INTRODUCTION

Sand storms arise very common in desert area and in Asia, it occurs in Gobi Desert, the Taklimakan Desert and interior China. Asian sand dust (ASD) spreads neighboring area including East China, Korean Peninsula, and Japan [1]. ASD is composed of various chemical components such as Sulfate ($SO_4^{2-}$) and Nitrate ($NO_3^-$) derived from air pollutants and microbial agents, including bacteria, fungi, fungal spores, and viruses [2]. ASD is known to be related with enhancement of ovalbumin induced lung eosinophilia, chronic pulmonary injury [3], and rhinovirus replication in human nasal epithelial cells [4]. Therefore, hazardous effects of ASD on human health are becoming a serious concern in East Asia.

The otitis media (OM) is multifactorial disease and the causative agent includes a viral and bacterial infection, biofilm formation, congenital anomaly, and environmental factor, such as smoking, and air pollution [5].

The epidemiologic studies have investigated the relationship between ambient air pollution and OM in human populations.
A study conducted in the Czech Republic showed that children living in areas with high concentrations of particulate matter (PM) and SO$_2$ have significantly higher rates of OM compared with those in the control area [6]. Rover et al. [7] showed that the level of SO$_2$ emission is one of the important risk factors of OM in many industrialized western countries. A large cohort study showed the close relationship between the prevalence of OM and air quality [8].

In our previous studies, we have shown that air pollutant such as urban particle, diesel exhaust particle and acrolein can induced inflammation and cause alteration of gene expression human middle ear epithelial cell (HMEEC) [9,10]. However, there is no study about the biological effect of ASD on middle ear epithelial cells.

The aim of this study is to evaluate the gene expression profile of ASD-treated HMEEC using microarray analysis.

**MATERIALS AND METHODS**

**Preparation of ASD**

ASD were collected on March 16, 2009 at outside the Gachon University building in Korea using a high volume air sampler (HV500F, Sibata, Tokyo, Japan) at a flow rate of 500 L/minute. These ASD were prefiltered to filter packs (prefilter AP, 124 mm; Millipore, Bedford, MA, USA) and then sieved on filter of 10-μm pore size after mixing with phosphate buffered saline in a 10-mL tube. The filtered ASD particles were sterilized in an autoclave at 121°C for 15 minutes and determined their mass and then collected in a 1.5-mL tube. The particle diameter of the samples (a total of 600 particles) was measured under a scanning electron microscope (JSM-5800 JEOL Ltd., Tokyo, Japan). The mean distribution peak of particle diameter in ASD was observed at 6 mm (Fig. 1). Chemical component of ASD particles were analyzed with X-ray fluorescence spectrometry (PHILIPS PW2404, PHILIPS Electronic Instruments, Amsterdam, the Netherlands) at Korea basic science institute.

**Cell culture and reagents**

HMEECs were maintained in a mixture of high glucose Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA, USA) and bronchial epithelial basal medium (Lonza, Walkersville, MD, USA) (1:1). The cells were maintained at subconfluence in a humidified atmosphere of 5% CO$_2$ and 95% air at 37°C. The cells were grown to 60% confluence in six-well culture plates. After starvation for 2 hours, medium was replaced to ASD containing medium and exposed to disperse ASD at a final concentration of 400 μg/mL for 24 hours. For the control group, ASD was not added.

**Gene expression microarray**

Total RNAs were extracted using Trizol reagent (Ambion, Carlsbad, CA, USA), and cDNA was synthesized by AccuPower reverse transcriptase premix (BIONEER, Daejeon, Korea). Three RNA samples were used for miRNA microarray analysis. The RNA samples were then labeled with Cyanine 3-CTP (Cy3) and Cyanine 5-CTP (Cy5) in an *in vitro* transcription reaction using a Low Input Quick Amp Labeling kit (Agilent Technologies, Santa Clara, CA, USA), in accordance with the manufacturer’s protocol. Labeled cRNA was hybridized on Human GE 4×44K v2 microarray (ID G2519-026652, Agilent Technologies), followed by manual washing, according to the manufacturer’s procedures. The array was scanned using the Agilent DNA MicroArray Scanner and probe signals were quantified using Agilent’s Feature Extraction ver. 10.10.1.1 (Agilent Technologies). Normalized data were analyzed using Subio platform v1.16.4376 (Subio Inc., Kagoshima, Japan).

