ISSN (Print) 1225−9918
ISSN (Online) 2287−3406
Journal of Life Science 2013 Vol. 23. No. 1. 116~124
DOI : http://dx.doi.org/10.5352/JLS.2013.23.1.116

Effect of Resistance Training on Skeletal Muscle Gene Expression in Rats: a Beadarray Analysis

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Received September 17, 2012 / Revised December 12, 2012 / Accepted January 4, 2013

The aim was to examine resistance exercise-related genes after 8 weeks of resistance training. Thirty-two male Sprague-Dawley rats were divided into four groups: 4 weeks sedentary (4 wks CON, n=8), 8 weeks sedentary (8 wks CON, n=8), 4 weeks exercise training (4 wks REG, n=8), and 8 weeks exercise training (8 wks REG, n=8). The rats were trained to climb a 1-m vertical incline (85-degree), with weights secured to their tails. They climbed 10 times, 3 days per week, for 8 consecutive weeks. Skeletal muscle was taken from the flexor halucis longus after the exercise training. After separating the total RNA, large-scale gene expression was investigated by beadarray (Illumina RatRef-12 Expression BeadChip) analysis, and qPCR was used to inspect the beadarray data and to analyze the RNA quantitatively. The detection p-value for the genes was p<0.01, the M-value {M=$\log_2$(condition)-$\log_2$(reference)} was >1.0, and the DiffScore was >20. In total, the expression of 30 genes significantly increased 4 weeks after the exercise training, and the expression of six genes decreased. At 8 weeks, the expression of five genes significantly increased and that of 12 decreased. Several genes are potentially involved in resistance exercise and muscle hypertrophy, including 1) regulation of cell growth (IGFBP1, PLA2G2A, OKL38); 2) myogenesis (CSRP3); 3) tissue regeneration and muscle development (MUSTN1, MYBPH); 4) hypertrophy (CYR61, ATF3, NR4A3); and 5) glucose metabolism (G6PC, PCK1). These results may help to explain previously reported physiological changes of the skeletal muscle and suggest new avenues for further investigation.

Key words : Resistance training, beadarray, gene expression

Introduction

The mature skeletal muscle is a remarkably adaptive tissue, able to demonstrate significant regeneration, hypertrophy and metabolic adaptation [9]. One of the most powerful stimuli for inducing skeletal muscle cellular re-organization is resistance training. In adults, depending on the type and duration of training, there can be appreciable changes in the size of the muscle mass and fiber-type composition, in addition to increased contractile activity and metabolic characteristics of the myofiber population [2].

Resistance training programs routinely produce increases in muscle cross-sectional area (CSA), muscular strength, and power, with gains occurring in both the slow type I and fast type II fibers [7, 21, 22, 24]. The increase in force and power appears to be primarily caused by exercise-induced muscle hypertrophy [7]. Hypertrophy of the skeletal muscle and its concomitant gains in power are of great interest to people from all walks of life, including the elite power athlete, patients rehabilitating from injury-induced atrophy, and the elderly with diminished mobility due to muscular weakness. Increased load on muscles drive hypertrophy through a number of signaling pathways, which ultimately increase the synthesis of proteins and decrease protein degradation [13].

Microarrays that rely on hybridization with DNA probes pioneered large-scale expression studies. The recent Illumina BeadChip has become a useful analysis tool for measuring the absolute expression level of independent samples. More than 22,000 different types of beads, each with a unique sequence, are represented on the RatRef-12 Expression BeadChip. Each bead contains hundreds of thousands of copies of covalently attached oligonucleotide probes. Beads are assembled into > 1.6 million pits, each measuring 3 μm in diameter, generating an average of 30-fold redundancy

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for each sequence represented on the array.

The expression of many skeletal muscle genes that encode for muscle hypertrophy and growth factors, as well as various signaling proteins, was upregulated by resistance training. However, no study to date has used beadarray analysis to assess the adaptive responses of gene expression in skeletal muscles of rats to resistance ladder training, nor has any previous study been designed to correlate the alterations in the expression of these exercise training-sensitive genes with relevant metabolic outcomes, such as muscle hypertrophy or growth factors.

