Toxicogenomics/proteomics Report

DNA microarray analysis of transcriptional responses of mouse spinal cords to physical exercise

Kazunori Hashimoto¹, Akiko Honda², Yuichi Hayashi¹, Takashi Inuzuka¹, Masahiko Satoh²
and Isao Hozumi¹

¹Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan

²Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University, 1-100 Kusumotocho, Chikusa-ku, Nagoya, Aichi 464-8650, Japan

(Received April 6, 2009; Accepted April 23, 2009)

ABSTRACT — We present herein transcriptional changes in mouse spinal cords in response to physical exercise on a treadmill using a DNA microarray. By 30-min exercise, the expression of 3 genes was enhanced and expression of 29 genes reduced. By continuous 2-week exercise (30-min exercise per day), the expression of 1 gene was enhanced and expression of 13 genes reduced.

Key words: Exercise, Treadmill, Spinal cord, DNA microarray, Amyotrophic lateral sclerosis

INTRODUCTION

Recent studies suggest that physical exercise has a beneficial effect on the progression of amyotrophic lateral sclerosis (ALS) (McCrane and Kaspar, 2008). In addition, regular exercise can increase the lifespan of a transgenic mouse model of familial ALS (FALS) (G93A SOD1) and delay the onset (Kirkinezos *et al.*, 2003). ALS is a progressive and fatal neurodegenerative disease, characterized by selective motor neuron degeneration. The majority of ALS cases are sporadic ALS (SALS), and approximately 10% of ALS cases are FALS, 10-20% of which are the result of mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Although the etiology of SALS remains unclarified, several possible mechanisms of motor neuron degeneration in SALS have been proposed, including oxidative stress, heavy metal toxicity, the conformational disorder of proteins and dysfunction of neurotrophic factors.

However, the mechanism by which exercise affects motor neurons is poorly understood. Herein, we examined how acute or continuous physical exercise affects gene expression in the spinal cords of normal mice on the 1st day and the 14th day.

MATERIALS AND METHODS

Animals and exercise

C57BL/6J male mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were housed in a temperature-controlled ($23 \pm 3^\circ\text{C}$) room with a 12-hr light/12-hr dark cycle. Food and water were provided *ad libitum* throughout this study. The treadmill running exercise program was administered as described previously (Kirkinezos *et al.*, 2003). Briefly, the male mice in the exercise groups ran on a treadmill machine (MK-680S, Muromachi, Tokyo, Japan) at 13 m/min and 30 min/day. The exercise program was carried out from 9 to 10 o'clock. A control group remained sedentary. The exercise was started at 7 weeks of age. For transcriptional change analysis following 30 min of exercise stimulation, mice were anesthetized and sacrificed, and their spinal cords removed 12 hr after exercise. On the other hand, for transcriptional change analysis following 2 weeks of 30-min daily exercise, mice were prepared 24 hr after the last exercise. The experimental designs and all procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the guidelines on the handling of experimental animals issued by the Japanese Association for Laboratory Animal Science and the Animal Experimental Committee of Gifu University. This study was approved by the Animal Experimental Committee of Gifu University.

Correspondence: Isao Hozumi (E-mail: metallo@gifu-u.ac.jp)