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**Fig. 1.** Analysis of Asian sand dust (ASD) constitution. (A) Scanning electron microscopic photomicrograph of ASD particles. (B) Distribution peak of particle diameter in ASD.
Pathway analysis

Molecular pathways, among the differentially expressed genes identified by the microarray, were dissected using Pathway Studio 9.0 (Ariadne Genomics, Rockville, MD, USA). This program used to facilitate integration of relevant information among the imported genes, consequently allowing identification of biological pathways, gene regulation networks and protein interaction maps.

Real-time reverse transcription polymerase chain reaction

Total RNA was extracted using Trizol according to the protocol of the manufacturer. Yield and purity was determined by spectrophotometry and total RNA was reverse transcribed to cDNA using PrimeScript 1st Strand cDNA Synthesis kit according to the manufacturer’s instructions (TaKaRa Bio Inc., Kusatsu, Japan). The quantitation of mRNA expression was carried out using the ABI Prism 7300 real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster, CA, USA). The PCR amplification performed with a 20-μL final reaction mixture and PCR reaction mixture were incubated at 95°C for 15 seconds and 60°C for 1 minutes, followed by amplification for 45 cycles. The relative level of gene expressions were normalized to endogenous glyceraldehyde-3-phosphate dehydrogenase and target mRNA expression in the experimental groups were calculated, relative to the control group.

The genes and primers used for RT-PCR were as follows: MUC1 (mucin 1, cell surface associated), MUC4 (mucin 4, cell surface associated), TNF (tumor necrosis factor [TNF superfamily, member 2]), TNFSF14 (tumor necrosis factor [ligand] superfamily, member 14), CCL5 (chemokine [C-C motif] ligand 5), CCL20 (chemokine [C-C motif] ligand 20), CXCL10 (chemokine [C-X-C motif] ligand 10), JUN (jun oncogene), ANGPT1 (angiopoietin 1), and TIMP1 (TIMP metallopeptidase inhibitor 1) (Table 1).

Statistical analysis

Data were expressed as mean±SD. Paired comparisons were performed using the Mann-Whitney test in SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant when P-values were <0.05. P-values less than 0.05 were considered significant.

RESULTS

The contents of elements in ASD

The most abundant component was 78.4% for SiO₂ among the contents of elements in ASD. Other ASD components were 9.35% for Al₂O₃, 2.52% for K₂O, 2.41% for Na₂O, 2.06% for Fe₂O₃, 1.74% for CaO, respectively. MgO, TiO₂, P₂O₅, and MnO composed less than 0.1% of the total (Table 2).

Table 1. Sequences of primers used for real-time reverse transcription polymerase chain reaction

| Gene name | Forward primer sequence | Reverse primer sequence | Size (bp) |
|-----------|-------------------------|-------------------------|-----------|
| MUC1      | CGCCGAAGAACACTGGCCAGCTG | CAAGTTGGCAGAAGTGTGGGCTGC | 207       |
| MUC4      | CTTACTCGGCAACTCTGTAGTG  | GAGAAGTTGGGGCTGACTGTC   | 470       |
| TNF       | AGGGCGAAGCCTGGTATG      | CCGGCCTGGTATG       | 91        |
| TNFSF14   | ATACAAAGCGAAGGTCTTCAGC  | CTGAGTCCTCCATAAGCGG   | 102       |
| CCL5      | CTGCTTGCTCTACATGCCC    | TCGGGTGCAAGAAGCAGCTG  | 103       |
| CCL20     | TGCTGTACCAAGAGITTGCTTC | CGCACACAGAACACTTCTTTT | 220       |
| CXCL10    | GTGGCATTCAGAGAGATCTCTC | TGATGGCCTCGATTGGATATT | 198       |
| JUN       | TCGACATGGAAGCTCCAGGA   | SGCGATCTCTCAGGCTCC    | 100       |
| ANG       | AGCGGCGAAGTCCAGAAAC    | TACTCTACGAGACGGTCCTTC | 108       |
| TIMP1     | AGAGTGTCTGGGGAATCTC    | CCAACAGTGAGGTCTTGAGT  | 169       |
| TERT      | CAAGCTGTGTTGGGGGAGTTC  | AGTCCACCGATGTCTCCGC   | 167       |

