.67 | −3.47 | −2.70 | −1.24 |
| Spleen tyrosine kinase | Syk | 1.38 | 2.39 | 2.52 | −3.13 | −2.92 | −1.68 |
| Coronin, actin binding protein 1C | Coro1c | 1.37 | 2.18 | 1.02 | −3.03 | −2.77 | −2.04 |
| Tensin 3 | Tns3 | 1.76 | 2.13 | 1.91 | −2.86 | −3.87 | −3.71 |
| adrenergic receptor, alpha 2b | Adra2b | 2.38 | 3.46 | 1.18 | −2.78 | −0.80 | −0.66 |
| KRIT1, ankyrin repeat containing | Krit1 | 0.82 | 0.69 | 1.05 | −2.75 | −3.72 | −1.05 |
| Rho guanine nucleotide exchange factor (GEF) 11 | Arhgef11 | 3.27 | 3.29 | 3.69 | −2.70 | −1.75 | −2.16 |
| somatostatin receptor 4 | Sstr4 | 3.45 | 1.54 | 3.19 | −2.44 | −1.08 | −2.20 |
| IQ motif and Sec7 domain 2 | Iqsec2 | 1.36 | 2.00 | 1.89 | −2.32 | −2.94 | −2.61 |
| TAO kinase 2 | Taok2 | 0.73 | 1.64 | 1.80 | −2.01 | −1.25 | −1.14 |
| Vav 3 oncogene | Vav3 | 0.70 | 0.92 | 0.62 | −1.78 | −5.36 | −1.17 |
| Protein tyrosine phosphatase, receptor type, N polypeptide2 | Ptprn2 | 3.61 | 3.92 | 3.83 | −1.64 | −2.48 | −1.34 |
| calcineurin binding protein 1 | Cabin1 | 0.79 | 0.70 | 0.70 | −1.59 | −2.00 | −1.52 |
| Protein tyrosine phosphatase, receptor type, D | Ptprd | 0.86 | 3.59 | 4.76 | −1.48 | −1.68 | −1.20 |
| Rap guanine nucleotide exchange factor (GEF) 2 | Rapgef2 | 0.74 | 1.68 | 1.38 | −1.40 | −1.51 | −0.59 |
| Wnt inhibitory factor 1 | Wif1 | 4.15 | 4.52 | 1.39 | −1.20 | −3.49 | −2.09 |
| phosphate regulating gene with homologies to endopeptidases on the X chromosome | Phex | 3.43 | 3.68 | 4.06 | −1.14 | −4.47 | −1.37 |
| Mastermind like 2 (Drosophila) | Maml2 | 2.12 | 1.83 | 2.44 | −1.12 | −3.49 | −3.72 |
| histamine receptor H 3 | Hrh3 | 2.00 | 2.20 | 2.22 | −1.07 | −2.57 | −2.60 |
| predicted gene, EG665317 | EG665317 | 1.46 | 2.16 | 2.34 | −1.07 | −0.66 | −1.20 |
| neuregulin 1 | Nrg1 | 2.27 | 3.29 | 0.85 | −1.05 | −3.08 | −0.97 |
| glial cell line derived neurotrophic factor family receptor alpha 2 | Gfra2 | 1.33 | 3.22 | 2.94 | −1.03 | −2.95 | −1.05 |
| RAS protein activator like 2 | Rasal2 | 1.55 | 0.71 | 2.18 | −0.92 | −0.88 | −2.14 |
| G-protein coupled receptor 12 | Gpr12 | 1.96 | 4.36 | 2.93 | −0.91 | −2.97 | −2.14 |
| a disintegrin and metallopeptidase domain 7 | Adam7 | 2.90 | 3.51 | 4.35 | −0.65 | −1.72 | −3.14 |
| <The others> | | | | | | | |
| spectrin beta 2 | Spnb2 | 3.04 | 2.70 | 1.42 | −4.43 | −3.17 | −0.88 |
| tubulin, beta 2b | Tubb2b | 3.45 | 1.70 | 4.51 | −4.36 | −0.52 | −0.66 |
| calbindin-28K | Calb1 | 4.25 | 5.41 | 5.27 | −2.99 | −4.69 | −5.12 |
| tropomyosin 2, beta | Tpm2 | 3.42 | 3.66 | 3.44 | −2.25 | −0.56 | −3.57 |
| TAO kinase 1 | Taok1 | 0.66 | 0.85 | 1.35 | −2.21 | −3.98 | −2.02 |
| dentin matrix protein 1 | Dmp1 | 2.88 | 3.37 | 4.32 | −2.14 | −2.19 | −0.58 |
| centromere protein E | Cenpe | 0.50 | 0.62 | 1.33 | −2.05 | −2.68 | −2.13 |
| zinc finger CCCH type, antiviral 1 | Zc3hav1 | 0.51 | 1.14 | 1.02 | −1.79 | −2.15 | −1.75 |
| SUMO1 activating enzyme subunit 1 | Sae1 | 0.68 | 0.58 | 0.98 | −1.74 | −3.74 | −1.68 |

