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Research Article

Comparative Analyses of Gene Expression Profiles Following Exercise - and Electrical Stimulation - Induced Improvement of Walking Performance in Rat Claudication Model

Momoko Shiragaki-Ogitani[1]*, Keita Kono[2], Futoshi Nara[3] and Atsushi Aoyagi[1]

[1]*Specialty Medicine Laboratories II, Daiichi Sankyo Co., Ltd., Tokyo, Japan*

[2]*Specialty Medicine Laboratories I, Daiichi Sankyo Co., Ltd., Tokyo, Japan*

[3]*Ube Industries. Ltd. Pharmaceuticals Research Laboratory, Japan*

***Corresponding Author:** Momoko Shiragaki-Ogitani, Specialty Medicine Laboratories II, Daiichi Sankyo Co., Ltd., Tokyo, Japan.

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Abstract

Exercise is considered to be the most efficacious therapeutic approach for improving walking capacity in patients with intermittent claudication (IC). Despite its enormous benefits, the underlying mechanisms remain unclear. To reveal the local mechanisms underlying exercise-induced improvement in walking performance, we previously developed an electrical stimulation (ES) model which exerts local limb muscle contraction that mimics exercise training. This prior study showed ES-induced improvement of walking performance in a rat IC model. In the present study, we confirmed gene expression profiles following hind-limb ischemia, and recovery following exercise or ES-intervention using GeneChip microarray in a rat IC model. Of the top 40 genes upregulated or downregulated following exercise and ES compared with hind-limb ischemia, most genes changed in opposite directions to sham-operated normal control. We further confirmed the gene expression changes using semi-quantitative real-time PCR, and identified several candidates that might potentially be involved in the improvement of walking performance following exercise or ES including *Dpp4, Nov*, and *Ptges*. Further analyses of gene expression profiles and characterization of individual genes will help in the identification of therapeutic targets for the treatment of patients with IC.

Keywords: Intermittent Claudication; Exercise; Electrical Stimulation; Mechanism; Transcriptional Analysis

Abbreviations

ES: Electrical Stimulation; EX: Exercise; IAO: Iliac Artery Occlusion; FAO: Femoral Artery Occlusion; PCR: Polymerase Chain Reaction; TA: Tibialis Anterior.

Introduction

Intermittent claudication (IC) is the most common symptom of peripheral arterial disease and has a significant impact on the quality of life of patients. IC presents with fatigability, numbness, spasm, or muscle pain resulting in deterioration of walking function due to insufficient blood supply to the affected limbs. Despite the large population of patients worldwide, there are few options for pharmaceutical treatment of IC; to date, cilostazol and pentoxifylline are the only drugs approved by the Food and Drug Administration, and both have limited therapeutic effects [1-3]. Thus, a novel, effective drug is urgently needed.

Exercise is considered the most effective therapy for improving the walking ability of patients with IC; it is far more effective than cilostazol treatment [4,5]. However, supervised exercise training, which is the preferred initial treatment for patients with IC is not feasible for all patients due to its limited provision [6]. Since exercise has potent and pleiotropic effects for the treatment of IC, identification of novel therapeutic approaches that mimic the beneficial effects of exercise is an attractive area of research.

Exercise exerts multiple effects, ranging from local effects on IC-affected limbs to cardiorespiratory effects and even effects on higher brain functions such as the learning process [7-9]. To understand the principal mechanisms underlying improved walking ability after exercise, development of a simplified model that reflects a key aspect of exercise would be of great benefit. As such, we postulated that motor nerve-mediated contraction of skeletal muscles during exercise is one of the key factors that might lead to functional improvement in IC-affected limbs, since exercise is known to induce various adaptations within active skeletal muscles [10-12]. In order to investigate the effects of skeletal muscle contraction on IC symptoms, in a previous study, we developed an electrical stimulation (ES) system and applied it to a rat IC model [13]. In this earlier study, we generated an IC condition by occluding the iliac artery and femoral artery, and implanted electrical stimulators that induced skeletal muscle contraction of the affected limbs. Both ex-

ercise and ES, respectively, significantly improved walking performance in the rat claudication model. Moreover, both exercise and ES increased the capillary-to-fiber ratio and exerted muscle fiber type shifts towards more oxidative types. These results suggested that ES could mimic at least some aspects of exercise, leading to an improvement in walking performance in rats with IC.

