Genomics Data 7 (2016) 32–37

Contents lists available at ScienceDirect

Genomics Data

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

Data in Brief

DNA microarray-based experimental strategy for trustworthy expression profiling of the hippocampal genes by astaxanthin supplementation in adult mouse

Jang Soo Yook [a], Junko Shibato [a,b], Randeep Rakwal [b,c], Hideaki Soya [a,*]

[a] Laboratory of Exercise Biochemistry and Neuroendocrinology, Faculty of Health and Sport Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8574, Ibaraki, Japan
[b] Global Research Center for Innovative Life Science, Peptide Drug Innovation, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 4-41 Ebara 2-chome, Shinagawa, Tokyo 142-8501, Japan
[c] Faculty of Health and Sport Sciences & Tsukuba International Academy for Sport Studies (TIAS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8574, Ibaraki, Japan

ARTICLE INFO

Article history:
Received 23 October 2015
Accepted 6 November 2015
Available online 7 November 2015

Keywords:
Astaxanthin supplementation
Dose-dependent
Hippocampal genes
Housekeeping gene, RT-PCR

ABSTRACT

Naturally occurring astaxantin (ASX) is one of the noticeable carotenoid and dietary supplement, which has strong antioxidant and anti-inflammatory properties, and neuroprotective effects in the brain through crossing the blood–brain barrier. Specially, we are interested in the role of ASX as a brain food. Although ASX has been suggested to have potential benefit to the brain function, the underlying molecular mechanisms and events mediating such effect remain unknown. Here we examined molecular factors in the hippocampus of adult mouse fed ASX diets (0.1% and 0.5% doses) using DNA microarray (Agilent 4 × 44 K whole mouse genome chip) analysis. In this study, we described in detail our experimental workflow and protocol, and validated quality controls with the housekeeping gene expression (*Gapdh* and *Beta-actin*) on the dye-swap based approach to advocate our microarray data, which have been uploaded to Gene Expression Omnibus (accession number GSE62197) as a gene resource for the scientific community. This data will also form an important basis for further detailed experiments and bioinformatics analysis with an aim to unravel the potential molecular pathways or mechanisms underlying the positive effects of ASX supplementation on the brain, in particular the hippocampus.

Organism/cell line/tissue	Mus musculus; hippocampus
Strains (s)	C57/BL6J
Sequencer or array type	Agilent whole mouse genome microarray G4122F
Data format	Raw
Experimental factors	Astaxanthin supplementation, 0.1% and 0.5%; Hippocampal genes expression; Housekeeping genes; Total RNA extraction; RT-PCR
Experimental features	Very brief experimental description
Consent	All the experimental procedures were performed in accordance with protocols approved by the University of Tsukuba Animal Experiment Committee guidelines for the Care and Use of Laboratory Animals (ID 15-054).

1. Direct link to deposited data

Deposited data can be found here:
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?targ=self&form=html&view=brief&acc=GSE62197

2. Experimental design, materials and methods

Astaxathin (ASX), a red natural pigment of the carotenoid family, has been suggested to play diverse biological roles in human health through its powerful antioxidant properties [1,2]. Recent studies have found that ASX could also possess neuroprotective properties and might be useful against neurological disorders and in preventing the decline of cognitive functions [3,4]. Nowadays, there has been a growing understanding that the influence of nutrient compound on health is complex and requires nutrigenomic studies to tease out the mechanism at the molecular level [5]. Hence, we initiated to investigate ASX-induced transcriptomic profiling on the adult hippocampus in a mouse model using a high-throughput DNA microarray approach.

Eleven-weeks-old male C57/BL6J mice were obtained from SLC, Co., Ltd. (Shizuoka, Japan). The mice were housed individually in standard cages with a 12-h light/dark cycle. After one-week of acclimatization rearing with standard powdered chow (an MF diet, Oriental Yeast, Tokyo, Japan) and water ad libitum, all mice were fed ASX or its placebo powder (AstaREAL powder 20F based on 2% content of astaxanthin from *Haematococcus pluvialis*; Fuji Chemical Industry, Toyama, Japan) at final consistency of 0.1%, 0.5% and 0% (control, hereafter abbreviated as CON) ASX dose for four-weeks (n = 6/group). All the experimental