In this context, the present investigation was designed to use gene expression profiling to identify genes in the flexor hallucis longus that respond to resistance ladder training in male Sprague-Dawley rats. This study examined resistance exercise-related genes after 4 weeks and 8 weeks of resistance training, and we investigated the large-scaled gene expression by beadarray using a BeadChip. We performed qPCR to inspect beadarray data and to conduct a quantitative analysis of mRNA.

Materials and Methods

Animals

Thirty-two male Sprague-Dawley rats (Semtako Ins., Korea) were obtained at 11 wks of age and separated into sedentary control (age-matched group; CON, n=16) and exercise-trained groups (resistance exercise group; REG, n=16). These groups were then further divided into 4 wk and 8 wk-treated groups. Thus, there were four groups: (1) the 4 wks sedentary group (4 wks CON, n=8); (2) 8 wks sedentary group (8 wks CON, n=8); (3) 4 wks exercise-trained group (4 wks REG, n=8); (4) 8 wks exercise-trained group (8 wks REG, n=8). Training began a week after familiarization. All animals were housed in a temperature-controlled room (21-23℃) with a 12:12-h light-dark cycle at the animal laboratory of Hanyang University in Korea. They were provided with rat chow and water *ad libitum*. All experimental protocols were approved by the Institutional Animal Ethics Committee of Hanyang University and were conducted according to the guidelines of the Korean Science Academy for the use and care of experimental animals.

Tissue collection

The rats were killed at least two days after training sessions to exclude any short-term effects of exercise. They were anesthetized with a mixture of Zoletil (50 mg/kg, IP) and Rompun (5-10 mg/kg) and euthanized by cervical dislocation. The tissue was collected from the flexor halucis longus of the agonist when they climbed the ladders [11, 13].

Resistance training protocol

The model of resistance exercise was chosen based on its efficacy in inducing skeletal muscle hypertrophy [11, 13, 25]. After the first week of familiarization, the rats in the exercise-trained group (4 wks REG, 8 wks REG) were trained for 3 days per week alternating days for 4weeks and 8 weeks, respectively. Resistance training was accomplished by using a 1 m high ladder with 2 cm grid steps and an 85° incline. In their first week, rats were familiarized with climbing up to the top cage with and without weight on their tails. Training sessions, from the second week, were commenced with intensity at 50% of each rat's body weight the weight in a conical tube was attached to the tail with a plastic belt and tape. The rats began climbing from the bottom of the ladder and were forced to climb to the top by touching. When they reached the top, 2 min of rest was given and the next trial followed. Subsequent trials were performed from the bottom, and 15% of the weight was added to the prior weight at every trial. If a rat was able to climb with the increasing weights for 10 times, a session of training was considered as being complete. In the case of failure to increase the weight as planned, the rat was forced to complete 10 trials with the last successful weight, with no further weight increase attempts.

Total RNA extraction and mRNA isolation

Total RNA was extracted from the muscle using a commercial Tri Reagent (Molecular Research Center, Inc. Cincinnati, OH, U.S.A.) following the manufacturer's instructions. Briefly, 50-100 mg of muscle was removed from the freezer and immediately immersed in 1 ml of Tri Reagent. The muscle was homogenized using a BioPulverizer (Biospec 59013N, U.S.A.), and the aqueous and organic phases were separated using 200 µl of chloroform. Total RNA was precipitated using 500 µl of isopropyl alcohol, washed three times with 75% ethanol, dissolved in 30 µl Nuclease-free water, and stored at -70℃. The concentration and purity of the RNA was determined using a Spectrophotometer (NanoDrop, U.S.A.) by measuring the absorbance at 260 nm and 280 nm.

Beadarray processing, data acquisition and statistical analysis

Total RNA (50-500 ng) was reverse transcribed and labeled with biotin using an Illumina RNA Amplification kit (Ambion, Austin, TX) according to the manufacturer's protocol. Briefly, the cRNA samples were mixed with the Hyb E1 hybridization buffer containing 37.5% (w/w) formamide, and the hybridization mix was dispensed on the center of the RatRef-12 Expression BeadChip (Illumina, U.S.A.), containing 22,523 genes represented in the consensus NCBI RefSeq database. Hybridization was conducted over 18 hours at 58℃. Array chips were then washed with the E1BC solution followed by 100% ethanol and E1BC solution again. Next, the chips were blocked with an E1 blocking buffer followed by staining with streptavidin-Cy3 and washing with E1BC solution.