Table 1. Changes in gene expression after single 30-min exercise

Accession No.	Gene Name	Definition	Function (GO process)	-fold changes
NM_198417	C030039L03Rik	RIKEN cDNA C030039L03 gene	regulation of transcription, DNA-dependent	2.05
NM_008760	Ogn	osteoglycin		2.41
NM_026172	Decr1	2,4-dienoyl CoA reductase 1, mitochondrial	metabolic process	2.02
NM_133362	Erdr1	erythroid differentiation regulator 1	biological process	0.49
NM_011176	Stl4	suppression of tumorigenicity 14 (colon carcinoma)	proteolysis, cell migration	0.47
NM_001081100	Morn1	MORN repeat containing 1	DNA methylation	0.49
NM_029850	Bcl7a	B-cell CLL/lymphoma 7A	biological process	0.43
NM_009405	Tnni2	troponin I, skeletal, fast 2	hydrogen transport	0.44
NM_009606	Acta1	actin, alpha 1, skeletal muscle	muscle contraction, cytoskeleton organization and biogenesis	0.37
NM_015745	Rbp3	retinol binding protein 3, interstitial	proteolysis, lipid metabolic process, transport, visual perception	0.46
BC070477	201031E24Rik	RIKEN cDNA 201031E24 gene		0.48
NM_016886	Gria3	glutamate receptor, ionotropic, AMPA3 (alpha 3)	transport, ion transport	0.46
XM_357781	Usp31	ubiquitin specific peptidase 31	ubiquitin-dependent protein catabolic process	0.44
NM_146176	Cnot3	CCR4-NOT transcription complex, subunit 3	transcription	0.47
NM_010466	Hoxc8	homeo box C8	transcription, regulation of transcription	0.34
NM_133818	AI597479	expressed sequence AI597479		0.46
NM_134142	Tmem109	transmembrane protein 109		0.45
NM_013868	Hspb7	heat shock protein family, member 7	protein folding	0.49
NM_172283	Fuk	fucokinase	metabolic process, phosphorylation	0.47
NM_181666	Mospd3	motile sperm domain containing 3	heart development	0.47
NM_030037	Pou2f1	POU domain, class 2, transcription factor 1	transcription	0.45
NM_178117	BC035947	cDNA sequence BC035947		0.49
NR_002843.1 (ensembl_refseq)	Rnu3b4	U3B small nuclear RNA 4		0.50
NM_017379	Tuba8	tubulin, alpha 8	microtubule cytoskeleton organization and biogenesis	0.49
NM_011119	Pa2g4	proliferation-associated 2G4	transcription	0.41
NM_134077	Rbm26	RNA binding motif protein 26	translational initiation	0.43
XM_140321	EG225468	predicted gene, EG225468		0.45

Table 1. (Continued)

Accession No.	Gene Name	Definition	Function (GO process)	-fold changes
NM_026816	Gtf2f2	general transcription factor IIF, polypeptide 2	transcription	0.46
BC048846	Sypl	synaptophysin-like protein		0.48
AC090431	Dock5	dedicator of cytokinesis 5		0.45
NM_177276	St8sia1	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	protein amino acid glycosylation, positive regulation of cell proliferation	0.45
NM_011374				
NM_144800	Mtss1	metastasis suppressor 1	cell motility, actin filament organization	0.50

Genes whose expression were altered more than two-fold are listed. The value indicates a ratio of the gene expression level in exercised mice to that in sedentary mice.

Table 2. Changes in gene expression after 2 weeks of 30-min daily exercise

Accession No.	Gene Name	Definition	Function (GO process)	-fold changes
NM_153457	Rtnl	reticulon 1	biological process	2.06
NM_013590	Lyz1	lysozyme 1	metabolic process, cell wall catabolic process	0.41
NM_022024	Gmfg	glia maturation factor, gamma		0.46
NM_010407	Hck	hemopoietic cell kinase	protein amino acid phosphorylation, intracellular signaling cascade	0.45
NM_146248	Ccher1	coiled-coil alpha-helical rod protein 1	protein export from nucleus	0.47
XM_483944	Atp2b1	ATPase, Ca++ transporting, plasma membrane 1	calcium ion transport	0.50
NM_009892	Chit3l3	chitinase 3-like 3	carbohydrate metabolic process	0.27
NM_017372	Lyz2	lysozyme 2	metabolic process, cell wall catabolic process	0.41
NM_030082	Hist3h2ba	histone cluster 3, H2ba	nucleosome assembly	0.41
NM_019731	Nme4	non-metastatic cells 4, protein expressed in	GTP biosynthetic process	0.47
NM_001003960				
NM_001003961	Dnmt3b	DNA methyltransferase 3B	DNA methylation	0.49
NM_001003963				
NM_010068				
NM_011538	Tbx6	T-box 6	mesoderm formation	0.49
NM_013650	S100a8	S100 calcium binding protein A8 (calgranulin A)	chemotaxis	0.17
NM_017379	Tuba8	tubulin, alpha 8	microtubule cytoskeleton organization and biogenesis	0.50

Genes whose expression were altered more than two-fold are listed. The value indicates a ratio of the gene expression level in exercised mice to that in sedentary mice.