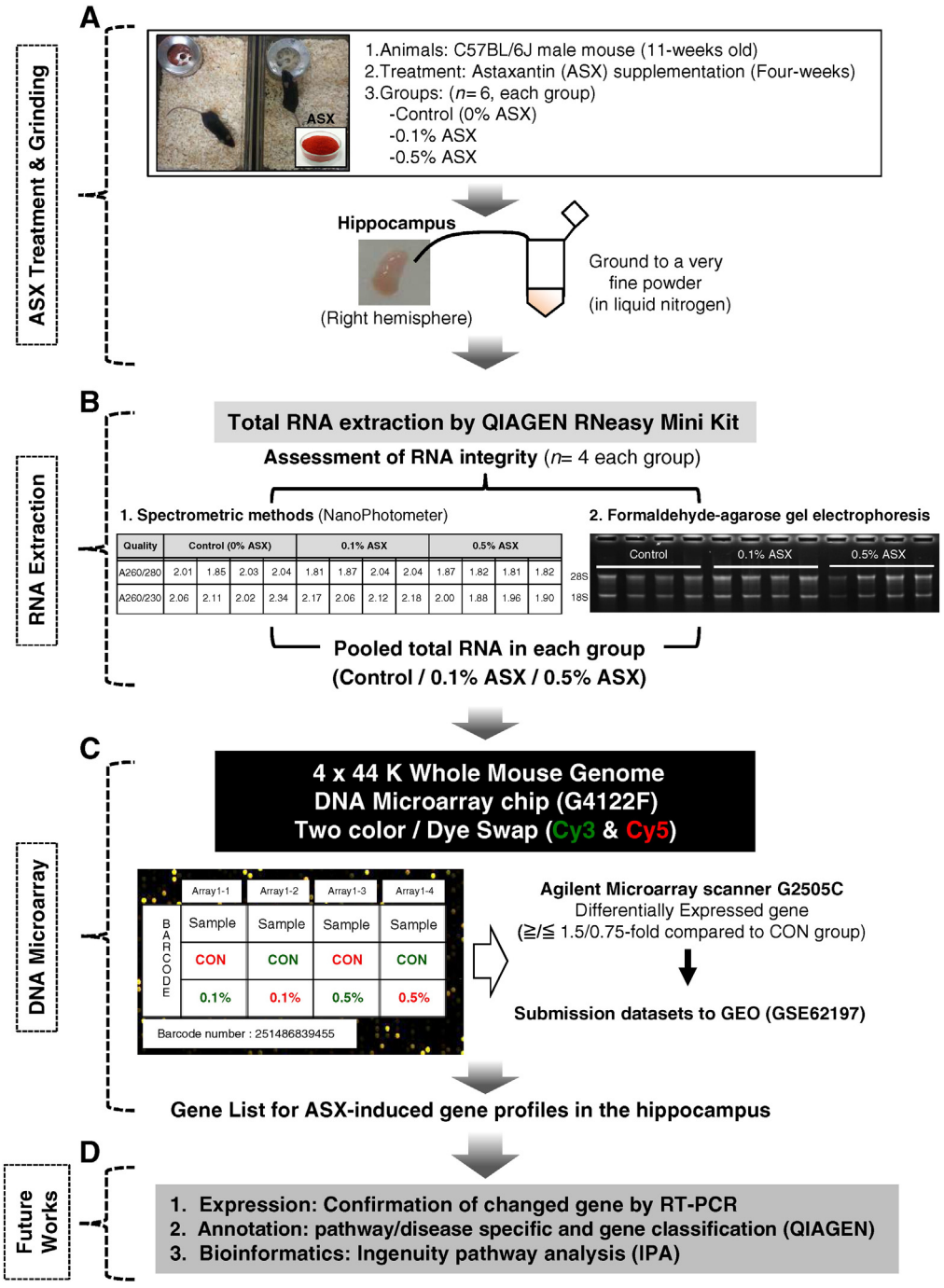
* Corresponding author.
E-mail address: hsoya@taiiku.tsukuba.ac.jp (H. Soya).

http://dx.doi.org/10.1016/j.gdata.2015.11.001

procedures were performed in accordance with protocols approved by the University of Tsukuba Animal Experiment Committee guidelines for the Care and Use of Laboratory Animals. The sampling was performed a day after the last day of ASX supplementation period to avoid acute influence. Following the dissection of the brain, hippocampus (right) were carefully removed and immediately deep frozen in liquid nitrogen and stored at −80 °C in a deep freezer. The hippocampi were individually ground in liquid nitrogen to make very fine powders, and then their powders were stored in aliquots at −80 °C till used for total RNA extraction (Fig. 1A).

