Biochemistry and Biophysics Reports 7 (2016) 63–69

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Establishment of combined analytical method to extract the genes of interest from transcriptome data

Akinori Ishihara [a,b], Hidenori Yamanaka [c], Reiko Takahashi [d], Tomomi Numajiri [d], Saki Kaneko [d], Yoko Ishizawa [d], Sakuji Koya [d], Kiyoshi Yamauchi [a,b,]*

[a] *Department of Biological Science, Faculty of Science, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan*
[b] *Green Biology Research Division, Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan*
[c] *Chemicals Assessment Center, Chemicals Evaluation and Research Institute, 1600, Shimo-Takano, Sugitomachi, Kitakatsushika-gun, Saitama 345-0043, Japan*
[d] *Wakanyaku Medical Institute, Ltd., 1193 Fujimimachi Akagiyama, Maebashi 371-0101, Japan*

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form
23 April 2016
Accepted 18 May 2016
Available online 19 May 2016

Keywords:
Proteome
Transcriptome
Crude drug
Pathway

ABSTRACT

Techniques for analyzing genome-wide expression profiles, such as the microarray technique and next-generation sequencers, have been developed. While these techniques can provide a lot of information about gene expression, selection of genes of interest is complicated because of excessive gene expression data. Thus, many researchers use statistical methods or fold change as screening tools for finding gene sets whose expression is altered between groups, which may result in the loss of important information. In the present study, we aimed to establish a combined method for selecting genes of interest with a small magnitude of alteration in gene expression by coupling with proteome analysis. We used hypercholesterolemic rats to examine the effects of a crude herbal drug on gene expression and proteome profiles. We could not select genes of interest by using standard methods. However, by coupling with proteome analysis, we found several effects of the crude herbal drug on gene expression. Our results suggest that this method would be useful in selecting gene sets with expressions that do not show a large magnitude of alteration.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Recently, methods for analyzing genome-wide expression profiles, such as microarrays and next-generation sequencing, have been developed. These methods enable us to obtain a large amount of gene expression data, easily and inexpensively. While the massive amount of data provides an overview of certain biological phenomena on the basis of suitable statistical analyses, there are several limitations. In general, analysis of genome-wide expression data is carried out by: (1) normalization of the expression data, (2) selection of genes of interest from the data, and (3) visualization of expression profiles [1]. The most important step is the selection of genes of interest and determining the biological significance of those selected gene set. In addition, most researchers select genes with statistically altered expression or when the magnitude of the fold change between groups is large, because it is difficult to limit the numbers of genes selected by using small thresholds [2–4]. However, such methods for the selection of genes often result in the loss of information because genes that demonstrate large variation in expression within groups or minor changes in expression between groups are not selected as genes of interest. Two-dimensional difference gel electrophoresis (2D-DIGE) is a new technique that compares the abundance of a large number of proteins, and many researchers have attempted to use this technique in various areas of research [5,6]. However, compared to studies utilizing model organisms that have sufficient genomic information, there are limitations in analyzing the proteome of non-model organisms whose genomes have not been sequenced [7], and the throughput of the experiment is lower than that of transcriptome analysis. Additionally, both proteome and transcriptome analyses have a similar problem in the selection of candidates, that is, loss of information. Thus, transcriptome and proteome analyses have both merits and demerits. It is necessary to develop new methods for analyzing transcriptome and proteome data without the loss of information.

In our previous study, we used microarray analysis to determine the effects of a combined extract of *Sasa albo-marginata* leaves (kumazasa; *Sasa kurinensis* Makino), Japanese red pine leaves (*Pinus densiflora* Sieb. et Zucc), and ginseng roots (*Panax*

* Corresponding author at: Green Biology Research Division, Research Institute of Green Science and Technology, Shizuoka University, Ohya 836 Suruga-ku, Shizuoka 422-8529, Japan.
E-mail address: yamauchi.kiyoshi@shizuoka.ac.jp (K. Yamauchi).

http://dx.doi.org/10.1016/j.bbrep.2016.05.015
2405-5808/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ginseng C.A. Meyer) (SJG) on high-cholesterol diet (HCD)-induced hypercholesterolemia [8]. In that study, we finally confirmed that expression of the gene encoding cytochrome P450 7A1 was altered in response to HCD and HCD with SJG, although we could not select the gene as a candidate gene of interest by using standard microarray analysis because of the minor change in gene expression [8]. This result suggests that it may be difficult to determine the molecular mechanism underlying the action of crude drugs using standard methods because changes in gene or protein expression caused by crude herbal drugs may be smaller than those caused by most pharmaceutical drugs. However, several plant polyphenols are known to play a major role in reducing risk of arteriosclerosis [9–13], suggesting that small molecules from plants have useful health effects. In this study, we aimed to select genes of interest whose transcript or protein expression profiles showed small changes by using transcriptome analysis coupled with proteome analysis and used crude herbal drug-treated rats as model animals.