Table 2. Elemental contents of Asian sand dust

| Component | Element fraction (wt%) |
|-----------|------------------------|
| SiO₂      | 78.4                   |
| Al₂O₃     | 9.35                   |
| K₂O       | 2.52                   |
| Na₂O      | 2.41                   |
| Fe₂O₃     | 2.06                   |
| CaO       | 1.74                   |
| MgO       | 0.69                   |
| TiO₂      | 0.29                   |
| P₂O₅      | 0.05                   |
| MnO       | 0.05                   |
| Loss on ignition | 1.74             |

*Total Fe.
increase in the gene expression involved in signal transduction and regulation of metabolism pathway.

A total of 1,274 genes were classified in up-regulated genes and down-regulated genes, it represents biological network of genes in HMEECs exposed to ASD. Up-regulated genes were mainly involved in cellular processes, including cell proliferation, apoptosis, cell differentiation, cell growth, cell death (Fig. 2). Down-regulated genes affected several cellular processes, including cell cycle, cell proliferation, apoptosis and cell growth (Fig. 3). Gene ontology analysis of microarray data also showed the biological functions of the differentially expressed gene signature in HMEECs treated ASD (Fig. 4).

Identification of potential signaling networks and key mediators associated with exposure to ASD in HMEECs

We investigated signaling networks using Pathway Studio 9.0 to determine potential molecular signaling networks by ASD exposure. Pathway Studio 9.0 represented that relevant components in the putative signaling pathways were chosen and incorporated into the established networks, based on a number of reliable studies.

A total of 47 genes were identified as crucial components in our signaling networks data containing 2-fold up regulated genes (Table 3, Fig. 2). Among them, 10 genes including ADM, CCL5, EDN1, EGR1, FOS, GHRL, JUN, SOCS3, TNF, and TNFSF10 were discovered as the key mediator genes among the up-regulated genes altered by ASD exposure. These 10 genes are shown to be related to diverse biological processes, including cell proliferation, cell differentiation, apoptosis, cell growth and cell death and these cell processes might lead to neoplasm, inflammation, infection, cancer, and death (Fig. 2).

On the other hand, a total of 38 genes were revealed as key modulators in the signaling pathway associated with 2-fold down regulated genes (Table 4, Fig. 3). Among 38 down-regulated genes, 11 genes, CSF3, DKK1, FOSL1, FST, TERT, MMP13, PTHLH, SPRY2, TGFBR2, THBS1, and TIMP1, were identified as the main modulator genes. These down-regulated gene sets showed distinct molecular pathway related to cell differentiation, cell cycle, apoptosis, cell proliferation, cell growth, which can induce neoplasm, neoplasm metastasis, inflammation, cancer, death, wounds and injuries (Fig. 3).

Validation of gene expression by real-time RT-PCR

We conducted real-time RT-PCR to determine the expression pattern of the genes of interest. The real-time RT-PCR and was compared with the results of the microarray experiments, which showed similar pattern (Table 5, Fig. 5).
Fig. 3. Dissection of responsive molecular network of down-regulated genes induced by Asian sand dust. Magnified blue circles indicate down-regulated genes in our microarray data; FST, TERT, SPRY2, PTHLH, MMP13, DKK1, TGFBR2, CSF3, FOSL1, THBS1, MAPK1, MAP1, SH202A, and TIMP1.