3. Workflow for optimal DNA microarray analysis through the quality assessment of total RNA

To obtain best results on the DNA microarray chip, the critical assessment of high-quality RNA is required. The following steps were sequentially performed to confirm total RNA quantity and quality. Total RNA was extracted from the powdered samples using the QIAGEN RNeasy Mini Kit (QIAGEN, Germantown, MD, USA) according to an optimized protocol [6–8]. Concentration and quality of total RNA were assessed a spectrometric method with NanoPhotometer (IMPLEN, München,



Quality	Control (0% ASX)				0.1% ASX				0.5% ASX			
A260/280	2.01	1.85	2.03	2.04	1.81	1.87	2.04	2.04	1.87	1.82	1.81	1.82
A260/230	2.06	2.11	2.02	2.34	2.17	2.06	2.12	2.18	2.00	1.88	1.96	1.90

Fig. 1. The experimental design for high-throughput DNA microarray analysis following astaxanthin (ASX) supplementation. (A) ASX supplementation was performed for four-weeks, and the mouse hippocampi was rapidly dissected and individually ground to a very fine powder in liquid nitrogen using pre-chilled mortar and pestle. (B) Total RNA was extracted from the right hippocampus using QIAGEN Rneasy Mini Kit, and their quality was confirmed both spectrometric method and formaldehyde-agarose gel electrophoresis. After this step, total RNA for each control and treatment (0.1% and 0.5% ASX) was pooled in each group ($n = 4$). (C) The design of DNA microarray chip was followed as per the dye-swap labeling (CON × 0.1% ASX and CON × 0.5% ASX). The differentially expressed gene list was generated according to ≧/≦1.5/0.75-fold compared to CON. (D) As next step after the microarray, further confirmation of changed gene by RT-PCR, and detailed annotation of data sets and bioinformatics using Ingenuity Pathway Analysis (IPA) will be performed (manuscript to be published elsewhere).

Germany) and further confirmed using formaldehyde-agarose gel electrophoresis (Fig. 1B). In this experiment, we assessed a good quality of total RNA in all samples as shown by a value of spectrophotometric absorbance (via $A_{260/280}$ and $A_{260/230}$ ratio) greater then 1.8, respectively. In addition, integrity of RNA that comprised the 28S and 18S ribosomal RNA is evaluated by formaldehyde-agarose gel electrophoresis stained with ethidium bromide as seen clearly on the gel images in Fig. 1B (right-hand side image). For DNA microarray experiment, an equal amount of total RNA (1000 ng) from four randomly chosen the powdered samples was pooled. A mouse whole genome 4 × 44 K DNA microarray chip (Agilent Technologies, CA, USA; G4122F) was used for global gene expression into the hippocampus, where the two-color (Cy3 and Cy5 labels) in conjunction with dye-swap method [9–11] was used (Fig. 1C). The gene expression data from this study on ASX supplementation effects in the adult mouse hippocampus were deposited in the Gene Expression Omnibus (GEO) database under the accession number GSE62197, and will be used for further analysis and study (Fig. 1D).

4. Confirmation of housekeeping gene expression between RT-PCR analysis and the obtained DNA microarray data

Here we performed the quality control procedure based on the expression of two housekeeping genes (*Gapdh* and *Beta-actin*) by semi-quantitative reverse transcription-PCR (RT-PCR) for all samples ($n = 4$/group) used in the DNA microarray analysis. For this, cDNA was synthesized from independent samples using AffinityScript QPCR cDNA Synthesis Kit (Agilent Technologies, La Jolla, CA, USA) according to the manufacturer's protocol. The reaction mixture for RT-PCR was prepared the EmeraldAmp PCR Master Mix (TaKaRa Shuzo, Shiga, Japan) and then performed on Thermal-cycling (C1000™ Thermal Cycler; Bio-Rad Laboratories, California, USA). We validated that both the housekeeping genes were expressed at almost no differences among the groups ($P > 0.05$) (Fig. 2A); the PCR amplified bands that were visualized and quantified using the Image Quant LAS 4000 mini (GE Healthcare, Tokyo, Japan) are shown in Fig. 2B. Furthermore, to achieve reliability of gene expression under dye-swap on the DNA microarray approach, we confirmed the expression of *Beta-actin* genes for all 10 probes on the level of signal intensity and fold change. The control (CON) and treated samples (0.1% and 0.5% ASX) were labeled with Cy3 and Cy5, or with reversed Cy5 and Cy3, respectively. This result showed that the fluorescent signal differences did not alter due to our dye-swap approach, and the fold change on *Beta-actin* probes appeared less than 1.5-fold (Fig. 2C), indicating that the expression of housekeeping gene for positive control is stable hybridization between reversed samples in the two-color DNA microarray approach.