Given the overlapping mechanisms of exercise and ES, we postulated that there are also common gene expression patterns in the affected limbs following exercise and ES. In the present study, we comprehensively analyzed gene expression profiles in the skeletal muscles following hind-limb ischemia, and examined the effects of exercise and ES. Intriguingly, of the top 40 genes upregulated or downregulated following exercise and ES compared with hind-limb ischemia, most genes changed in opposite directions to sham-operated normal control. Further analyses of common gene expression patterns associated with exercise and ES, and characterization of individual genes, would lead to a more developed understanding of the local mechanisms underlying the beneficial effects of exercise; in turn, this could lead to the discovery of new therapeutic approaches for patients with IC.

Materials and Methods

Animals

Eight- to nine-week-old male F344/DuCrlCrlj rats were purchased from Charles River Laboratories Japan Inc. The animals were housed in a room with 12:12 h light-dark cycle, and had access to water and normal chow diet ad libitum. Animals were maintained in an AAALAC-accredited facility in accordance with the Guide for the Care and Use of Laboratory Animals. All animal experiments were approved by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering.

Surgical procedures of hind-limb ischemia

The surgical procedures for inducing hind-limb ischemia were performed as previously described [13]. Briefly, the animals were anesthetized via inhalation of 2% isoflurane throughout the surgery. The right iliac artery was occluded using a silk suture approximately 5 mm below the bifurcation from the aorta. Two weeks after iliac artery occlusion, the right femoral artery was occluded below the branching of the arteria profunda femoris. Sham-operated rats were treated in the same manner aside from occlusion.

Exercise and ES protocol

The animals in the exercise group ran on a treadmill apparatus (Osaka Microsystems, Osaka, Japan, TM-R-N1) twice daily for two weeks. Each day, the animals exercised both in the morning and afternoon, with at least 4 h between the two exercise sessions. The exercise training condition was at a 15° incline, 15 m/min for 20 min.

Each animal in the ES group underwent implantation of a custom-designed preprogrammed stimulator (Bio Research Center Co. Ltd., Tokyo, Japan) under the skin of their back at the same time as femoral artery occlusion; the stimulator was connected to electrodes sutured in the vicinity of the right peroneal nerve so as to stimulate the tibialis anterior (TA) muscle. Each set of stimulations was programmed to stimulate at 10 Hz (pulse width: 0.3 ms, intensity: 3 V) for 15 min with a rest period of 85 min; there were seven sets per day. Both exercise and ES were commenced three days after femoral artery occlusion surgery and were implemented five days a week for two consecutive weeks.

On the day after the last day of exercise or ES, the animals were euthanized, and TA muscle samples were collected.

GeneChip microarray expression analysis

TA muscle samples were stored at -80°C until RNA extraction. Snap frozen tissue samples were powdered by TissueLyser II (Qiagen, Hilden, Germany, 85300). Total RNA isolation was performed using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany, 74704) according to the manufacturer's instructions. For GeneChip analysis, an equal amount of total RNA from all animals in each group was pooled. Microarray processing was conducted according to the GeneChip Expression Analysis Technical Manual (Affymetrix, CA, USA). To identify differentially expressed transcripts in our analysis the GeneChip Rat Genome 230 2.0 Array (Affymetrix, CA, USA) was employed. The data sets are accessible through the National Center for Biotechnology Information Gene Expression Omnibus database repository (GSE140629).