Fig. 4. Biological processes involving genes that are altered in response to Asian sand dust exposure at human middle ear epithelial cells are shown in graph.
Table 3. List of genes up-regulated 2 folds in human middle ear epithelial cell exposed to Asian sand dust

| Gene symbol | Gene name                                               | Fold change | P-value       |
|-------------|---------------------------------------------------------|-------------|---------------|
| CCL11       | chemokine (C-C motif) ligand 11                         | 19.2        | 0.0070259     |
| IGFBP3      | insulin-like growth factor binding protein 3            | 12.3        | 1.15E-20      |
| PROC        | protein C (inactivator of coagulation factors Va and VIIIa) | 6.3        | 0.0071087     |
| NDRG1       | N-myel downstream regulated 1                          | 6.2         | 0.0069414     |
| MUC4        | mucin 4, cell surface associated                        | 6.1         | 0.0229008     |
| EGRI        | early growth response 1                                 | 6.0         | 0.0070178     |
| TEK         | TEK tyrosine kinase, endothelial                        | 5.7         | 0.0258073     |
| ANGPT1      | angiopoietin 1                                          | 5.3         | 0.0126616     |
| INPP5D      | inositol polyphosphate-5-phosphatase, 145kDa           | 5.3         | 0.0075098     |
| ADM         | adrenomedullin                                          | 5.3         | 1.64E-19      |
| COL2A1      | collagen, type II, alpha 1                             | 5.1         | 0.0438108     |
| ALOX5       | arachidonate 5-bpoxynase                                | 5.0         | 2.9E-10       |
| IL1RN       | interleukin 1 receptor antagonist                       | 4.9         | 0.0078352     |
| MUC1        | mucin 1, cell surface associated                        | 4.8         | 0.0078951     |
| ATF3        | activating transcription factor 3                       | 4.5         | 0.0082827     |
| CCL5        | chemokine (C-C motif) ligand 5                          | 4.5         | 0.0084039     |
| GHRL        | ghrelin/obestatin prepropeptide                         | 4.4         | 6.90E-05      |
| TNFSF14     | tumor necrosis factor (ligand) superfamily, member 14   | 4.1         | 0.0095794     |
| S100A8      | S100 calcium binding protein A8                         | 3.8         | 0.0017427     |
| NGFR        | nerve growth factor receptor (TNFR superfamily, member 16) | 3.5       | 0.0114835     |
| TNFSF10     | tumor necrosis factor (ligand) superfamily, member 10   | 3.4         | 0.010262      |
| IL24        | interleukin 24                                          | 3.3         | 0.0145976     |
| FOSL2       | FOS-like antigen 2                                      | 3.2         | 0.011863      |
| ESR1        | estrogen receptor 1                                     | 3.1         | 2.03E-08      |
| PDCD4       | programmed cell death 4 (neoplastic transformation inhibitor) | 3.1       | 0.013895      |
| CEBPA       | CCAAT/enhancer binding protein (C/EBP), alpha           | 3.0         | 0.0026859     |
| CXCL10      | chemokine (C-X-C motif) ligand 10                       | 2.9         | 0.0137745     |
| DPP4        | dipeptidyl-peptidase 4                                  | 2.7         | 0.0146426     |
| CTSK        | cathepsin D                                            | 2.7         | 0.0138585     |
| FGFR3       | fibroblast growth factor receptor 3                     | 2.7         | 0.0067294     |
| TNF         | tumor necrosis factor (TNF superfamily, member 2)       | 2.6         | 0.0211606     |
| PTGS1       | prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) | 2.6       | 0.014793      |
| IL11        | interleukin 11                                          | 2.5         | 0.0153152     |
| EDN1        | endothelin 1                                            | 2.4         | 0.0028692     |
| JUN         | jun oncogene                                            | 2.3         | 0.0175072     |
| CCL20       | chemokine (C-C motif) ligand 20                         | 2.3         | 0.0182421     |
| TIMP2       | TIMP metallopeptidase inhibitor 2                       | 2.3         | 0.0175299     |
| COL18A1     | collagen, type XVIII, alpha 1                           | 2.3         | 0.0181303     |
| HSPB1       | heat shock 27kDa protein 1                              | 2.3         | 0.0181494     |
| CLU         | clusterin                                              | 2.2         | 0.0193858     |
| MAP3K8      | mitogen-activated protein kinase kinase 8               | 2.2         | 0.0189869     |
| NOTCH1      | Notch homolog 1, translocations-associated (Drosophila) | 2.1         | 0.0036407     |
| SOCS3       | suppressor of cytokine signaling 3                      | 2.1         | 0.0219293     |
| TLR3        | toll-like receptor 3                                    | 2.1         | 0.0222069     |
| F2R         | coagulation factor II (thrombin) receptor               | 2.0         | 0.0255691     |
| TP73        | tumor protein p73                                       | 2.0         | 0.0336533     |
| CSF1R       | colony stimulating factor 1 receptor                    | 2.0         | 0.002476      |