5. Hybridization, normalization, and scatter plot distribution of intensity values

It is well known that an accurate outcome of differentially expressed genes from a microarray data requires the critical normalization of the hybridization intensity before the differentially expressed genes (among treatments) can be analyzed. In this study, we used the filtering with replication based on the value of $\log_2$ ratio that can determine a systematic dependence on intensity spot in the two-color and dye-

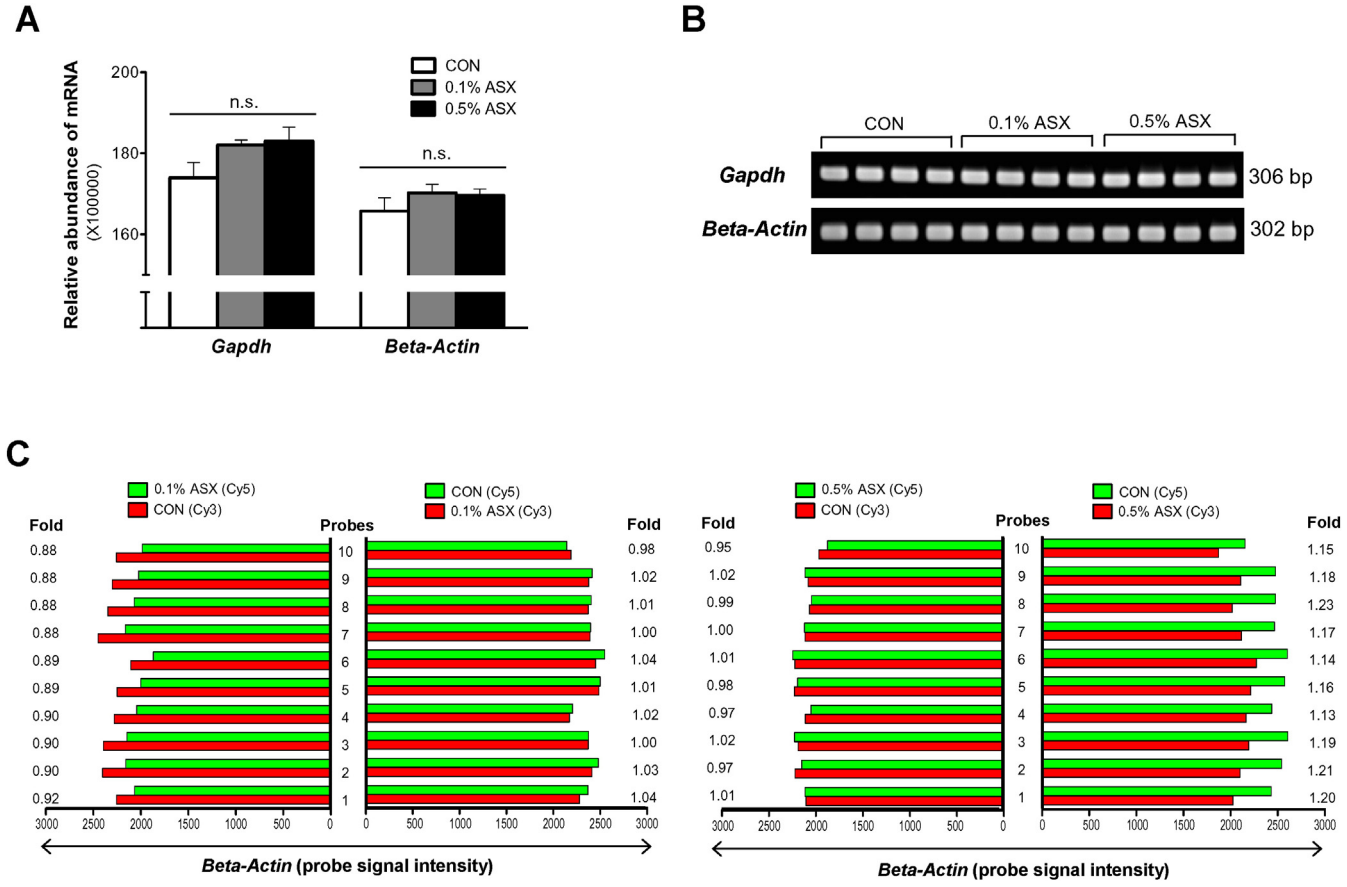


Fig. 2. Expression of housekeeping genes, glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) and *Beta-actin*, using RT-PCR and DNA microarray experiments. (A) Relative abundance of *Gapdh* and *Beta-actin* mRNA as a positive control was confirmed by RT-PCR. The data from RT-PCR are presented as the means ± SEM, and there are no significant differences among the groups (One-way ANOVA, $n = 4$ each group). (A) Graphical presentation of the band intensities and (B) the gel image showing the RT-PCR product bands of all samples. (C) As an example, the *Beta-actin* gene, the signal intensity in Cy3 and Cy5 labeling for all 10 probes with the dye-swap DNA microarray are presented in the hippocampus at 0.1% and 0.5% ASX supplementation.