Preparation of RNAs for DNA microarray analysis

Total RNAs were isolated from each of 4 or 5 mice spinal cords per exercised or sedentary group using the RNeasy Lipid Tissue Mini Kit (Qiagen GmbH, Hilden, Germany). Pooled total RNA (5 µg each, 4 or 5 mice per group) was applied to OpArray™ Mouse V4.0 (Operon Technologies, Alameda, CA, USA), in which 35,852 genes are registered. We used Low RNA Fluorescent Linear Amp Kit PLUS (Agilent Technologies Inc., Santa Clara, CA, USA) to synthesize double-stranded (ds) cDNA from the total RNA and then synthesize cRNA from the ds cDNA as a template. Total RNA was reverse-transcribed with a T7 Promoter primer. After second-strand synthesis, we used the ds cDNA as a template for Cyanine (Cy) 3/Cy5-labeled cRNA synthesis. The OpArray slide was hybridized with 20-25 µg of each labeled cRNA and washed with the OpArray Hybridization Buffer Kit (Operon). A fluorescent image of the OpArray slide was then recorded with CRBIO (Hitachi Software Engineering, Tokyo, Japan). The digitized image data were processed with DNASIS Array software (Hitachi Software Engineering). The ratios of intensity of Cy5 to that of Cy3 were calculated. Information on each gene on the slide was obtained from the National Center for Biotechnology Information (NCBI) database.

RESULTS AND DISCUSSION

This study demonstrated that single 30-min or 2-week exercise resulted in specific gene expression changes in mouse spinal cords using microarrays. By 30-min exercise, the expression of 3 genes was enhanced, and the expression of 29 genes reduced (Table. 1). By continuous 2-week exercise, the expression of 1 gene was enhanced, and the expression of 13 genes was reduced (Table. 2). *Gria3* is glutamate receptor called *GluR3*, whose antisense peptide nucleic acid targeting *GluR3* delayed disease onset and progression in the SOD1 G93A mouse model of familial ALS (Rembach *et al.*, 2004). The elimination of *St8sia1* coding GD3 synthase improved memory and reduced amyloid-beta plaque load in Alzheimer disease model mice (Bernardo *et al.*, 2008). Reticulon family members including *Rtn1* coding reticulon1 modulated BACE1 activity and amyloid-beta peptide generation (He *et al.*, 2004). This study will provide a valuable clue on the molecular basis of exercise-induced neuroprotective effects in spinal cords.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Basic Research (B) 19390151 (to I.H.), Young Scientists (B) 19790603 (to Y.H.)).

REFERENCES

- Bernardo, A., Harrison, F.E., McCord, M., Zhao, J., Bruchey, A., Davies, S.S., Jackson, Roberts, L.2nd., Mathews, P.M., Matsuoka, Y., Ariga, T., Yu, R.K., Thompson, R. and McDonald, M.P. (2008): Elimination of GD3 synthase improves memory and reduces amyloid-beta plaque load in transgenic mice. *Neurobiol. Aging*. [Epub ahead of print]
- He, W., Lu, Y., Qahwash, I., Hu, X.Y., Chang, A. and Yan, R. (2004): Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. *Nat Med.*, **10**, 959-965.
- Kirkinezos, I.G., Hernandez, D., Bradley, W.G. and Moraes, C.T. (2003): Regular exercise is beneficial to a mouse model of amyotrophic lateral sclerosis. *Ann Neurol.*, **53**, 804-807.
- McCrane, M.E. and Kaspar, B.K. (2008): Physical activity and neuroprotection in amyotrophic lateral sclerosis. *Neuromol. Med.*, **10**, 108-117.
- Rembach, A., Turner, B.J., Bruce, S., Cheah, I.K., Scott, R.L., Lopes, E.C., Zagami, C.J., Beart, P.M., Cheung, N.S., Langford, S.J. and Cheema, S.S. (2004): Antisense peptide nucleic acid targeting *GluR3* delays disease onset and progression in the SOD1 G93A mouse model of familial ALS. *J Neurosci Res.*, **77**, 573-582.