**Table 2.** Continued.

| Gene description | Gene symbol | Regulation profile and ratio | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPS | | | FS | | |
| | | 30 m | 1 hr | 3 hr | 30 m | 1 hr | 3 hr |
| ubiquitously transcribed tetratricopeptide repeat gene, X chromosome | Utx | 1.88 | 1.62 | 1.37 | −1.62 | −1.75 | −0.66 |
| heat shock protein 14 | Hspa14 | 2.33 | 2.24 | 2.09 | −0.56 | −1.61 | −1.39 |
| <Transport-related genes> | | | | | | | |
| potassium channel, subfamily V, member 1 | Kcnv1 | 3.91 | 1.67 | 2.39 | −3.95 | −0.57 | −1.05 |
| RAN binding protein 5 | Ranbp5 | 1.05 | 1.51 | 0.90 | −3.84 | −1.85 | −1.68 |
| solute carrier family 2 (facilitated glucose transporter), member 12 | Slc2a12 | 1.12 | 0.70 | 1.02 | −3.38 | −2.16 | −2.30 |
| potassium large conductance calcium-activated channel, subfamily M, alpha member 1 | Kcnma1 | 3.51 | 2.79 | 2.29 | −3.01 | −2.45 | −2.79 |
| synaptotagmin IX | Syt9 | 1.68 | 1.22 | 2.46 | −2.87 | −0.89 | −4.24 |
| RNA binding motif protein 6 | Rbm6 | 0.79 | 0.85 | 0.89 | −1.90 | −5.55 | −1.80 |
| glutamate receptor, ionotropic, delta 2 | Grid2 | 1.88 | 1.32 | 3.04 | −1.83 | −2.19 | −0.57 |
| Kinesin 2 | Kns2 | 0.77 | 0.61 | 0.79 | −1.83 | −3.51 | −1.81 |
| procollagen, type IV, alpha 6 | Col4a6 | 2.22 | 2.06 | 2.74 | −1.79 | −1.80 | −1.75 |
| Syntaxin 6 | Stx6 | 0.50 | 0.88 | 1.32 | −1.68 | −1.87 | −1.94 |
| aldhehyde dehydrogenase family 5, subfamily A1 | Aldh5a1 | 5.14 | 3.22 | 4.70 | −1.68 | −3.28 | −1.06 |
| Atpase, class I, type 8B, member 2 | Atp8b2 | 3.22 | 3.35 | 3.09 | −1.60 | −2.17 | −2.23 |
| nucleoporin 160 | Nup160 | 1.03 | 0.82 | 1.12 | −1.12 | −3.69 | −2.22 |
| <Metabolism-related genes> | | | | | | | |
| Ubiquitin specific peptidase 48 | Usp48 | 2.65 | 2.00 | 2.46 | −5.11 | −3.96 | −0.65 |
| Glucosamine-phosphate N-acetyltransferase 1 | Gnpnat1 | 5.03 | 4.21 | 4.48 | −5.05 | −3.12 | −1.15 |
| Phosphatidylinositol-4-phosphate 5-kinase, type II, alpha | Pip5k2a | 2.09 | 2.54 | 1.19 | −4.72 | −3.45 | −1.97 |
| Lipase, member H | Liph | 2.73 | 3.40 | 3.03 | −4.51 | −0.98 | −2.26 |
| phosphatase, orphan 1 | Phospho1 | 2.40 | 1.86 | 2.54 | −4.34 | −4.25 | −1.63 |
| ATP-binding cassette, subfamilyC (CFTR/MRP), member 9 | Abcc9 | 3.34 | 1.76 | 4.34 | −4.28 | −1.20 | −3.25 |