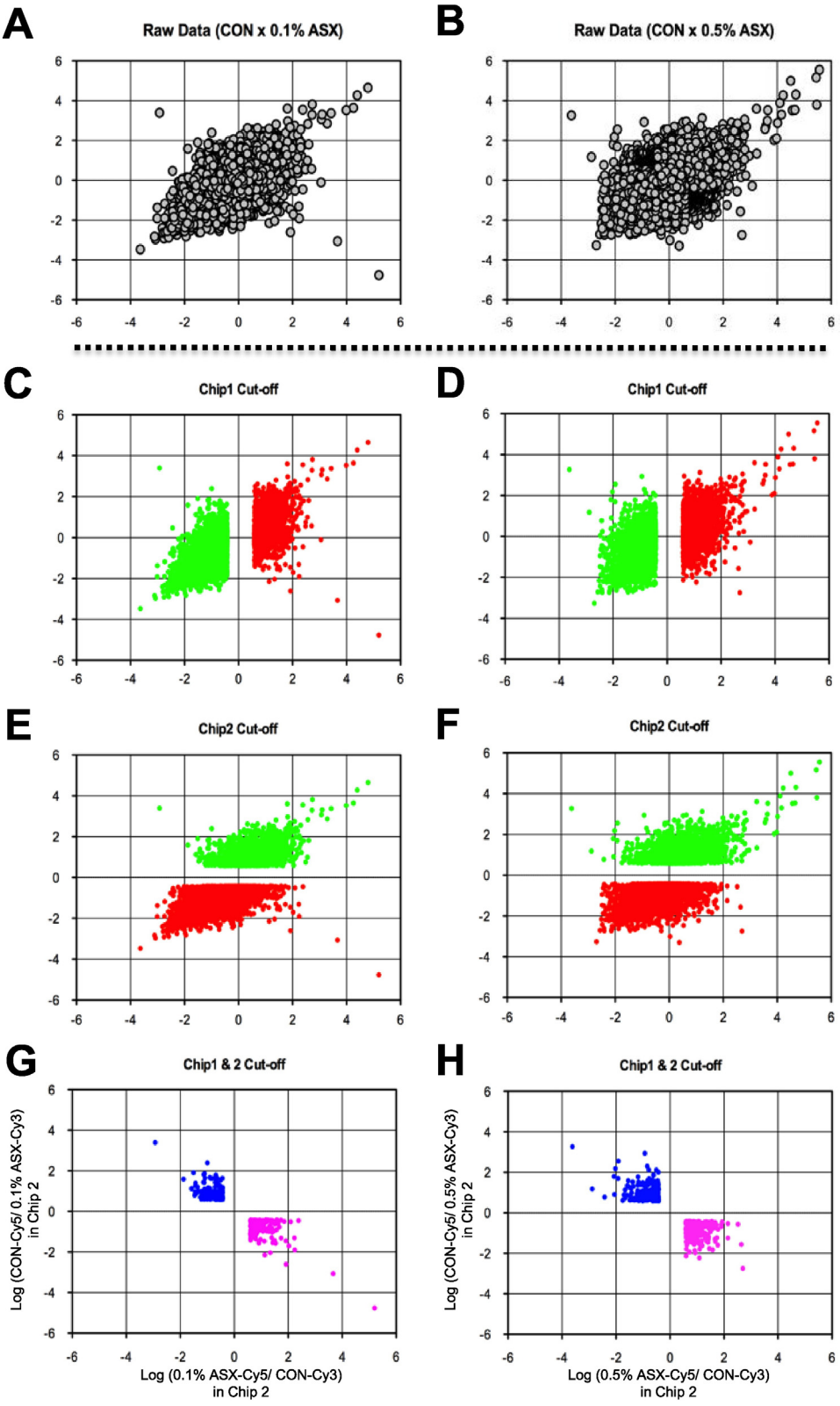


Fig. 3. Intensity scatter plot of the two-color DNA microarray chip. All spots on the graph are calculated by the logarithms ($\log_2$) value of the Cy5/Cy3 ratio for a single gene probe. (A, B) Gray spots, which represent all genes, in upper two graphs show the raw data from 0.1% and 0.5% ASX supplementation compared to CON. (C-F) Four graphs in the center indicate the dye-swap analysis using chip 1 and chip 2. The cut-off for chip 1 and chip 2 is applied by an up- and down-regulated fold ratio of greater than or equal to 1.5 (indicated by red color) and less than or equal to 0.75 (indicated by green color). Non-changes in expression genes are indicated by the vertical and horizontal spaces between the both colors on around the zero line. For the dye-swap to take place (selection), the genes showing an up-regulated fold-change in chip 1 (red) need to show a corresponding down-regulated value in chip 2 (green) and vice versa for the