**DISCUSSION**

Recently, the environmental factor, such as air pollution and PM have been evaluated for hazardous effects on human health and most of these studies are elucidating the correlation between environmental factors and human disease [1]. In the present study, we focused on the effects of ASD on HMEECs to determine the relationship between ASD and the pathogenesis of OM in human. ASD are mainly composed of various chemical components.
### Table 4. List of genes down-regulated 2 folds in human middle ear epithelial cell exposed to Asian sand dust

| Gene symbol | Gene name                                                                 | Fold change | P-value       |
|-------------|---------------------------------------------------------------------------|-------------|---------------|
| TGFBR2      | transforming growth factor, beta receptor II (70/80kDa)                    | 0.5         | 0.0420199    |
| SPRY1       | sprouty homolog 1, antagonist of FGF signaling (Drosophila)                | 0.5         | 0.049248     |
| NEFH        | neurofilament, heavy polypeptide                                           | 0.5         | 0.0393223    |
| GRB14       | growth factor receptor-bound protein 14                                    | 0.5         | 0.0385258    |
| RUNX1       | runt-related transcription factor 1                                        | 0.5         | 0.0407238    |
| TNFRSF10A   | tumor necrosis factor receptor superfamily, member 10a                     | 0.5         | 0.0451189    |
| ADAM19      | ADAM metallopeptidase domain 19 (melrin beta)                              | 0.5         | 0.0433274    |
| SNAI1       | snail homolog 1 (Drosophila)                                               | 0.5         | 0.0388442    |
| SSH1        | slingshot homolog 1 (Drosophila)                                          | 0.5         | 0.041561     |
| FKB4        | FK506 binding protein 4, 59kDa                                             | 0.5         | 0.0392789    |
| IFRD1       | interferon-related developmental regulator 1                               | 0.5         | 0.0396027    |
| NT5E        | 5'-nucleotidase, ecto (CD73)                                               | 0.5         | 0.0408103    |
| HMGA2       | high mobility group AF-hook 2                                              | 0.4         | 0.0385285    |
| WNT5B       | wingless-type MMTV integration site family, member 5B                      | 0.4         | 0.0402535    |
| SERPINE2    | serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 | 0.4         | 0.0396555    |
| FUT4        | fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)   | 0.4         | 0.0438824    |
| TNFRSF8     | tumor necrosis factor receptor superfamily, member 8                       | 0.4         | 0.0383572    |
| ESX1        | ESX homeobox 1                                                             | 0.4         | 0.0409304    |
| NF2         | neurofibromin 2 (merlin)                                                   | 0.4         | 0.0382844    |
| SPRY2       | sprouty homolog 2 (Drosophila)                                             | 0.4         | 0.0420374    |
| PLCB2       | phospholipase C, beta 2                                                    | 0.4         | 0.0392296    |
| FOSL1       | FOS-like antigen 1                                                         | 0.4         | 0.0382001    |
| LHX1        | LIM homeobox 1                                                             | 0.4         | 0.0484611    |
| TERT        | telomerase reverse transcriptase                                            | 0.4         | 0.0332238    |
| SPRED1      | sprouty-related, EVH1 domain containing 1                                  | 0.4         | 0.0416513    |
| PTHLH       | parathyroid hormone-like hormone                                           | 0.4         | 0.0402907    |
| WWTR1       | WW domain containing transcription regulator 1                             | 0.4         | 0.0382663    |
| THBS1       | thrombospondin 1                                                           | 0.4         | 0.0395504    |
| SLC8A1      | solute carrier family 8 (sodium/calcium exchanger), member 1               | 0.4         | 0.0447106    |
| TIMP1       | TIMP metallopeptidase inhibitor 1                                          | 0.4         | 0.0389436    |
| PHLD1       | pleckstrin homology-like domain, family A, member 1                        | 0.4         | 0.0477989    |
| FST         | follistatin                                                                | 0.4         | 0.0347311    |
| IL23A       | interleukin 23, alpha subunit p19                                          | 0.3         | 0.0403809    |
| DUSP6       | dual specificity phosphatase 6                                              | 0.3         | 0.043029     |
| DKK1        | dickkopf homolog 1 (Xenopus laevis)                                        | 0.3         | 0.0434005    |
| MMP1        | matrix metallopeptidase 1 (interstitial collagenase)                       | 0.3         | 0.0266174    |
| DCRD2       | discoidin, CUB and LCC1 domain containing 2                                | 0.3         | 0.0466419    |
| CSF3        | colony stimulating factor 3 (granulocyte)                                  | 0.2         | 0.0483729    |