| Ring finger protein 11 | Rnf11 | 1.25 | 1.01 | 1.13 | −4.24 | −4.79 | −2.47 |
| Niemann Pick type C2 | Npc2 | 0.76 | 0.97 | 1.54 | −3.71 | −0.58 | −0.74 |
| thymidylate synthase | Tyms | 3.13 | 2.58 | 2.46 | −2.67 | −1.93 | −1.74 |
| UDP-N-acetyl-alpha-D-galactosamine | Galnt4 | 3.46 | 3.52 | 3.72 | −2.63 | −2.66 | −0.98 |
| pancreatic lipase-related protein 2 | Pnliprp2 | 1.16 | 1.36 | 1.32 | −2.61 | −2.49 | −2.45 |
| Phosphorylase kinase alpha 2 | Phka2 | 2.33 | 3.01 | 2.28 | −1.73 | −1.15 | −0.56 |
| <Transcription-related genes> | | | | | | | |
| X (inactive)-specific transcript, antisense | Tsix | 1.88 | 2.14 | 1.95 | −4.57 | −1.85 | −1.28 |
| SERTA domain containing 2 | Sertad2 | 1.97 | 2.75 | 2.90 | −3.87 | −2.37 | −0.92 |
| homeo box D10 | Hoxd10 | 2.18 | 2.04 | 1.34 | −3.63 | −2.22 | −1.24 |
| Transcription factor 12 | Tcf12 | 0.97 | 0.78 | 0.99 | −3.61 | −2.44 | −3.46 |
| Forkhead box P1 | Foxp1 | 1.03 | 1.67 | 0.73 | −2.90 | −3.72 | −3.07 |
| bromodomain adjacent to zinc finger domain, 2A | Baz2a | 0.69 | 0.84 | 1.14 | −2.85 | −2.45 | −2.06 |
| naked cuticle 2 homolog (Drosophila) | Nkd2 | 4.10 | 2.51 | 2.24 | −2.56 | −1.91 | −2.53 |
| interferon regulatory factor 6 | Irf6 | 0.77 | 0.87 | 1.54 | −1.74 | −1.82 | −1.64 |
| Tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2 | Tanc2 | 1.35 | 1.00 | 1.24 | −1.68 | −1.76 | −0.78 |
| zinc finger protein 612 | Zfp612 | 0.74 | 1.40 | 0.75 | −1.68 | −3.76 | −1.04 |
| distal-less homeobox 6 | Dlx6 | 3.54 | 2.75 | 1.78 | −1.65 | −1.52 | −0.50 |
| reproductive homeobox 9 | Rhox9 | 3.77 | 1.81 | 2.25 | −1.60 | −1.19 | −1.03 |
| SRY-box containing gene 17 | Sox17 | 3.39 | 4.33 | 2.83 | −1.55 | −5.31 | −3.48 |
| Zinc finger protein 672 | Zfp672 | 1.26 | 1.10 | 2.06 | −1.53 | −2.61 | −1.73 |
| zinc finger and BTB domain containing 7C | Zbtb7c | 2.06 | 3.48 | 3.44 | −1.49 | −2.34 | −2.87 |
| Transcription factor 12 | Tcf12 | 0.55 | 1.04 | 1.33 | −1.44 | −4.25 | −1.73 |
| Arginine/serine-rich coiled-coil 1 | Rsrc1 | 2.05 | 2.89 | 3.76 | −1.27 | −0.62 | −1.75 |
| transducin (beta)-like 1X-linked receptor 1 | Tbl1xr1 | 0.84 | 1.38 | 0.77 | −1.15 | −7.23 | −1.74 |
| NF-kappaB repressing factor | Nkrf | 0.65 | 2.45 | 4.13 | −0.59 | −2.46 | −1.32 |