down-regulations (red). (G, H) The lower two graphs finally show the dye-swap scatter plots which manifest reliable/confident gene expression on the basis of integration with chip 1 and 2 data analyses. The common regulated genes are shown; up-regulation for the pin color, down-regulation for the blue color.

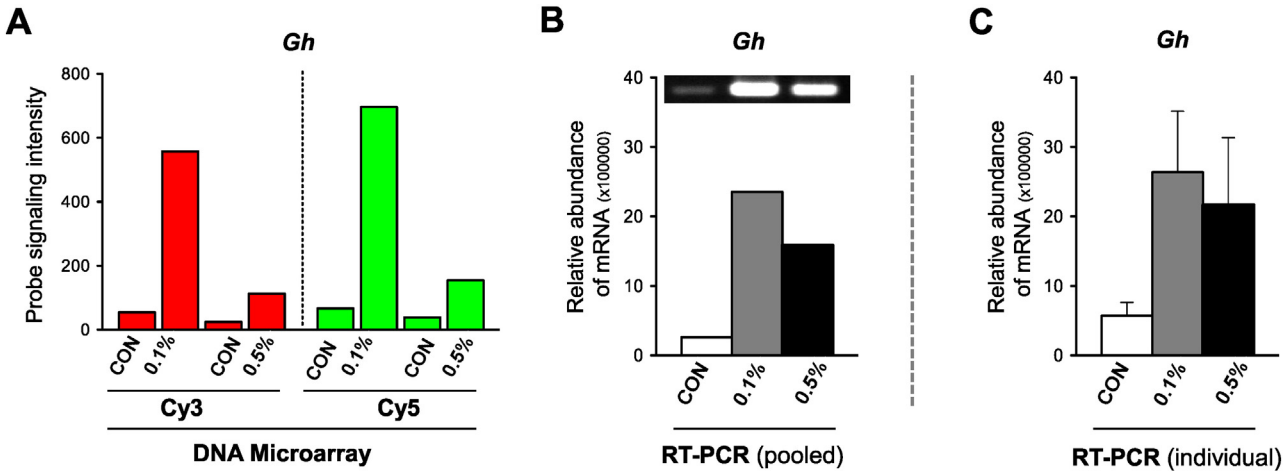


Fig. 4. Validation of a differently expressed gene, *growth hormone* (*Gh*) obtained from the DNA microarray experiment by RT-PCR. (A) Probe signal intensity of *Gh* gene by 0.1% and 0.5% ASX is presented graphically on the both labels (Cy3 and Cy5). (B) RT-PCR result is confirmed for relative abundance of *Gh* mRNA, from the pooled sample cDNAs similar to that for the microarray analysis. (C) RT-PCR result is confirmed for relative abundance of *Gh* mRNA for each individual sample cDNA. The data in (C) are presented as the means ± SEM, and there are no significant differences among the groups (One-way ANOVA, $n = 4$ each group).