We analyzed the Illumina RatRef-12 Expression BeadChip using the manufacturer's BeadArray Reader, and collected primary data using the supplied Scanner software. Beadarray data analysis was performed over three stages. First, expression intensities were calculated for each gene probed on the array for all twelve hybridization experiments using Illumina's Beadstudio software. Second, intensity values were controlled and normalized. Quality control was carried out using the Illumina Beadstudio detection p set to <0.01 as a cutoff. Then, we selected genes with M-value {$M=\log_2$ (condition)-$\log_2$ (reference)} to >1.0 and DiffScore to >20. Especially, the M-value (an expression ratio; exercise group vs. sedentary group) of ≥ 1 or ≤ -1 was considered to reflect a significant difference between the two groups.

Real-time quantitative PCR (qPCR)

For the confirmation of the beadarray gene expression findings, we carried out quantitative real-time PCR (qPCR) using SYBR Green. This was performed on an iCycler real-time PCR system (Bio Rad, U.S.A.) in the iQ SYBR Green Supermix (Bio Rad, U.S.A.), according to the manufacturer's instructions with target gene primers and probe and internal standard gene primers and probe in the same reactions. We selected five genes on the basis of the bead array data. Primers and probes for each target gene were designed based on the cDNA sequence in PrimerBank (http://pga.mgh.harvard.edu/primerbank), with primer 3 designer (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The primer sequences are given in Table 1.

qPCR data refers to the ratio of relative gene expression (2^n-dC_t) using the relative value (dC_t=gene-GAPDH), compared with GAPDH, which is the housekeeping gene. DNA was amplified by an initial incubation at 94℃ for 1 min followed by 37 cycles of 95℃ for 40 sec, 58℃ for 40 sec, 70℃ for 40 sec, and a final extension at 72℃ for 3 min. In addition, the PCR products were then separated by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining for band size and product purity.

Results

Beadarray screen identifies differential gene expression in skeletal muscle after resistance training

To investigate the resistance exercise-related genes, the expressional patterns of the genes were compared and analyzed in the exercise-trained group (4 wks REG, 8 wks REG) in reference to the sedentary group (4 wks CON, 8 wks CON). Genes whose Detection P-value was 0.01 or less were selected from a total of 22,523 genes (Fig. 1A), and the genes whose M-value was 1.0 or greater or -1.0 or less and whose DiffScore was 20 or higher were selected as the group of genes exhibiting significant differences (Fig. 1B).

On the whole, there were genes that increased or decreased through resistance training, and in particular, there

Table 1. Primer sequences for amplification of qPCR

Genes	Forward primer sequences	Reverse primer sequences	Product size (bp)
GAPDH	gggtgtgaaccacgagaaat	atggcatggactgtggtcat	146
HSPB7	atccagtgcaaaaacccttg	cctcccctccctatctgaag	164
IGFBP1	gcggtagtgcctagaacgag	tgggattcgatgaggaagtc	107
MUSTN1	gggagtctgtccaagaacca	ttgggcttctcgaagactgt	137
MYBPH	accccaagtatcgtgctgtc	ctccttgtcccagtctctgc	193
SERPINA1	tcaacaggccagacagtgag	tgagtcggcaaagttgacag	143

GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HSP7, heat shock 27 kD protein family member 7; IGFBP1, insulin-like growth factor binding protein 1; MUSTN1, musculoskeletal/embryonic nuclear protein 1; MYBPH, myosin binding protein H; SERPINA1, serine (or cysteine) proteinase inhibitor clade A member 1