**Table 2.** Continued.

| Gene description | Gene symbol | Regulation profile and ratio | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPS | | | FS | | |
| | | 30 m | 1 hr | 3 hr | 30 m | 1 hr | 3 hr |
| <Development-related genes> | | | | | | | |
| sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain and short cytoplasmic domain, 5A | Sema5a | 1.09 | 1.40 | 1.72 | −2.02 | −1.53 | −0.75 |
| interferon induced transmembrane protein 5 | Ifitm5 | 2.39 | 3.27 | 4.19 | −1.95 | −1.94 | −2.50 |
| plexin A2 | Plxna2 | 0.67 | 1.17 | 1.23 | −1.87 | −2.36 | −0.99 |
| sema domain, transmembrane domain, and cytoplasmic domain, 6C | Sema6c | 1.67 | 1.92 | 1.48 | −1.57 | −1.12 | −0.75 |
| tolloid-like 2 | Tll2 | 1.00 | 2.79 | 2.16 | −1.42 | −0.68 | −2.00 |
| Exostoses (multiple) 1 | Ext1 | 1.81 | 1.99 | 2.72 | −1.41 | −2.56 | −2.57 |
| Fukuyama type congenital muscular dystrophy homolog | Fcmd | 1.39 | 0.77 | 1.28 | −1.32 | −1.75 | −1.66 |
| neuregulin 1 | Nrg1 | 2.27 | 3.29 | 0.85 | −1.05 | −3.08 | −0.97 |
| <Cell adhesion/migration-related genes> | | | | | | | |
| Protocadherin 11 X-linked | Pcdh11x | 4.62 | 4.24 | 4.34 | −4.31 | −3.36 | −5.18 |
| biregional cell adhesion molecule-related/down-regulated by oncogenes (Cdon) binding protein | Boc | 1.80 | 1.10 | 2.46 | −3.57 | −0.78 | −2.24 |
| LIM homeobox transcription factor 1 beta | Lmx1b | 1.46 | 1.56 | 2.08 | −3.24 | −2.73 | −2.88 |
| neurexin III | Nrxn3 | 3.25 | 3.38 | 2.89 | −2.52 | −3.31 | −0.66 |
| chondroitin sulfate proteoglycan 2 | Cspg2 | 2.80 | 0.60 | 2.56 | −2.09 | −1.24 | −2.05 |
| cadherin 7, type 2 | Cdh7 | 2.65 | 3.34 | 1.10 | −1.78 | −0.76 | −0.78 |
| contactin 2 | Cntn2 | 2.40 | 3.41 | 2.19 | −0.65 | −3.48 | −0.58 |
| <Biological process-related genes> | | | | | | | |
| Anthrax toxin receptor 2 | Antxr2 | 5.56 | 6.36 | 6.64 | −4.01 | −3.90 | −3.89 |
| aarF domain containing kinase 2 | Adck2 | 2.94 | 3.43 | 5.06 | −3.56 | −4.02 | −5.17 |
| Zinc finger protein 407 | Zfp407 | 0.74 | 0.98 | 0.78 | −2.41 | −3.88 | −0.99 |
| cyclic AMP-regulated phosphoprotein, 21 | Arpp21 | 3.25 | 3.46 | 3.47 | −1.84 | −0.80 | −3.75 |
| BAT2 domain containing 1 | Bat2d | 0.68 | 0.53 | 0.93 | −1.75 | −2.50 | −0.72 |
| Autism susceptibility candidate 2 | Auts2 | 4.93 | 4.61 | 3.37 | −1.22 | −4.10 | −1.19 |
| <Translation-related genes> | | | | | | | |
| cytoplasmic polyadenylation element binding protein 2 | Cpeb2 | 0.54 | 0.55 | 0.87 | −4.94 | −3.29 | −3.15 |
| Ribosomal protein L22 like 1 | Rpl22l1 | 4.18 | 3.49 | 4.66 | −4.75 | −2.51 | −1.48 |
| <Inflammation & immune response-related genes> | | | | | | | |
| guanylate binding protein 6 | Gbp6 | 1.58 | 0.76 | 4.04 | −3.01 | −1.44 | −0.70 |
| T-cell receptor beta, variable 13 | Tcrb-V13 | 3.98 | 3.61 | 3.51 | −2.47 | −1.98 | −1.62 |
| <Inflammation & immune response-related genes> | | | | | | | |
| Phosphoprotein associated with glycosphingolipid microdomains 1 | Pag1 | 0.91 | 0.60 | 1.00 | −2.45 | −1.49 | −0.53 |
| <Proteolysis-related genes> | | | | | | | |
| elastase 3, pancreatic;similar to elastase 3B, pancreatic | Ela3 | 2.75 | 3.02 | 3.07 | −4.92 | −2.06 | −2.97 |
| cathepsin M | Ctsm | 1.02 | 1.11 | 2.00 | −3.66 | −4.01 | −1.61 |
| fibroblast activation protein | Fap | 4.69 | 2.34 | 4.47 | −3.26 | −1.18 | −2.56 |
| protease, serine, 3 | Prss3 | 4.06 | 4.96 | 4.86 | −2.62 | −1.01 | −3.55 |