swap microarray analysis [12]. Differentially up- and down-regulated genes using the cut-off values (≧/≦1.5/0.75-fold versus CON) were measured by CON with Cy5 dye and ASX with Cy3 dye in chip 1, followed by a swap of the dye labeling in the chip 2. Post-dye-swap and applied normalization, the number of overall genes is gradually reduced from the raw datasets (0.1% ASX; 44,480 genes, 0.5% ASX; 43,379 genes) to a high confidence of differentially expressed gene list (Fig. 3). As can be seen in the representative scatter plots in Fig. 3, the pattern of distribution is similar between 0.1% and 0.5% ASX supplementation. Furthermore, the step of hybridization and washes with 60-mer probes was carried out based on the process in the Agilent manufacturer's guide, and the Agilent scanner (G2505C) was used to detect the signals on the hybridized microarrays slide. Subsequently, the data for differential expression of genes was generated by Agilent Feature Extraction software (version 11.0.1.1, Agilent Technologies). As microarrays have been known to show several systematic errors including labeling bias, irregular feature morphologies, mismatched sample concentration and cross hybridization, in the Agilent system these issues have been addressed by the development of the Feature Extraction algorithms [13].

6. Validation of DNA microarray data by re-confirmed RT-PCR result

To verify differentially expressed genes, as an example, we selected one gene, namely growth hormone (*Gh*) gene that is highly up-regulated in both 0.1% (10.29-fold) and 0.5% ASX supplementations (4.29-fold) and confirmed the validation for the expression between the employed two-color microarray approach (Fig. 4A) and RT-PCR experiment using the pooled samples and individual samples (Fig. 4B and 4C). Similar tendency of its expression levels was observed between the two experimental methods. To note, in the RT-PCR result using individual samples ($n = 4$/group), there was no significant difference among three groups from One-way ANOVA ($P > 0.05$), because it may be influenced by small number of samples and slight variation in each group (Fig. 4C). Nevertheless, a clear trend in RT-PCR is shown to be similar to the Cy3 and Cy5 values of the microarray method, which is mentioned a good relevance for positive control gene before [8]. This result demonstrates that a validation procedure using a discrete tool needs to recognize the quality of gene expression obtained in the microarray experiment. Thus, we sought to present and discuss the deposited microarray data set as a highly reliable dataset to understand the effect of ASX supplementation on the hippocampus in our mouse model.

7. Discussion

Nutritional/dietary compounds may play an important role for brain structure and its function, and these effects would be mediated by changes in the genes [14]. DNA microarray analysis is a powerful approach to identify genome-wide gene expression in response to intervention of nutrients on the central neuronal system referred to as nutrigenomics [15]. We performed this study with a long-term goal to investigate unique hippocampal gene profiling on the hippocampus of adult mouse affected by four-weeks of ASX supplementation using whole-genome DNA microarray analysis with the dye-swap methods (Agilent Technologies). In this study, we show that the ASX-induced genes have sufficient validity and reliability through a validation process including the quality control with housekeeping genes and the dye-swap based normalization. Therefore, our deposited dataset could be recognized as a first ASX-influenced transcriptome analysis on the hippocampus of adult mouse. Furthermore, these large numbers of gene inventories will also provide a promising basis for understanding the complex molecular mechanisms that underlie neurobiological effect of ASX on the hippocampus, the critical region for learning and memory, through highlighting potential candidate genes.

Acknowledgments

This research was supported in part by the Japan Society for the Promotion of Science (Grants-in-aid for Scientific Research A 23240091) and the special funds for Education and Research of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) entitled "Global Initiative for Human High Performance (HHP) Research Project (No. 1111501004)" and "Global Initiative for Sports Neuroscience (GISN): For Development of Exercise Prescription Enhancing Cognitive Functions (HFH27016)". Authors declare no conflict of interest.