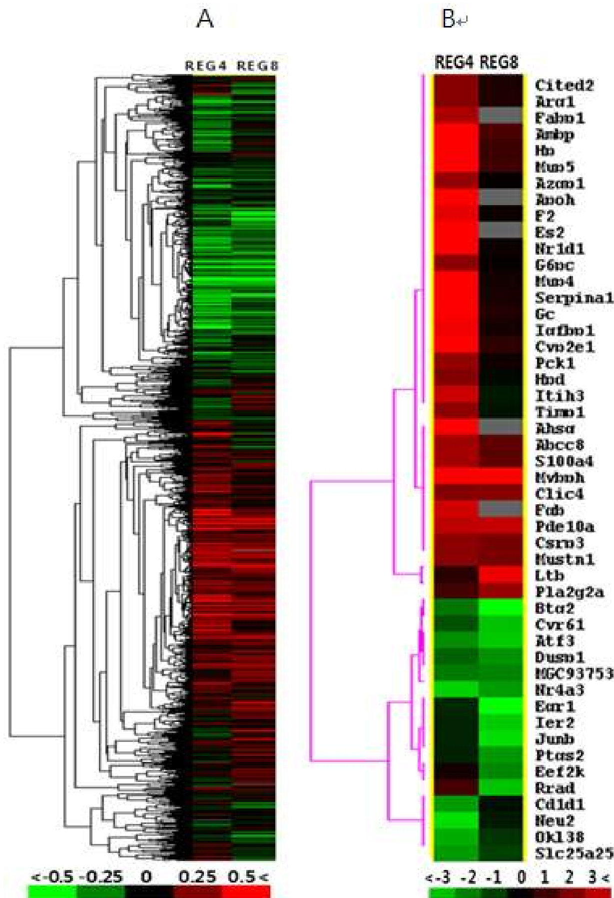

Fig. 1. Expressed genes of resistance exercised group in contrast with sedentary control group; (A) Whole data, (B) Significant data. Beadarray data represents the relative difference in gene expression of resistance exercise group compared to control group. Increased genes are shown in progressively brighter shades of red, and decreased genes are shown in progressively brighter shades of green. Values unit are expressed as fold.

were many genes increased after 4 weeks of resistance training and decreased after 8 weeks of resistance training. The results of the beadarray analysis are as shown in Table 2.

Our beadarray analyses detected that the mRNA expression of 32 genes was differentially increased after resistance training, while that of 16 was decreased among the 22,523 genes. After 4 weeks of resistance training, the expression of 30 genes was differentially increased and that of 6 was decreased. After 8 weeks of resistance training, the expression of 5 genes was differentially increased and that of 12 was decreased. The expression of 3 genes significantly increased at both 4 and 8 weeks, and the expression of 2 genes significantly decreased at all-time points. Thus, changes of several genes related to resistance exercise were found in the present beadarray study.

In the 4 weeks exercise-trained group (4 wks REG), IGFBP1 increased by 3.76-fold and OKL38 decreased by 2.55-fold. These are the genes involved in the regulation of cell growth. In the 8 wks exercise-trained group (8 wks REG), PLA2G2A showed an increase of 2.91-fold, indicating significant expression. Also, CSRP3, which is involved in the myogenesis pathway, exhibited an increase of 2.16-fold in the 4 weeks exercise-trained group (4 wks REG). MUSTN1, involved in tissue regeneration, showed an increase of 2.11-fold in the 4 weeks exercise-trained group (4 wks REG). MYBPH, involved in muscle development and striated muscle contraction, exhibited an increase of 13.09-fold in the 4 weeks exercise-trained group (4 wks REG) and an increase of 5.35-fold in the 8 weeks exercise-trained group (8 wks REG), representing the highest increase of gene expression.

Table 2. Expressed genes of resistance exercised group in contrasts with 4 weeks and 8 weeks sedentary control groups