cells (Table 2).

*Ddah1* belongs to the dimethylarginine dimethylaminohydrolase (*DDAH*) gene family. *DDAH* is widely distributed in rat tissues, NO-producing cells and human tissues. The encoded enzyme plays a role in nitric oxide generation by regulating cellular concentrations of methylarginines, which in turn inhibit nitric oxide synthase activity[19]. Leiper *et al.*[20] suggested that DDAH inhibition could be harnessed therapeutically to reduce the vascular collapse associated with sepsis. The results of this study revealed that *Ddah1* was down-regulated in FS-treated BV-2 microglial cells (Table 2).

Taken together, these results indicate that FS may have potential efficacy for the treatment of inflammation disease and other neurodegenerative diseases

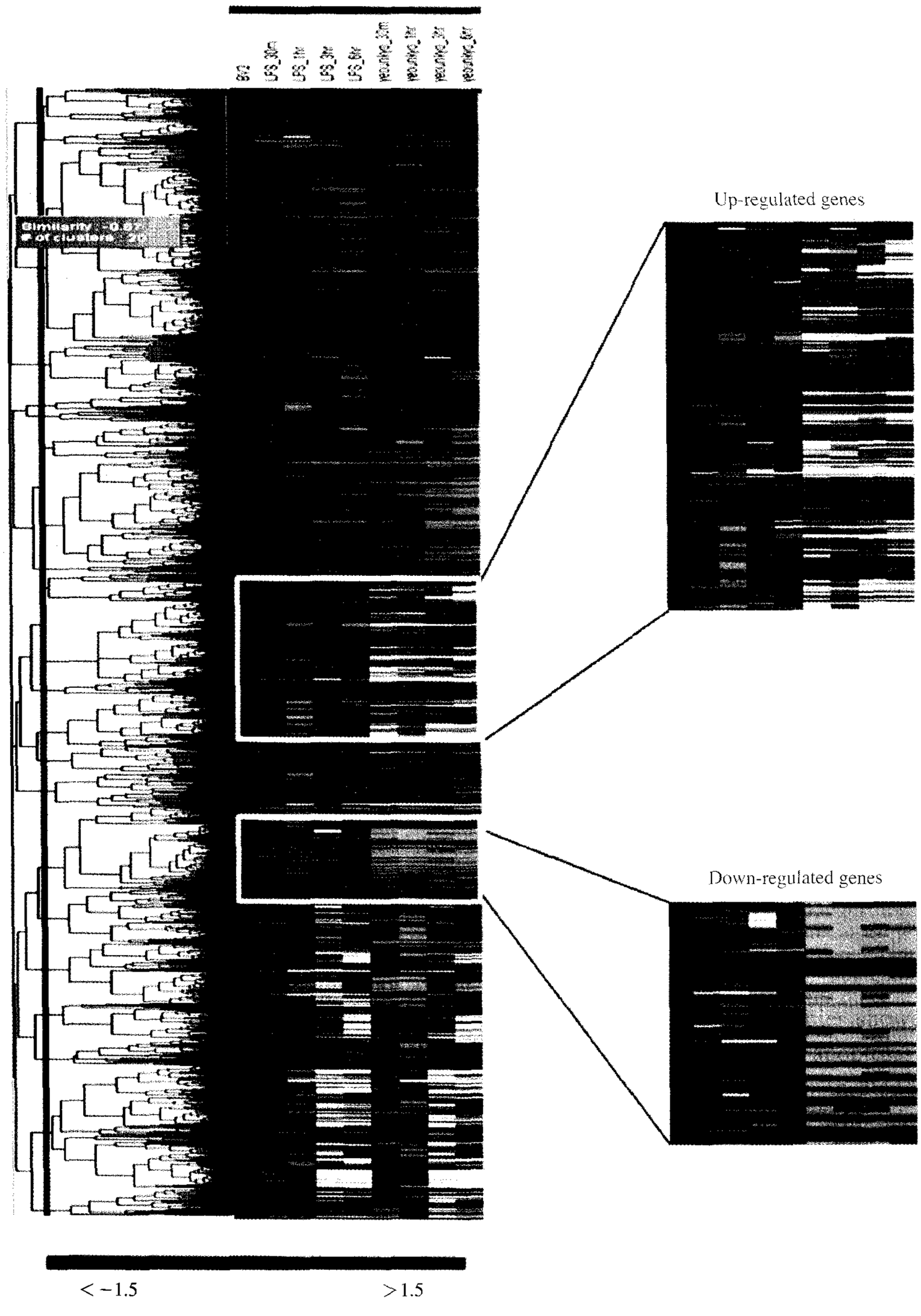


**Figure 2.** Clustergram of up- and down-regulated gene in BV-2 microglial cells. Microarray data from control (non-treated BV-2 microglial cells) and experimental (LPS or LPS plus FS-treated BV-2 microglial cells) group were combined and clustered. Each gene is represented by a single row of clusted boxes; each experimental sample is represented by a single column. The entire clusterd image is shown on the left. These clusters contain uncharacterized genes and genes not involved in these processes.

through anti-neuroinflammation by inhibiting MAPK pathway. The microarray-based genomic survey has been of interest in the study of herbal-medicines because it can quickly identify herbs with the potential for use for treatment of specific diseases based on their characteristic expression profiles and the generated profiles can also be used to identify putative mechanisms of action.

## Materials and Methods

### Preparation of FS

FS that was purchased from Sun Ten Pharmaceutical (Taipei, Taiwan) was powdered to 0.1 g and then extracted by stirring in 10 mL of DW overnight at room temperature. The sample was then centrifuged for 10 min at 3,000 rpm, after which the supernatant

was removed and sterilized by passing it through a 0.22 μm syringe filter. The filtered supernatant was then used as a stock FS for all experiments conducted for this study.