Real-time reverse transcription-polymerase chain reaction (Real-time RT-PCR)

The extracted RNA was used to synthesize first-strand cDNA with SuperScript VILO Master mix (Life Technologies, CA, USA, 11755-500) and polymerase chain reaction (PCR) was performed with TaqMan Fast Advanced Master mix (Life Technologies, CA, USA, 4444558) and TaqMan Custom Arrays (Life Technologies, CA, USA) according to the manufacturer's instructions. The relative amount of the specific mRNA of interest was normalized to reference gene (Rplp, 50S ribosomal protein L16). Analysis of the obtained data was performed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analyses were performed with SAS System Release 9.2 (SAS Institute Inc., Cary, NC, USA). Comparison of animal data between multiple groups were performed using Dunnett's test as previously described [13]. Comparison of real-time PCR results between multiple groups were performed using Tukey's test. A value of $P < 0.05$ was considered statistically significant.

Results

Animal characteristics and physiological parameters

The previously reported study scheme and the parameters analyzed following exercise (EX) and electrical stimulation (ES) in the rat IC model are shown in Figure 1 and Table 1, respectively [13]. We analyzed each parameters among Sham, hind-limb ischemia (Isch), Isch + EX, Isch + ES groups. Two weeks after the onset of exercise, walking distance was improved to a level similar to that of the sham group. ES also improved walking distance to a level half of the sham group. Plantar blood flow under anesthesia was not changed by either exercise or ES. The capillary-to-fiber ratio in TA skeletal muscle was increased by both exercise and ES.

Figure 1: Schematic Diagram of Exercise (EX) and Electrical Stimulation (ES).

(A) For development of rat ischemia model (Isch), femoral artery occlusion (FAO) was applied ipsilaterally two weeks after unilateral iliac arterial occlusion (IAO). Exercise (EX) and electrical stimulation (ES) were commenced three days after FAO and were implemented five days per week for two consecutive weeks. Four weeks after IAO, muscle samples were collected and RNA was isolated later. Isolated RNA samples were subjected to microarray analysis and real-time PCR.

(B) A photograph of electrical stimulator. Bar = 20mm.

(C) The animals in Isch + ES group had preprogrammed stimulators implanted under the skin of their backs; the stimulators were connected to electrodes sutured in the vicinity of the right peroneal nerve so as to stimulate the tibialis anterior (TA) muscle.

	Walking distance (m)	Blood flow (ratio of control leg)	Capillary-to-fiber ratio
Sham	373 ± 18	1.15 ± 0.082	1.1 ± 0.065
Isch	86 ± 24	0.48 ± 0.061	1.2 ± 0.056
Isch + EX	319 ± 33***	0.35 ± 0.074	1.3 ± 0.020*
Isch + ES	186 ± 8.7*	0.46 ± 0.047	1.5 ± 0.027**

Table 1: Summary of parameters in each group.

Previously reported parameters in each group. Isch, ischemia group; Isch + EX, ischemia + exercise group; Isch + ES, ischemia + ES group. Values are presented as mean ± SE and statistical significance was determined using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Isch group.

DNA microarray analysis

To explore the key genes in the skeletal muscle that are associated with improved walking performance in the IC model, we analyzed gene expression profiles in each group. There were 419 genes in Isch + EX group and 915 genes in Isch + ES group that were upregulated more than 1.5 times compared with Isch group. On the contrary, 576 genes from Isch + EX group and 853 genes from Isch + ES group were downregulated more than 1.5 times compared with Isch group. Since we aimed to explore the genes that mimic the local effects of exercise, we extracted the genes whose expression were altered in both Isch + EX and Isch + ES groups. As shown in Figure 2, 80 genes were upregulated (up) and 81 genes were downregulated (down) in both Isch + EX and Isch + ES groups compared with Isch group. The top 40 of upregulated or downregulated genes in Isch + EX and Isch + ES groups are listed in Table 2 and Table 3, respectively, in the order of extent which exercise group varied. Interestingly, 70 of 80 genes in those groups changed expression in the opposite directions to the sham-operated normal control.