Accession number	Symbol	Definition	Class / Function	Expression ratio	
				R4:C4	R8:C8
NM 013144	Igfbp1	insulin-like growth factor binding protein 1	Regulation of cell growth	**1.91**	0.18
NM 031598	Pla2g2a	phospholipase A2, group IIA	Regulation of cell growth	0.53	**1.19**
NM 138504	Okl38	pregnancy-induced growth inhibitor	Regulation of cell growth	**-1.35**	-0.40
NM 057144	Csrp3	cysteine-rich protein 3	Myogenesis	**1.11**	0.96
NM 181368	Mustn1	musculoskeletal, embryonic nuclear protein 1	Tissue regeneration	**1.08**	0.94
NM 031813	Mybph	myosin binding protein H	Muscle development Striated muscle contraction	**3.71**	**2.42**
NM 031327	Cyr61	cysteine rich protein 61	Hypertrophy model	-0.65	**-1.53**
NM 012912	Atf3	activating transcription factor 3	Hypertrophy model	**-1.11**	**-1.58**
NM 031628	Nr4a3	nuclear receptor subfamily 4, group A, member 3, transcript variant 1	Hypertrophy model	**-1.64**	**-1.22**
NM 013098	G6pc	glucose-6-phosphatase, catalytic	Guconeogenesis	**1.12**	0.07

Table 2. Continued

Accession number	Symbol	Definition	Class / Function	Expression ratio	
				R4:C4	R8:C8
NM 198780	Pck1	phosphoenolpyruvate carboxykinase 1	Guconeogenesis Adipogenesis	**1.15**	0.14
NM 012551	Egr1	early growth response 1	Insulin signaling	-0.32	**-2.77**
NM 053338	Rrad	Ras-related associated with diabetes	Insulin signaling	0.52	**-1.53**
NM 013039	Abcc8	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	Transport	**1.27**	0.75
NM 012556	Fabp1	fatty acid binding protein 1, liver	Transport	**1.35**	-
NM 012564	Gc	group specific component	Transport	**2.79**	0.27
NM 031543	Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1	Electron transport	**3.11**	0.37
NM 031818	Clic4	chloride intracellular channel 4	Ion transport	**1.03**	**1.09**
NM 053769	Dusp1	dual specificity phosphatase 1	Cell cycle	-0.77	**-1.15**
NM 021836	Junb	Jun-B oncogene	Cell cycle Oxidative stress	-0.27	**-1.74**
NM 012898	Ahsg	alpha-2-HS-glycoprotein	Acetylcholine synthesis	**2.56**	-
NM 022924	F2	coagulation factor 2	Regulation of actin cytoskeleton	**1.81**	0.1
NM 012582	Hp	haptoglobin	Proteolysis and peptidolysis	**2.27**	0.46
NM 012901	Ambp	alpha 1 microglobulin/bikunin	Anti-inflammatory response	**3.16**	0.61
NM 012826	Azgp1	alpha-2-glycoprotein 1, zinc	Immune response	**1.18**	0.06
NM 053698	Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	Central nervous system development	**1.05**	0.23
NM 145775	Nr1d1	nuclear receptor subfamily 1, group D, member 1	Regulation of transcription	**2.31**	0.10
NM 020071	Fgb	fibrinogen, B beta polypeptide	Complement activation, classical pathway	**1.64**	-
NM 022519	Serpina1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	Complement activation, classical pathway	**2.65**	0.20
NM 053819	Timp1	tissue inhibitor of metalloproteinase 1	Matrix metalloporteinases	**1.12**	-0.15
NM 012947	Eef2k	eukaryotic elongation factor-2 kinase	Translation factors	0.16	**-1.06**
NM 017232	Ptgs2	prostaglandin-endoperoxide synthase 2	Eicosanoid synthesis	-0.26	**-1.19**
NM 017134	Arg1	arginase 1	Response to wounding	**1.06**	0.26
NM 017233	Hpd	4-hydroxyphenylpyruvic acid dioxygenase	Tyrosine catabolism	**1.04**	-0.07
NM 022236	Pde10a	phosphodiesterase 10A	Signal transduction	**1.50**	**1.54**
NM 017079	Cd1d1	CD1d1 antigen	Positive regulation of innate immune response	**-1.22**	-0.12
NM 017130	Neu2	neuraminidase 2	Carbohydrate metabolism	**-1.74**	-0.21
NM001009626	Apoh	apolipoprotein H	Unknown	**2.89**	-
NM 017004	Es2	esterase 2	Unknown	**2.40**	-
NM 017351	Itih3	inter-alpha trypsin inhibitor, heavy chain 3	Unknown	**1.59**	-0.19
NM 212507	Ltb	lymphotoxin B	Unknown	0.32	**1.87**
NM 198784	Mup4	major urinary protein 4	Unknown	**2.36**	0.16
NM 203325	Mup5	major urinary protein 5	Unknown	**2.38**	0.49
NM 012618	S100a4	S100 calcium-binding protein A4	Unknown	**1.29**	0.75
NM 017259	Btg2	B-cell translocation gene 2, anti-proliferative	Unknown	-0.93	**-2.06**
NM001009541	Ier2	immediate early response 2	Unknown	-0.27	**-1.59**
NM001004236	MGC93753	tetraspan 1	Unknown	-0.95	**-1.04**
NM 145677	Slc25a25	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Unknown	**-1.21**	-0.50