## Cell Culture

The immortalized murine BV-2 microglial cell line, which exhibits both the phenotypic and functional properties of reactive microglia cells, was grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), streptomycin, and penicillin (Invitrogen Life Technologies, Rockville, USA). BV-2 microglial cells were first cultured in a 100 mm dish ($1 \times 10^7$/dish) for 24 hr, and then pretreated with 1 μg/mL FS or left untreated and incubated for 30 min. Next, 1 μg/mL LPS was added to the samples, and the cells were then reincubated at 37°C for 30 min, 1 hr and 3 hr.

## RNA Preparation

RNA was isolated from the BV-2 microglial cells using an Rneasy® mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The isolated RNA was then quantified using NanoDrop (NanoDrop Technologies, Inc ND-1000; Wilmington, DE USA).

## Oligonucleotide Chip Microarray

An oligonucleotide chip microarray was performed using single round RNA amplification protocols, following Affimetrix's specifications (Affimetrix GeneChip Expression Analysis Technical Manual). Briefly, 3 micrograms of total RNA were used to synthesize the first-strand complementary DNA (cDNA) using oligonucleotide probes with 24 oligo-dT plus T7 promoter as a primer (Proligo LLC, Boulder, CO) and the Superscript Choice System (Life Technologies, Invitrogen, Milan, Italy). After double-stranded cDNA synthesis, the products were purified by phenol-chloroform extraction, and biotinylated antisense complimentary RNA (cRNA) was generated through *in vitro* transcription using the BioArray RNA High-Yield Transcript Labeling kit (ENZO Life Sciences Inc., Farmingdale, NY). Next, the biotinylated labeled cRNA was fragmented, and 10 μg of the total fragmented cRNA was then hybridized to the Affymetrix Mouse 430 2.0 GeneChip array (P/N900470, Affymetrix Inc., USA). The Affimetrix Fluidics Station 400 was then used to wash and stain the chips, which removed the nonhybridized target. Next, the chips were incubated with a streptavidin-phycoerythrin conjugate to stain the biotinylated cRNA. The staining was then amplified using goat IgG as blocking reagent and biotinylated antistreptavidin antibody (goat), followed by a second staining step with a streptavidin-phycoerythrin conjugate. Fluorescence was detected using the Genechip System Confocal Scanner (Hewlett-Packard), and data analysis of each GeneChip was conducted through the GeneChip 3.1 software from Affymetrix, using the standard default settings. For comparison between different chips, global scaling was used with all probe sets scaled to a user-defined target intensity of 150.

## Data Analysis

The MAS5 algorithm was used for expression summary and signal calculation of the Affymetrix Mouse 430 2.0 data. Global scaling normalization was performed and the normalized data were then log-transformed with base 2. Fold change was applied to select the differentially expressed genes (DEGs): the fold change threshold used was 1.5-fold and the significance level was $P<0.05$. Furthermore, the probe sets with all A calls in the compared groups were removed to filter false positives. Each probe set of the Affymetrix GeneChip data has a detection call: P, present call considered as good quality; M, marginal call considered intermediate quality; A, absent call considered to be of relatively low reliability. The 1.5-fold DEGs were clustered using hierarchical clustering with Pearson's correlation as a similarity measure and complete linkage as a linkage method. GenPlex™ v2.3 software (ISTECH Inc., Korea) was utilized for cluster analysis. The gene ontology significance analysis was then performed to investigate the functional relationships among the 1.5-fold DEGs using high-throughput GoMiner. The 1.5-fold DEGs were mapped to the relevant pathways using GenPlex™ v2.4 software (ISTECH Inc., Korea). The pathway resources are provided by the KEGG database.

# Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R13-2007-00000-0).