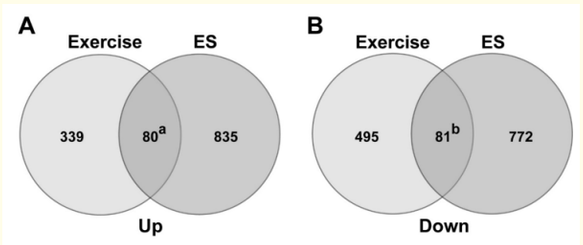

Figure 2: Venn Diagrams Based on Genes of the DNA Microarray.

a for a list of top40 genes see Table 2. b for a list of top40 genes see Table 3. (Left) The number of genes upregulated by exercise or electrical stimulation (ES) groups compared with ischemia (Isch) group [FC (fold change) > 1.5]. (Right) The number of genes downregulated by exercise or ES compared with Isch (FC < 1/1.5).

Real-time PCR analysis

Real-time PCR analysis was performed to confirm the results of the DNA-microarray. Genes shown in Table 2 and Table 3 were analyzed using individual mRNA samples of each group. The analysis revealed that *Dpp4, Nov*, and *Ptges* were significantly downregulated in both Isch + EX and Isch + ES groups compared with Isch group (Figure 3).

Discussion

In the present study, we have shown changes in a number of genes in skeletal muscles as a result of exercise and ES interventions, respectively. Among them, *Dpp4, Nov,* and *Ptges* were significantly decreased by both exercise and ES following hind-limb ischemia. To our knowledge, this is the first study that comparatively analyzed gene expression profiles following exercise- and ES-induced improvement in walking performance in a rat IC mod-

Probe Set ID	Gene Name	Gene symbol	Fold change					
			Isch		Isch+EX		Isch+ES	
			vs. Sham		vs. Isch		vs. Isch	
1393625_at	Intersectin 2	Itsn2	0.02		50.66		1.96	
1368734_at	Cholinergic receptor, nicotinic, delta	Chrnd	0.07		20.70		3.79	
1372865_at	Zinc finger protein 364	Zfp364	0.10		14.80		1.86	
1380236_at	Integrin alpha 9	Itga9	0.11		12.41		1.78	
1370165_at	Small muscle protein, X-linked	Smpx	0.17		12.05		2.25	
1386937_at	ATPase, Na+/K+ transporting, beta 1 polypeptide	Atp1b1	0.11		11.77		2.76	
1397867_at	A kinase (PRKA) anchor protein 6	Akap6	0.06		10.95		8.92	
1390937_at	Similar to chromosome 14 open reading frame 50	RGD1309051	0.11		10.51		1.92	
1389265_at	Glucan (1,4-alpha-), branching enzyme 1	Gbe1	0.10		9.80		3.31	
1389737_at	Dystrobrevin alpha	Dtna	0.13		8.05		2.60	
1387059_at	Serine/threonine kinase 39, STE20/SPS1 homolog (yeast)	Stk39	0.17		7.43		1.96	