Differential expression of genes in the rat skeletal muscle after 4 and 8 weeks of resistance ladder exercise as revealed by beadarray analysis. An expression ratio (exercise group vs. sedentary group) of ≥ 1 or ≤ -1 was considered to reflect a significant difference between the two groups. Symbol, unigene symbol; C4, 4 wks sedentary group; C8, 8 wks sedentary group; R4, 4 wks exercise-trained group; R8, 8 wks exercise-trained group; **bold**: statistically significant, M>1 or M<-1 (fold >2).

However, CYR61, which is known to be involved in hypertrophy, showed a decrease of 2.89-fold in the 8 weeks exercise-trained group (8 wks REG), and the ATF3 and NR4A3 genes showed a decrease of 2.16-fold and 3.12-fold, respectively, in the 4 weeks exercise-trained group (4 wks REG) and a decrease of 2.99-fold and 2.33-fold, respectively, in the 8 weeks exercise-trained group (8 wks REG).

The result of quantitative real-time PCR (qPCR) analysis

qPCR was used to verify changes in gene expression as demonstrated by beadarray. We selected 5 genes the expressions of which were different between the two groups in the sedentary state and two whose expressions were different in the trained state. These included heat shock 27 kD protein family member 7 (HSP7), insulin-like growth factor binding protein 1 (IGFBP1), musculoskeletal/embryonic nuclear protein 1 (MUSTN1), myosin binding protein H (MYBPH), and serine (or cysteine) proteinase inhibitor clade A member 1 (SERPINA1). qPCR data represents the relative difference (2^{n}-dC_{t}) in gene expression compared to GAPDH, considering the 5 genes selected for verifying beadarray data and quantitatively analyzing RNA in the sedentary (4 wks CON, 8 wks CON) and exercise-trained groups (4 wks REG, 8 wks REG). Fig. 2 compares qPCR and the beadarray data, which is the average signal value for each gene probe.

As a result, similar patterns were exhibited in both data in the sedentary group as well as in the exercise-trained

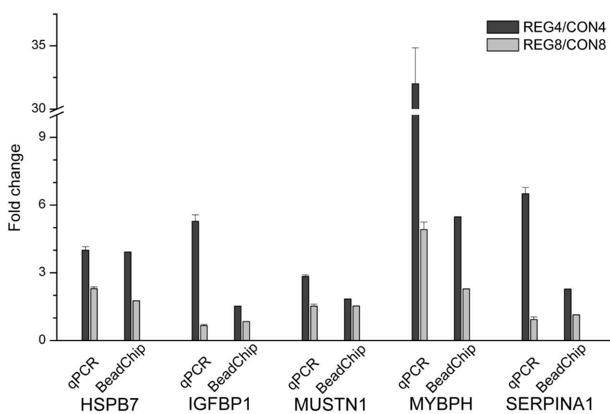

Fig. 2. Comparison of qPCR data with beadarray data. Comparison of quantitative PCR (qPCR) and beadarray expression data for 5 genes: HSBP7, IGFBP1, MUSTN1, MYBPH, and SERPINA1. The amount of mRNA is shown relative to GAPDH expression. Both qPCR and beadarray results showed concordant directional as well as proportional changes. Values are mean±SD.

group as in Fig. 4. In particular, a clear increase of expressions was exhibited in the 4 wks exercise-trained group.