### Table 5. Quantification of gene expression using real-time reverse transcription polymerase chain reaction (RT-PCR)

| Gene symbol | Gene name                                                                 | Fold change | Quantitative RT-PCR | Microarray |
|-------------|---------------------------------------------------------------------------|-------------|---------------------|-----------|
| MUC1        | mucin 1, cell surface associated                                          | 3.9         | 4.8                 |
| MUC4        | mucin 4, cell surface associated                                          | 5.7         | 6.1                 |
| TNF         | tumor necrosis factor (TNF superfamily, member 2)                        | 2.2         | 2.6                 |
| TNFRSF14    | tumor necrosis factor (ligand) superfamily, member 14                    | 3.0         | 4.1                 |
| CCL5        | chemokine (C-C motif) ligand 5                                            | 4.7         | 4.5                 |
| CCL20       | chemokine (C-C motif) ligand 20                                           | 3.0         | 2.3                 |
| JUN         | jun oncogene                                                             | 2.8         | 2.3                 |
| ANG         | angiotensin 1                                                            | 4.7         | 5.3                 |
| TIMP1       | TIMP metallopeptidase inhibitor 1                                         | 0.4         | 0.4                 |
| TERT        | telomerase reverse transcriptase                                          | 0.2         | 0.4                 |
as well as biological materials such as bacterial, fungi and viruses. The composition of ASD mix can change during their long-range transport and it can cause numerous harmful health effect according to materials of ASD mix [11]. Recent studies reported that ASD exposure is associated with inflammatory reaction via cytokine production [12]. Also, it has been reported that acute and chronic pulmonary toxicity was induced by intratracehally instilled ASD [3]. However, specific molecular signaling network for ASD-induced toxic response in human middle ear epithelium is not investigated yet. We, therefore, analyzed gene expression profile using microarray to understand molecular mechanism between ASD and OM. Visualization of signaling pathways among differentially expressed genes enables to create their own pathways and understand complicated signaling network easily [13]. Additionally, Pathway Studio 9.0 was applied for investigation of molecular signaling network triggered by ASD in human cells.