1390830_at	Hypothetical protein LOC499602	LOC499602	0.24		7.13		1.56	
1377869_at	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	Ccrn4l	0.19		7.11		3.83	
1367660_at	Fatty acid binding protein 3, muscle and heart	Fabp3	0.17		6.96		1.66	
1379833_at	Leucine rich repeat and Ig domain containing 4 /// RAR-related orphan receptor C	Lingo4 /// Rorc	0.18		6.60		1.57	
1387036_at	Hairy and enhancer of split 1 (Drosophila)	Hes1	0.23		5.93		4.56	
1369843_at	Cholinergic receptor, nicotinic, alpha 1 (muscle)	Chrna1	0.25		5.86		3.43	
1398245_at	Synuclein, gamma (breast cancer-specific protein 1)	Sncg	0.25		5.70		2.44	
1378745_at	Period homolog 3 (Drosophila)	Per3	0.21		5.06		4.01	
1392952_at	Acyl-CoA synthetase family member 2	Acsf2	0.25		4.83		1.74	
1387795_at	Polymerase (DNA directed), alpha 2	Pola2	0.49		4.78		3.81	
1372595_at	Actinin alpha 2	Actn2	0.25		4.25		3.05	
1372107_at	Four and a half LIM domains 1	Fhl1	0.45		3.79		1.77	
1397536_at	Similar to KIAA0833 protein	Camta1	0.31		3.59		1.86	
1370856_at	Actin, alpha, cardiac muscle 1	Actc1	0.33		3.55		1.79	
1370390_at	Coronin 6	Coro6	0.92		3.52		4.23	
1369590_a_at	DNA-damage inducible transcript 3	Ddit3	0.38		3.45		2.26	
1381798_at	LIM domain 7	Lmo7	0.24		3.08		1.60	
1387874_at	D site of albumin promoter (albumin D-box) binding protein	Dbp	0.70		3.04		2.42	
1367576_at	Glutathione peroxidase 1	Gpx1	0.37		2.91		1.92	
1370026_at	Crystallin, alpha B	Cryab	0.35		2.82		1.58	
1398664_at	GRAM domain containing 3	Gramd3	0.29		2.76		2.46	
1388802_at	Brain expressed gene 1	Bex1	0.29		2.73		1.79	
1375941_at	BAI1-associated protein 2-like 1	Baiap2l1	0.33		2.62		2.69	
1372091_at	MID1 interacting protein 1 (gastrulation specific G12 homolog (zebrafish))	Mid1ip1	0.49		2.62		1.80	
1389622_at	Solute carrier family 25, member 13 (citrin)	Slc25a13	0.49		2.53		2.28	
1391030_at	Similar to KIAA0833 protein	Camta1	0.41		2.52		2.31	
1386965_at	Lipoprotein lipase	Lpl	0.44		2.49		1.52	
1370157_at	Phospholamban	Pln	0.61		2.46		1.53	
1372626_at	Tumor protein D52-like 1	Tpd52l1	0.63		2.40		1.94	