Discussion

The purpose of this study was to examine resistance exercise-related genes after 8-weeks of resistance training of rats. We investigated large-scale gene expression. Generally, gene array technology has provided a rapid and efficient way to screen a large number of mRNAs in order to identify potential targets and pathways for further research [16]. Especially, microarray technology has greatly enhanced research in exploring transcriptional contributions to cellular events [6, 10]. This technology enables researchers to screen thousands of mRNAs simultaneously and define whole genome transcriptional responses, enhancing the capacity to discover novel genes and characterizing entire programs of differential mRNA expression [17]. Recently, several groups have successfully used microarrays to examine mRNA expression related to muscle repair, inflammation, protein synthesis and cellular control in skeletal muscles after various damaging and hypertrophic stimuli [1, 3-5, 20, 26]. To date, however, there have been no studies to have examined global mRNA expression from resistance exercise using beadarray. This was the first study to use beadarray to examine global mRNA expression in skeletal muscles after resistance training. There were genes that exhibited differences in expression after the resistance ladder training that used beadarray.

Beadarray results identified 3 genes related to the regulation of cell growth that were differentially regulated between CON and REG. IGFBP1 was upregulated 3.76-fold in the 4 wks exercise-trained group (4 wks REG) compared to the 4 wks sedentary group (4 wks CON). OKL38, however, was downregulated 2.55-fold. PLA2G2A was upregulated 2.91-fold in the 8 wks exercise-trained group (8 wks REG) compared to the 8 wks sedentary group (8 wks CON). Among these genes, IGFBP1 was quantitatively analyzed using qPCR, and 5.28-fold higher expression was exhibited after 4 weeks of resistance ladder training.

CSRP3, which is known to be involved in the myogenesis pathway, is the contraction-induced muscle damage marker gene, and acute eccentric exercise bouts are known to cause the increase of CSRP3 expression [23]. Generally, CSRP3 is expressed at low levels in other tissues than in striated muscles during development [19]. Additionally, CSRP3 has been

shown to promote the terminal differentiation of skeletal muscle myoblasts. Mice knockout models have produced evidence of involvement of CSRP3 in the muscle hypertrophy and regeneration processes. In this study, CSRP3 increased 2.16-fold after 4 weeks of resistance ladder training.

This study also showed an increase in the expression of genes related to muscle development and striated muscle contraction, such as the muscle-specific gene. MYBPH was upregulated 13.09-fold in the 4 wks exercise-trained group (4 wks REG) over the sedentary group (CON), and 5.35-fold in the 8 wks exercise-trained group (8 wks REG). We carried out qPCR to confirm the mRNA of this gene. Results identified a novel resistance exercise-based difference in the mRNA of MYBPH as a muscle-specific gene. A study by Barash and colleagues [1], which analyzed the patterns of gene expression in skeletal muscles using Affymetrix MGU74Av2 microarray after acute eccentric exercise and isometric exercise using mice and electric stimulation, also reported a 4.65-fold increase of MYBPH. A study analyzing the patterns of gene expression in skeletal muscles using Whole Rat Genome CodeLink microarray after 4 weeks of treadmill exercise using zucker rats also reported that MYBPH expression increased 1.51-fold [18].

In normal adult tissues, MUSTN1 expression was predominantly detected in skeletal muscles and tendons, suggesting that it represent a musculoskeletal-specific gene [14]. MUSTN1, as a musculoskeletal-specific gene, was involved in bone development and regeneration [15] as well as chondrogenesis [8]. Liu and colleagues [14] described its expression during skeletal muscle development and, more importantly, its critical role in the myogenic progenitor cell function. The same authors also investigated the effects of an acute bout of resistance training on gene expression [14]. They found that MUSTN1 expression upregulated with eccentric contractions, but not concentric contractions, suggesting that MUSTN1 may be involved in the repair of eccentric-exercise-induced muscle damage [12]. MUSTN1, the musculoskeletal-specific gene involved in tissue regeneration, increased 2.11-fold after 4 weeks of resistance ladder training and qPCR results also show a 2.83-fold increase.

Also, we found three genes related to hypertrophy model that was downregulated between CON and REG. CYR61 showed a 2.89-fold decrease in the 8 wks exercise-trained group (8 wks REG), while ATF3 and NR4A3 genes showed 2.16-fold and 3.12-fold decreases, respectively, in the 4 wks exercise-trained group (4 wks REG), and 2.99-fold and 2.33-fold decreases, respectively, in the 8 wks exercise-trained group (8 wks REG).

