EXPERIMENTAL STUDY

Genome wide expression analysis of the effect of Socheongryong Tang in asthma model of mice

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Abstract

OBJECTIVE: To investigate the molecular effect of Socheongryong Tang (SCRT, Xiaoqinglong Tang in Chinese) on whole genome level in asthma mouse model by microarray technology.

METHODS: Asthma was induced by intranasal instillation of ovalbumin in mouse. After administration of SCRT on asthma-induced mouse, the expression of genes in lung tissue was measured using whole genome microarray. The functional implication of differentially expressed genes was performed using ontological analysis and the similarity of promoter structure of genes was also analyzed.

RESULTS: Treatment of SCRT restored expression level of many up- or down-regulated genes in asthma model, and this recovery rate means SCRT could regulate a set of genes having specific TFBS binding sites.

CONCLUSION: In this study, we identified a set of genes subjected to similar regulation by SCRT in asthma model in mice.

INTRODUCTION

Socheongryong Tang (SCRT, Xiaoqinglong Tang in Chinese; Sho-Seiryu-To in Japanese) is a well-known traditional herbal medicine for allergic rhinitis, bronchitis, bronchial asthma and cold symptoms in Korea.1,2 Recently, SCRT was known to have an anti-allergic activity on airway inflammation in a mouse model.3 In addition, in vitro analysis shows several anti-allergic activity of SCRT including inhibition of histamine release,4 proliferation of eosinophils and basophils.5,6 Interestingly, SCRT was shown to decrease the expression of the interleukin (IL)-4 mRNA, which suppresses Th2 cell development1,2 which have relevance to our previous report.8 These evidences indicate SCRT would exert various immuno-suppressive effects in vivo and in vitro although the molecular mechanism has not been clearly demonstrated. Throughout the world, asthma is a serious public health problem affecting people of all ages, and is an inflammatory disease of the airways which may be worsened due to numerous extrinsic factors. The most common trigger is continuous exposure to allergens.9 Allergic asthma is characterized by reversible airway obstruction, increased mucus production, infiltration of cosin-
ophylin, and nonspecific airway hyper-responsiveness, and its development is mediated by the over-expression of Th2-mediated or Th1-mediated cytokines, including IL-4, IL-5, IL-8, and tumor necrosis factor-α.\textsuperscript{10,11} The pathophysiologic mechanism of asthma is still unclear despite the increasing prevalence of this disease, and current treatments are not satisfactory. Severe asthma accounts for only 5% to 10% of patients with asthma, but it accounts for a considerable portion of the health care costs associated with this disease, and patients with severe asthma are particularly difficult to treat.\textsuperscript{12-15} Current therapies incompletely control symptoms of asthma and even intensive treatment having little effect on health care utilization.\textsuperscript{15} Consequently, effort should be made to identify new remedies, preferably of natural origin, for mitigating these disorders. We previously reported the strong possibility of SCRT as a complementary or alternative drug to western drug also demonstrated that regulation of Th1/Th2 imbalance may be one of mechanism contributed to treatment for respiratory disease by SCRT.\textsuperscript{1}

However, it is difficult to elucidate pharmaceutical mechanism of mixed herbal formula such as SCRT because of complex nature of herbal components. Recent development of microarray makes it possible to investigate the effect of chemicals in molecular level. By applying microarray technology, expression levels of thousands of genes can be measured simultaneously. Therefore, in this study, the effect of SCRT on asthma model in mouse was investigated on whole genome level.

**MATERIALS AND METHODS**

**Animal**

Eight week-old female Balb/c mice (Orient Bio, Sungnam, Korea) were housed in polypropylene cages at (24 ± 3) °C under 12 h light and dark cycle for at least 2 weeks prior to the experiment. They were fed with standard pellet diet and water ad libitum. All experiments were approved by Institutional Animal Care committee of Seoul National University and conducted in accordance with the guidelines of Seoul National University.

**Preparation of SCRT**

Eight species of dried medicinal herbs composing SCRT were supplied by H-Max Pharmaceuticals Ltd. (Seoul, Korea), and were authenticated by one of the authors (SIC, an experienced Pharmacognost) at the School of Korean Medicine, Pusan National University, where voucher specimens (No. SKM-SCRT-201-208) were deposited. As shown in Table 1, SCRT was composed of eight species of medicinal herbs and the mixture of ingredients was boiled in 1300 mL of distilled water using Herb Extractor (Dae Woong, Seoul, Korea) for 3 h to make the final volume of 500 mL. The extract was centrifuged at 2200 × g for 20 min, and then it was filtered using Whatman filter paper No. 3. The filtrate was condensed using Vacuum Evaporator (EYELA, Tokyo, Japan) and then stored at -20 °C until use.