Table 2: Top 40 genes upregulated in Isch + EX and Isch +ES groups compared with Isch group.

The top 40 genes with altered expression from Isch + EX and Isch + ES groups are listed in the order of the extent of upregulation in Isch + EX group. The genes downregulated more than 1.5 times in Isch group compared with Sham group are shown in the red column. The genes upregulated more than 1.5 times in Isch + EX and Isch + ES groups compared with Isch group are shown in the green column.

Probe Set ID	Gene Name	Gene symbol	Fold change					
			Isch		Isch+EX		Isch+ES	
			vs. Sham		vs. Isch		vs. Isch	
1390119_at	Secreted frizzled-related protein 2	Sfrp2	7.43		0.09		0.30	
1396614_at	Secreted frizzled-related protein 2	Sfrp2	4.05		0.23		0.37	
1377086_at	C1q and tumor necrosis factor related protein 3	C1qtnf3	3.87		0.27		0.62	
1388116_at	Collagen, type I, alpha 1	Col1a1	3.26		0.29		0.58	
1373401_at	Tenascin C	Tnc	2.71		0.33		0.47	
1368172_a_at	Lysyl oxidase	Lox	3.00		0.34		0.63	
1384063_at	Collagen triple helix repeat containing 1	Cthrc1	3.3		0.34		0.60	
1378586_at	Cytokine inducible SH2-containing protein	Cish	1.19		0.35		0.30	
1368394_at	Secreted frizzled-related protein 4	Sfrp4	3.24		0.36		0.32	
1368171_at	Lysyl oxidase	Lox	3.42		0.37		0.59	
1368014_at	Prostaglandin E synthase	Ptges	1.88		0.41		0.50	
1371815_at	Microfibrillar-associated protein 2	Mfap2	2.35		0.43		0.67	
1371232_a_at	Versican	Vcan	1.94		0.43		0.64	
1398350_at	Hypothetical protein LOC100364588	LOC100364588	2.32		0.43		0.66	
1387947_at	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	Mafb	1.55		0.43		0.66	
1370382_at	RT1 class II, locus Db1	RT1-Db1	2.18		0.44		0.56	
1393129_at	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide III	P4ha3	2.67		0.46		0.40	
1388792_at	Growth arrest and DNA-damage-inducible, gamma	Gadd45g	0.93		0.47		0.53	
1384437_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	Smarca1	1.86		0.47		0.63	
1383708_at	Integrin, beta-like 1	Itgbl1	2.25		0.48		0.54	
1381572_at	Similar to RIKEN cDNA 1810065E05	RGD1565787	1.69		0.48		0.65	
1370042_at	Stathmin-like 2	Stmn2	1.89		0.48		0.54	
1370892_at	Complement component 4, gene 2 /// complement component 4B (Chido blood group)	C4-2 /// C4b	2.51		0.49		0.59	
1374616_at	Platelet-derived growth factor receptor-like	Pdgfrl	1.93		0.49		0.56	
1388265_x_at	versican	Vcan	1.42		0.49		0.52	
1370869_at	Branched chain aminotransferase 1, cytosolic	Bcat1	2.71		0.49		0.55	
1373148_at	Carboxypeptidase X (M14 family), member 2	Cpxm2	1.96		0.51		0.35	
1372646_at	Similar to RIKEN cDNA 1500015O10	RGD1305645	1.88		0.51		0.47	
1368490_at	CD14 molecule	Cd14	1.81		0.51		0.65	
1370384_a_at	Prolactin receptor	Prlr	1.84		0.51		0.57	
1368813_at	CCAAT/enhancer binding protein (C/EBP), delta	Cebpd	1.29		0.51		0.52	
1371754_at	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Slc25a25	0.69		0.52		0.40	
1368163_at	Dipeptidylpeptidase 4	Dpp4	1.36		0.52		0.40	
1392265_s_at	Matrix metallopeptidase 23	Mmp23	1.96		0.52		0.57	
1368237_at	Tenomodulin	Tnmd	2.5		0.52		0.36	
1369152_at	Protein phosphatase 3, regulatory subunit B, alpha isoform	Ppp3r1	1.32		0.53		0.40	

Table 3: Top 40 genes downregulated in Isch + EX and Isch +ES groups compared with Isch group.

The top 40 genes with altered expression from Isch + EX and Isch + ES groups are listed in the order of the extent of downregulation in Isch + EX group. The genes upregulated more than 1.5 times in Isch group compared with Sham group are shown in the green column. The genes downregulated more than 1.5 times in Isch + EX and Isch + ES groups compared with Isch group are shown in the red column.

Figure 3: Expression Analyses of Dpp4, Nov, and Ptges.

The relative amounts of Dpp4 (A), Nov (B), and Ptges (C) were normalized to reference gene (Rplp, 50S ribosomal protein L16). Values are presented as ratios of mean values of sham group. Values are presented as mean ± SE and statistical significance was determined using Tukey's test. *P < 0.05, **P < 0.01 vs. Isch group.

el. Our ES model is particularly useful for investigating the local mechanisms underlying the beneficial effects of exercise in the IC affected limbs. The genes identified in the current study might have contributed to the improvement in walking performance via effects on local limbs following exercise. Further analyses are underway to identify the connection between the gene expression changes and the functional outputs of the skeletal muscles.

To explore the genes that were locally affected in the ischemic limbs following exercise, we also extracted the genes that changed following ES. Intriguingly, when we examined the genes that were upregulated in both Isch + EX and Isch + ES groups compared with Isch group, we found they were downregulated by the ischemic condition, and vice versa (with the exception of some genes). These results suggest that skeletal muscle contraction itself might have the potential to restore the damage following ischemia. These changes could have contributed in the improvement in walking performance. The following three genes are especially worthy of discussion.