References

[1] G. Hussein, U. Sankawa, H. Goto, K. Matsumoto, H. Watanabe, Astaxanthin, a carotenoid with potential in human health and nutrition. J. Nat. Prod. 69 (2006) 443–449.
[2] R.R. Ambati, S.M. Phang, S. Ravi, R.G. Aswathanarayana, Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. Mar. Drugs 12 (2014) 128–152.
[3] Y.P. Lu, S.Y. Liu, H. Sun, X.M. Wu, J.J. Li, L. Zhu, Neuroprotective effect of astaxanthin on H(2)O(2)-induced neurotoxicity in vitro and on focal cerebral ischemia in vivo. Brain Res. 1360 (2010) 40–48.
[4] D. Zanotta, S. Puricelli, G. Bonoldi, Cognitive effects of a dietary supplement made from extract of *Bacopa monnieri*, astaxanthin, phosphatidylserine, and vitamin E in

subjects with mild cognitive impairment: a noncomparative, exploratory clinical study. Neuropsychiatr. Dis. Treat. 10 (2014) 225–230.
[5] V. García-Cañas, C. Simó, C. León, A. Cifuentes, Advances in nutrigenomics research: novel and future analytical approaches to investigate the biological activity of natural compounds and food functions. J. Pharm. Biomed. Anal. 51 (2010) 290–304.
[6] A. Yoshikawa, T. Nakamachi, J. Shibato, R. Rakwal, S. Shioda, Comprehensive analysis of neonatal versus adult unilateral decortication in a mouse model using behavioral, neuroanatomical, and DNA microarray approaches. Int. J. Mol. Sci. 12 (2014) 22492–224517.
[7] M.C. Lee, R.R. Rakwal, J. Shibato, K. Inoue, H. Chang, H. Soya, DNA microarray-based analysis of voluntary resistance wheel running reveals novel transcriptome leading robust hippocampal plasticity. Physiol. Rep. 2 (2014) e12206.
[8] M. Hori, J. Shibato, T. Nakamachi, R. Rakwal, T. Ogawa, S. Shioda, S. Numazawa, Two-color dye-swap DNA microarray approach toward confident gene expression profiling in PMCAO mouse model for ischemia-related and neuropeptide PACAP38-influenced genes. Genomics Data 3 (2015) 148–154.
[9] B.A. Rosenzweig, P.S. Pine, O.E. Domon, S.M. Morris, J.J. Chen, F.D. Sistare, Dye bias correction in dual-labeled cDNA microarray gene expression measurements. Environ. Health Perspect. 112 (2004) 480–487.
[10] K. Inoue, M. Okamoto, J. Shibato, M.C. Lee, T. Matsui, R. Rakwal, H. Soya, Long-term mild, rather than intense, exercise enhances adult hippocampal neurogenesis and greatly changes the transcriptome profile of the hippocampus. PLoS ONE 10 (2015), e0128720.
[11] H. Kubo, J. Shibato, T. Saito, T. Ogawa, R. Rakwal, S. Shioda, Unraveling the rat intestine, spleen and liver genome-wide transcriptome after the oral administration of lavender oil by a two-color dye-swap DNA microarray approach. PLoS ONE 10 (2015), e0129961.
[12] I.V. Yang, E. Chen, J.P. Hasseman, W. Liang, B.C. Frank, S. Wang, V. Sharov, A.I. Saeed, J. White, J. Li, N.H. Lee, T.J. Yeatman, J. Quackenbush, Within the fold: assessing differential expression measures and reproducibility in microarray assays. Genome Biol. 3 (2002) (research0062).
[13] G. Delenstarr, H. Cattel, C. Chen, A. Dorsel, R. Kincaid, K. Nguyen, N. Sam-pas, S. Schidel, K. Shannon, A. Tu, P. Wolber, Estimation of the confidence limits of oligo nucleotide array-based measurements of differential expression. Proc. SPIE, Microarrays: Optical TEchnologies and Informatics, 4266 2001, pp. 120–131.
[14] M.J. Dauncey, Genomic and epigenomic insights into nutrition and brain disorders. Nutrients 3 (2013) 887–914.
[15] K. Gohil, A.A. Chakraborty, Applications of microarray and bioinformatics tools to dissect molecular responses of the central nervous system to antioxidant micronutrients. Nutrition 1 (2004) 50–55.