# References

1. Zheng, L. T., Ock, J., Kwon, B. M. & Suk, K. Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. *Int Immunopharmacol* **8**:484-494 (2008).
2. Ock, J. *et al.* Regulation of Toll-like receptor 4 expression and its signaling by hypoxia in cultured microglia. *J Neurosci Res* **85**:1989-1995 (2007).
3. Kim, W. K. *et al.* A new anti-inflammatory agent

KL-1037 represses proinflammatory cytokine and inducible nitric oxide synthase (iNOS) gene expression in activated microglia. *Neuropharmacology* **47**:243-252 (2004).
4. Nagai, A. *et al.* Immortalized human microglial cell line: phenotypic expression. *J Neurosci Res* **81**:342-348 (2005).
5. Seo, W. G. *et al.* Inhibitory effect of ethyl acetate fraction from Cudrania tricuspidata on the expression of nitric oxide synthase gene in RAW 264.7 macrophages stimulated with interferon-gamma and lipopolysaccharide. *Gen Pharmacol* **35**:21-28 (2000).
6. Reynolds, A. D. *et al.* Nitrated alpha-synuclein and microglial neuroregulatory activities. *J Neuroimmune Pharmacol* **3**:59-74 (2008).
7. Skaper, S. D. The brain as a target for inflammatory processes and neuroprotective strategies. *Ann N Y Acad Sci* **1122**:23-34 (2007).
8. Hou, R. C., Chen, H. L., Tzen, J. T. & Jeng, K. C. Effect of sesame antioxidants on LPS-induced NO production by BV2 microglial cells. *Neuroreport* **14**: 1815-1819 (2003).
9. Guo, H., Liu, A. H., Li, L. & Guo, D. A. Simultaneous determination of 12 major constituents in Forsythia suspensa by high performance liquid chromatography-DAD method. *J Pharm Biomed Anal* **43**: 1000 -1006 (2007).
10. Abebe, W. Herbal medication: potential for adverse interactions with analgesic drugs. *J Clin Pharm Ther* **27**:391-401 (2002).
11. Stickel, F. & Schuppan, D. Herbal medicine in the treatment of liver diseases. *Dig Liver Dis* **39**:293-304 (2007).
12. Kim, C. S. *et al.* Effect of various implant coatings on biological responses in MG63 using cDNA microarray. *J Oral Rehabil* **33**:368-379 (2006).
13. Wang, Y. *et al.* Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide microarrays. *BMC Genomics* **7**:59 (2006).
14. Raines, K. W. *et al.* Nitric oxide inhibition of ERK1/2 activity in cells expressing neuronal nitric-oxide synthase. *J Biol Chem* **279**: 3933-3940 (2004).
15. Sim, S. *et al.* NADPH oxidase-derived reactive oxygen species-mediated activation of ERK1/2 is requir-ed for apoptosis of human neutrophils induced by Entamoeba histolytica. *J Immunol* **174**:4279-4288 (2005).
16. Akundi, R. S. *et al.* Signal transduction pathways regulating cyclooxygenase-2 in lipopolysaccharide-activated primary rat microglia. *Glia* **51**:199-208 (2005).
17. Zaragoza, C. *et al.* Activation of the mitogen activated protein kinase extracellular signal-regulated kinase 1 and 2 by the nitric oxide-cGMP-cGMP-dependent protein kinase axis regulates the expression of matrix metalloproteinase 13 in vascular endothelial cells. *Mol Pharmacol* **62**:927-935 (2002).
18. Fang, M. *et al.* Dexras1: a G protein specifically coupled to neuronal nitric oxide synthase via CAPON. *Neuron* **28**:183-193 (2000).
19. Kimoto, M. *et al.* Purification, cDNA cloning and expression of human NG,NG-dimethylarginine dimethylaminohydrolase. *Eur J Biochem* **258**:863-868 (1998).
20. Leiper, J. *et al.* Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat Med* **13**:198-203 (2007).