Dpp4

Dipeptidyl peptidase 4 (*Dpp4*) is found in many tissues and cells, and known to impair insulin signaling in fat and skeletal and smooth muscle cells [14]. In addition to its insulin regulation role, *Dpp4* is also implicated in angiogenesis. *Dpp4* silencing and Dpp4 inhibitors have been shown to inhibit apoptosis of endothelial cells under hypoxic condition and promoted angiogenesis [15-17]. In our previous study, we observed an increased capillary-to-fiber ratio in skeletal muscles with both exercise and ES, respectively. In relation to this finding, decreased expression of *Dpp4* might be involved in the increase in microvessels, thus contributing to improved walking ability. Although it has reported that *Dpp4* inhibitors are associated with a lower risk of peripheral arterial disease in patients with type 2 diabetes mellitus [18], to date, there is no study investigating whether *Dpp4* inhibitors directly affect walking ability in patients with IC or in an animal model of IC. Therefore, future research should investigate the impact of *Dpp4* inhibitors on walking ability in IC through the view of both insulin regulation and angiogenesis.

Nov

Nephroblastoma overexpressed gene (*NOV*) is a member of the CCN gene family and interacts with multiple cell surface receptors including Notch and integrins [19,20]. With targeted disruption of the *Nov* gene, it was shown that *NOV* is a regulator of skeletal muscle and cardiac development, and is implicated in various disease processes including cardiomyopathy and muscle atrophy [21]. Because *NOV* is upregulated upon hypoxic treatment, accompanied by stabilization of hypoxia-inducible factor-1α in extravillous trophoblast [22], IC might also upregulated *NOV* expression in skeletal muscle. In myogenic cells, elevated expression of *NOV* led to downregulation of MyoD and myogenin, resulting in inhibition of myotube formation [19,23]. In our study, both exercise and ES downregulated *NOV* expression. The results suggest that both the ES and exercise interventions may have promoted myogenic differentiation and mitigated skeletal muscle injury, leading to improvement in walking distance. On the other hand, it has also been reported that *NOV* is an angiogenic factor and supports wound healing [20,24]. This does not align with our results that showed that exercise and ES produced an increase in microvessels. Cell type-specific gene expression analyses might provide a better understanding and resolve these inconsistencies.

Ptges (mPGES-1)

Microsomal prostaglandin E synthase- 1 (mPGES-1) is an enzyme responsible for the conversion of prostaglandin H2, produced by cyclooxygenase (COX) enzymes, into the biologically active PGE2. mPGES-1 expression is induced under proinflammatory conditions similar to COX-2 expression [25]. Gene deletion studies have confirmed the involvement of mPGES-1 in various disease models such as collagen-induced arthritis, atherosclerosis, and stroke [26-28]. In a mice stroke model, it was suggested that induction of mPGES-1 together with COX-2 expression is required for post-ischemic PGE2 production, which causes inflammation and ischemia-induced neuronal death. Recently, mPGES-1 inhibitor

have been suggested as potential tools for the treatment of pain and inflammation [29,30]. Assuming that exercise and ES might have downregulated mPGES-1 expression and suppressed inflammation in the ischemic hind-limb, mPGES-1 inhibitors may have the potential to improve walking distance in the rat IC model. However, PGE2 and its receptors EP3, and EP4 have various roles other than inflammation, including myogenic differentiation and angiogenesis [31-33]. Further investigation on the role of mPGES-1 in ischemic hind-limb is needed.

Conclusion

In conclusion, the present study described a transcriptional response of skeletal muscle following exercise and ES which lead to an improvement in walking performance in a rat IC model. Our final goal is to determine the underlying mechanisms by which exercise improves walking ability in patients with IC, and to identify novel therapeutic targets. Further analyses of common gene expression patterns between exercise and ES, and characterization of affected genes would lead to the discovery of new therapeutic approaches for patients with IC.

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