G6PC and PCK1, which are involved in glucose metabolism, increased 2.17-fold and 2.11-fold, respectively, after 4 weeks of resistance ladder training.

According to the above results, 8 weeks of resistance ladder training has an influence on the partial signaling pathway of male Sprague-Dawley rats. Although the expression of several skeletal muscle genes was altered by resistance ladder training, the changes could not explain all the health benefits resulting from training. Therefore, mechanisms other than altered gene expression may be involved in such training-induced adaptation. Future analysis of expression and activity at the protein level will be necessary to obtain additional information.

In conclusion, we used the beadarray to study the effects of resistance training for 8 weeks on skeletal muscle gene expression in rats. In total, the expression of 30 genes significantly increased 4 weeks after exercise and 6 genes were decreased, including genes with unknown functions. At 8 weeks, the expression of 5 genes significantly increased and 12 genes were decreased. Several of these genes are potentially important genes involved in resistance exercise and muscle hypertrophy, including those involved in: 1) the regulation of cell growth (IGFBP1, PLA2G2A, OKL38); 2) myogenesis (CSRP3); 3) tissue regeneration and muscle development (MUSTN1, MYBPH); 4) hypertrophy (CYR61, ATF3, NR4A3); and 5) glucose metabolism (G6PC, PCK1). These results may help to explain previously reported physiological changes of the skeletal muscle, and may open and suggest new avenues for further investigation.

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초록 : 저항성 운동이 골격근 유전자 발현에 미치는 영향: Beadarray 분석

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본 연구의 목적은 저항성 운동 후 골격근에서 저항성 관련 유전자를 규명하는 것이다. 연구 목적을 달성하기 위하여 총 32두의 Sprague-Dawley계 수컷 흰쥐를 분양 받은 후 4주차 통제군(4 wks CON, n=8), 8주차 통제군(8 wks CON, n=8), 4주차 운동군(4 wks REG, n=8), 8주차 운동군(8 wks REG, n=8)으로 집단을 분류하였다. 저항성 운동군은 꼬리에 무게를 달고 동물용 사다리(1-m vertical, 85 degree incline)를 오르는 저항성 사다리 운동을 1회 10번, 주당 3일, 4주와 8주간 점증적으로 실시하였으며, 골격근 조직은 저항성 운동 후 장무지굴근(flexor hallucis longus; FHL)을 적출하여 분석에 이용하였다. 적출한 골격근에서 total RNA를 분류한 후, 대규모 유전자 발현 분석을 위하여 Illumina RatRef-12 Expression BeadChip을 이용한 Beadarray를 시행하였으며, Beadarray 결과를 확인하기 위해 qPCR (real-time quantitative PCR)를 실시하였다. 유의성 검증은 Beadstudio software를 이용하여 실시하였으며, Beadarray 데이터 중 Detection *p*-value to <0.01, M-value {M= log2 (condition)-log2 (reference)} to >1.0, DiffScore to >20인 유전자만을 통계적으로 의미 있는 유전자로 선택하였다. 4주차 저항성 운동 후 통제 집단에 비해 2배 이상 유의하게 발현이 증가한 유전자는 30개였으며, 6개의 유전자가 감소하였다. 8주차 저항성 운동 후에는 5개의 유전자가 발현이 증가하였으며, 12개의 유전자가 유의하게 감소하였다. 연구결과 다음의 유전자를 포함한 저항성 운동과 근비대와 관련 후보 유전자를 도출하였다; 1) 세포 성장 조절(IGFBP1, PLA2G2A, OKL38); 2) 근육발생(CSRP3); 3) 조직 재생과 근육 발달(MUSTN1, MYBPH); and 4) 비대 모델(CYR61, ATF3, NR4A3); and 5) 당대사(G6PC, PCK1). 이러한 연구결과는 차후 저항성 운동과 관련된 다양한 생리학적 변인을 연구하는데 있어서 기초 자료를 제공할 것으로 생각된다.