2. Materials and methods

2.1. Reagents

Immobilized pH gradient strip (pH 3–10), pharmalytes, drystrip cover fluid, bromophenol blue, agarose, Cy2, Cy3, and Cy5 were purchased from GE Healthcare (Little Chalfont, UK). Protease inhibitor cocktail (Pefabloc SC and Pefabloc SC protector) was purchased from Roche (Mannheim, Germany). Sequence-grade trypsin was obtained from Promega UK (Southampton, Hants, UK). All other chemicals used in this study were of the highest grade available and were purchased from GE Healthcare, Dojin Chemical Japan (Osaka, Japan), Sigma (St. Louis, MO), Wako (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), or Kanto (Tokyo, Japan). The crude herbal drug SJG was prepared by Wakanyaku Medical Institute, Ltd. (Maebashi, Japan). SJG is composed of a water extract of kumazasa leaves and ethanol extracts of Japanese red pine leaves and ginseng roots in the ratio 8:1:1 [14]. SJG was supplied as a liquid preparation and diluted using tap water to 50% (v/v). We previously analyzed the component of SJG and found that SJG contained several compounds like tricin, *p*-coumaric acid, ginsenoside [8].

2.2. Animals and treatment

Twenty male Wistar rats (age, 13 weeks) were placed in a room with controlled lighting (12-h light/dark cycle), temperature (23 ± 1 °C), and humidity (55 ± 10%). All rats were provided with food and tap water or 50% SJG ad libitum throughout the experiment. We divided the rats into four groups (Groups 1, 2, 3, and 4). After 1 week of habituation, all rats were fed a standard diet (23% protein, 5% fat, and 55% nitrogen-free extract; MF, Oriental Yeast Co., Tokyo, Japan) and given water (Groups 1 and 3) or 50% SJG (Groups 2 and 4) for 12, 30, or 52 weeks (experimental phase I, Fig. 1). The rats were then provided with food and water as follows: standard diet and water, Group 1; standard diet and 50% SJG, Group 2; HCD and water, Group 3; and HCD and 50% SJG, Group 4 for three weeks (experimental phase II, Fig. 1). HCD was the standard diet supplemented with 0.5% cholesterol and 0.5% cholic acid. Then, the rats were anesthetized with diethyl ether and sacrificed. The plasma was collected for measuring the levels of biochemical parameters, such as glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, cholesteryl ester, free cholesterol, triglyceride (TG), free fatty acid (FFA), phospholipids, and total lipids, and the livers were collected for RNA extraction. The animal experiments were approved by the Animal Research Committee of Wakanyaku Medical Institute, Ltd., and performed in accordance with the Guidelines for Care and Use of Laboratory Animals at Wakanyaku Medical Institute, Ltd. and the Guidelines for Proper Conduct of Animal Experiments from Science Council of Japan.

2.3. Plasma biochemical parameters

Blood samples were obtained from the postcaval vein by using a vacuum blood collection tube containing heparin. Plasma levels of glucose, total cholesterol, HDL cholesterol, LDL cholesterol, cholesteryl ester, free cholesterol, TG, FFA, phospholipids, and total lipids were determined using the Glucose test Wako (Wako), L-type Wako cholesterol (Wako), Cholestest N HDL (Sekisui Medical Co., Ltd, Tokyo, Japan), Cholestest LDL (Sekisui), L-type Wako cholesterol (Wako) and L-type Wako free cholesterol (Wako), L-type Wako free cholesterol (Wako), L-type Wako TG (Wako), NEFA-SS Eiken (Eiken Chemical Co., Ltd, Tokyo, Japan), L-type Wako phospholipids (Wako), and Total lipids reagent Kokusai (Sysmex, Kobe, Japan), respectively.

2.4. RNA isolation and microarray analysis

Isolation of total RNA from the liver and microarray analyses were performed according to the methods described in our previous study [8]. Total RNA was extracted from frozen rat livers by using the High Pure RNA Isolation Kit (Roche) and subjected to oligonucleotide array analysis (Agilent Microarray Design ID 028279; Agilent, Santa Clara, CA, USA) by using a single-color labeling system (Low Input Quick Amp Labeling Kit for One Color; Agilent). We performed *t* tests or Benjamini and Hochberg's method to compare signals between two groups, and a Venn diagram was created to extract genes whose expression was altered by the intake of HCD, and the expression was further modified by SJG. All analyses for microarray data were performed using linear models for microarray data (limma) package [15] or Subio

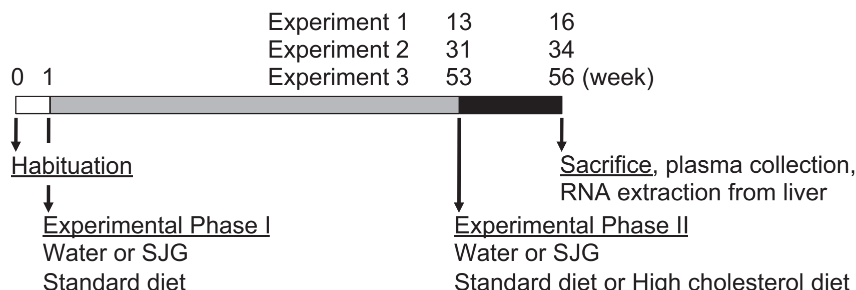

Fig. 1. Scheme of the experimental schedule. All rats were acclimatized for a week and fed a standard diet throughout habituation and the experimental phase I (12, 30, and 52 weeks). After habituation, the rats were divided into four groups. Groups 1 and 3 received water throughout the experimental phase, while groups 2 and 4 received 50% SJG. In experimental phase II (3 weeks), groups 3 and 4 were fed a high-cholesterol diet. Finally, all the rats were sacrificed, and plasma and liver were collected for analysis.

Download English Version:

https://daneshyari.com/en/article/1941601

Download Persian Version:

https://daneshyari.com/article/1941601

Daneshyari.com