**Induction of asthma**

Twenty one mice were divided into three groups (7 mice for naive, 7 mice for induction of asthma and 7 mice for induction of asthma with SCRT administration). Two weeks after intraperitoneal injection of 100 mL of phosphate buffer saline (PBS) for naïve or emulsion containing 100 μg of ovalbumin (OVA) and 2 mg of alum for induction of asthma for 3 consecutive days (Days 1-3), mice were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and rompun (10 mg/kg). Mice were then treated with intranasal instillation of 30 mL of PBS containing 25 μg of OVA for 2 days (Days 18 and 19). Three days later, intranasal instillation was conducted again for 2 days (Days 23 and 24). SCRT was given on the last day of first intranasal instillation period and continued for 6 days (Day 19-24). The dose of SCRT was 4 times higher than that for human adults. The dose was determined after consideration of basal metabolic rates and body weight as in our previous experiments.

<table>
<thead>
<tr>
<th>Herbal name</th>
<th>Standard materials</th>
<th>Botanical name</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizoma Pinelliae</em></td>
<td>Homogentistic acid</td>
<td><em>Pinelia ternata</em></td>
<td>16.86</td>
</tr>
<tr>
<td><em>Herba Ephedrae</em></td>
<td>Ephedrine</td>
<td><em>Ephedra sinica</em></td>
<td>16.86</td>
</tr>
<tr>
<td><em>Radix Paeoniae</em></td>
<td>Paeonilinor</td>
<td><em>Paeonia lactiflora</em></td>
<td>16.86</td>
</tr>
<tr>
<td><em>Fructus Schisandrae</em></td>
<td>Schizandrin</td>
<td><em>Schizandra chinensis</em></td>
<td>16.86</td>
</tr>
<tr>
<td><em>Herba Asari</em></td>
<td>Asarone</td>
<td><em>Asarum sieboldii</em></td>
<td>11.25</td>
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<tr>
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<td>6-Gingerol</td>
<td><em>Zingiber officinale</em></td>
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</tr>
<tr>
<td><em>Ramusus Cinnamonomi</em></td>
<td>Cinnamaldehyde</td>
<td><em>Cinnamomum cassia</em></td>
<td>11.25</td>
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<tr>
<td><em>Radix Glycyrrhiza</em></td>
<td>Glycyrrhetic acid</td>
<td><em>Glycyrrhiza uralensis</em></td>
<td>11.25</td>
</tr>
</tbody>
</table>

**RNA isolation**

After treatment with extracts, mice were sacrificed by intraperitoneal injection of sodium pentobarbital. The lung tissues were then surgically resected and immediately frozen in liquid nitrogen. The total RNA was then isolated using a Qiagen RNeasy Kit according to the manufacturer’s instructions (Qiagen Korea Ltd., Seoul, Korea). RNA quality was then checked using agarose gel electrophoresis in which the ratio of 28S/18S RNA was measured to be approximately 1.6.

**Microarray experiment**

RNA from the 7 mice in each group was pooled prior to analysis to eliminate individual variability. A microarray consisted of approximately 45 000 oligo-spots (Agilent Technologies Co., Santa Clara, CA, USA). 3DNA array detection system was used for probe preparation and hybridization according to the manufacturer’s protocol (Genisphere, PA, USA) in which 20 g of total RNA was used to produce fluorescently labeled cDNA. Total RNA from normal and vehicle-treated mice was used as reference. Microarray was then scanned to obtain image file using a ScanArray scanner (Perkin-Elmer, Boston, USA).

**Data analysis**

Primary raw data obtained using IMAGENE 4.0 (Biodiscovery, Hawthorne, CA, USA) were normalized by Lowess method. Only spots having intensity greater than 1.4 times to that of the background were selected. Then, for analysis, we selected only genes having well-measured ratio in all samples. The expression ratios were then hierarchically clustered using CLUSTER and then visualized using TREEVIEW (M.B. Eisen, http://rana.lbl.gov). Ontology analysis was performed using GOSTAT algorithms. To identify genes having similar promoters, TFBS in promoter region of a gene, which was predicted by TOUCAN and CONFAC program, were converted to matrix format in which columns correspond to specific promoters of genes and rows to the number of presence of individual TFBS. We used 1000 bp upstream and 500 bp downstream of putative transcription start site obtained using DBTSS as promoter sequence of each gene. The TFBS matrix was then converted to similarity matrix using Jaccards algorithm as previously reported. The identification of genes having similar promoters was analyzed by hierarchical clustering of similarity matrix.