A total of 1,274 genes were 2 folds differentially expressed by ASD exposure. Among them, 1,138 genes were 2 folds up-regulated, whereas 136 genes were 2 folds down-regulated. Our signaling network among 2-fold up-regulated genes suggested several cellular processes including apoptosis, cell death, cell differentiation, cell growth, and cell proliferation might be regulated by ASD exposure. Also, this cell processes may lead to cancer, death, inflammation, infection, and neoplasm. Our signaling network data suggested the key modulators based on integration of relevant information among imported genes, consequently allowing identification of biological pathways, gene regulation networks, and protein interaction maps. The 10 genes including ADM, CCL5, EDN1, EGR1, FOS, GHRL, JUN, SOCS3, TNF, and TNFSF10 were identified as main modulators in up-regulated genes. TNF family genes are a multifunctional proinflammatory cytokine. Regarding PM, few of studies have been reported recently. TNF-α production was enhanced by yellow sand dust exposure in RBL-2H3 cells [14]. Another study reported increase of TNF-α in bronchoalveolar lavage under ASD exposure [15]. Our data also showed up-regulation of TNF genes under ASD exposure, therefore, this gene can be a critical biomarker for ASD.

Yanagisawa et al. [16], examined the enhancement of inflammatory response-related genes in the murine lung by ASD using microarray analysis. Inflammation is a critical for OM, and uncontrolled inflammation in the middle ear can cause the development and progression of OM. In our data, inflammatory response-related genes including CCL5, CCL20, and CXCL10 are increased after exposure to ASD to HMEEC compared to the control group, indicating that ASD-induced inflammatory cytokines can affect to the development of OM. One of the key modulators in up-regulated genes, JUN gene, is known to be regulated by peptide growth factors, proinflammatory cytokines, oxidative and other forms of cellular stress with dimerization of AP-1 [17]. In our data, JUN was up-regulated by ASD, indicating that it may act as a main mediator to induce OM via regulation of inflammatory cytokines and oxidative stress in middle ear epithelial cells.

Mucins are known to play an important role for the protection and function of the underlying middle ear epithelium. In this study, MUC1 and MUC4 expression level were higher after ASD exposure. It can relate to mucous production for protection of epithelium by ASD. Wei et al. [18], reported interaction of MUC1 with p53 results in inhibition of p53-mediated apoptosis and cell cycle arrest. In our data, MUC1 was increased in HMEECs exposed ASD compared to the control group. It means that MUC1 may contribute to a defense mechanism to prevent cell death against ASD exposure.

Other genes, ADM, CCL5, EDN1, EGR1, FOS, GHRL, SOCS3, and TNFSF10, were firstly identified and suggested as responsible genes toward ASD exposure. Therefore, further studies are warranted for being reliable biomarker.

We also depicted molecular signaling network among 2-fold down-regulated genes. We found that 11 genes including CSF3, DKK1, FOSL1, FST, TERT, MMP13, PTHLH, SPRY2, TGFB2, THBS1, and TIMP1 acted as main components of pathway associated with apoptosis, cell cycle, cell growth, cell differentiation, and cell proliferation against ASD exposure. Among them, FOSL1 was significantly up-regulated against PM 2.5 in A549 cells [19]. Although our data showed down-regulation of FOSL1, this gene might be considered as a candidate ASD biomarker.

Telomerase reverse transcriptase (TERT) has been known to prevent of aging following multiple round of replication. Recent study demonstrated that TERT partially protect HSCs from reactive oxygen species-induced apoptosis via p38MAPK activation [20]. Expression of TERT was decreased compared to the control group in our present study. Therefore, down-regulation of TERT might affect reactive oxygen species-induced apoptosis and block cell survival in HMEECs after ASD exposure. In addition, down-regulation of TIMP1 has been suggested as key mod-

Fig. 5. Quantitative reverse transcription polymerase chain reaction analysis of selected genes at 24 hours after exposure to Asian sand dust (ASD). The results are shown as means±SD. All data are determined P-value. P<0.05 vs. the control group.
CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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