**RESULTS**

**Clustering pattern of gene expression**

The effects of administration of SCRT on gene expression in lung tissue of mouse were measured by microarray analysis. Of a total of approximately 45 000 oligo-spots on the microarray, genes that were up (2 fold)- or down (0.5 fold)-regulated in either injured lung or SCRT treated tissue were clustered according to gene expression levels. Figure 1A shows expression profile for the effect of SCRT on injured lung tissue. Specifically, 1438 genes were up-regulated and 806 genes were down-regulated in asthma-induced lung tissue (Figure 1B). The effects of SCRT on the expression ratio of these altered genes were depicted on Figure 2. By treatment of SCRT on mice, 50.1% (721/1438) of up-regulated genes and 72.5% (584/806) of down-regulated genes were restored to normal level. In addition, it was measured that many genes were newly up- or down-regulated by SCRT treatment (Figure 1C).

**Ontological analysis**

Because the recovery rates of altered genes by SCRT-treatment in injured lung were different between up- and down-regulated genes, we measured the functional differences of these genes by analyzing gene ontology. Table 2 indicates that recovered genes from up-regulation in injured lung would be mainly involved with metabolic process. Whereas recovered genes from down-regulation in injured lung was implicated with various biological processes. The differential functional distribution between up- and down-regulated genes means selective recovery of functions by SCRT.
Promoter analysis

To identify genes that would be regulated by common signalling pathway, promoter regions of altered genes in injured lung tissue were analyzed. One thousand base pairs upstream and 500 bp downstream of transcription start site were used as promoter region of each gene. We used TOUCAN and CONFAC program to identify putative transcription factor binding site (TFBS) in promoter region. The positions of TFBS for some selected genes were shown in Figure 3. After identification of putative TFBS by CONFAC algorithm, the similarity matrix for TFBS among genes were generated and hierarchically clustered. Promoter regions of initial 553 up-regulated genes and 232 down-regulated genes in injured lung were analyzed. The total numbers of TFBS in matrix were 269 and 268 for up- and down-regulated genes, respectively. The similarity profile of these genes on TFBS was shown in Figure 4. In both cases of up- and down-regulated genes in asthma mice model, we could identify correlated cluster of genes clearly. The cluster includes 120 up-regulated genes and 47 down-regulated genes. The top 10 list of these genes were depicted in Table 3. The recovery rates of these genes having similar TFBS were 66.7% and 85.1% for up- and down-regulated genes, respectively in asthma model in mice (Figure 5). When compared with recovery rate obtained using all altered genes (50.1% and 72.5% for up and down-regulation, each), significant increase of recovery rate was measured, that is, 66.7% and 85.1% for up- and down-regulated genes, respectively.
DISCUSSION

Although SCRT has been traditionally used to treat allergic disease such as allergic rhinitis, bronchitis, and bronchial asthma in Asian traditional medicine, the mechanism of pharmaceutical effect has not been clearly reported yet. In recent, in vivo and in vitro studies showed anti-allergic activity of SCRT in which many kinds of immune cells and cytokines is regulated by treatment of SCRT. In this study, we analyzed the effect of SCRT on asthma model in mice. As shown in Figure 1 and 2, expression level of many up- or down-regulated genes in asthma model is restored by treatment of SCRT. Whereas recovered genes from up-regulation in injured lung are mainly implicated with metabolic process, recovered genes from down-regulation is implicated with various functions such as pallium development, signaling cascade and regulation of cytokine secretion. Because the recovered genes by treatment with SCRT were composed of functionally different genes, we then tried to refine these recovered genes based on TFBS similarity. For recovered genes from up or down-regulation in injured lung, major distinctive cluster could be discriminated (Figure 4 and Table 3). When the recovery rate was re-measured with these genes having similar TFBS binding sites, 66.7% and 85.1% of recovery rate were obtained from up- and down-regulation, respectively. Compared to overall recovery rate obtained using all genes (50.1% and 72.5% for up- and down-regulation, respectively), refined recovery rate increased about 16% (for up-regulation) and 13% (for down-regulation) (Figure 5).
increase of recovery rate means that SCRT could regulate a set of genes having specific TFBS binding sites. Although having similar TFBS sites, these genes were implicated with diverse biological processes. The functional relationship among these genes should be studied further in detail.

The increased recovery rate by treatment with SCRT does not mean the recovery of asthma itself in mice. In our previous report, the measurement of biochemical parameters showed that some sorts of cytokines such as IL-4, IL-5, and IL-17 were significantly reduced or even restored to normal level by SCRT in asthma model in mice and the same data were obtained in this study (data not shown). But these recovery rates of biochemical factors also do not reflect the recovery rate of asthma directly. Therefore, to measure the exact recovery rate of asthma by SCRT, molecular evidence as well as biochemical parameters should be considered simultaneously.

In conclusion, by applying microarray technology, the effect of SCRT on asthma model in mouse was investigated on whole genome level. We identified a set of genes subjected to similar regulation by SCRT in asthma model in mice. The recovery rate using these genes was more improved than using all genes. Therefore, the functional implication of these genes on asthma should be studied more in detail.

**REFERENCES**


