Spinal Cord (2017) 55, 441–446

www.nature.com/sc

ORIGINAL ARTICLE

Molecular mechanisms underlying the positive role of treadmill training in locomotor recovery after spinal cord injury

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Objectives: This study aimed to investigate the molecular mechanisms underlying the positive role of treadmill training (TMT) in locomotor recovery.
Methods: GSE52763 microarray data were downloaded from GEO database, which was collected from the lumbar spinal cord samples of three groups of mice: mice subjected to contusive injury and killed 1 week after injury (I1), mice subjected to injury and killed 3 weeks after injury (I3), and mice subjected to injury and TMT beginning at week 1 and lasting until week 3 (T3). Differential expression analysis between I3 and I1, between T3 and I1 and between T3 and I3 were performed by *T*-test using R/LIMMA. Genes with $|\log_2 FC \text{ (fold change)}| > 0.58$ and *P*-value < 0.05 were considered as differentially expressed genes (DEGs). Specific I3 vs I1 DEGs and T3 vs I1 DEGs were screened. Then TMT-induced specific DEGs were subject to functional and pathway enrichment analysis using DAVID online tool. Protein–protein interaction (PPI) analysis was also carried out using the STRING database.
Results: Finally, 82 upregulated DEGs and 297 downregulated DEGs were found specifically induced by TMT. Specific upregulated DEGs were mostly enriched in response to organic substance and morphogenesis-related events, and specific downregulated DEGs were related to positive regulation of transcription. *ATP2A1*, *PRKACA*, *ITPR2* and so on had high connection degree in the PPI network of the specific upregulated DEGs; *FOS*, *GSK3B* and so on had high degrees in the PPI network of the specific downregulated DEGs.
Conclusion: *ATP2A1*, *C-FOS* and *GSK3B* may have critical roles in the positive role of TMT in locomotor recovery.
Spinal Cord (2017) **55,** 441–446; doi:10.1038/sc.2016.134; published online 6 December 2016

INTRODUCTION

Spinal cord injury (SCI) refers to any injury to the spinal cord that can cause sensory and motor dysfunctions, with symptoms varying from pain to paralysis even to incontinence. Aside from lifetime deficits in locomotion, patients with SCI also have to bear huge financial burden.[1] However, no satisfactory strategies for locomotor recovery have been presented so far.

Physical exercise has been applied for the recovery of motor function after SCI. Forssberg *et al.*[2] first reported that the treadmill training (TMT) notably promoted the locomotor recovery in spinalized cats following SCI. Rossignol and collaborators have undertook many studies on TMT, especially the kinematics and the electromyographic activity during treadmill locomotion, mainly in cats.[3–5] In a systematic review of exercise applied to locomotor recovery, Battistuzzo *et al.*[6] have demonstrated that TMT has superior performance in locomotor recovery as compared with other exercise types (body weight-supported TMT, voluntary wheel running and swimming) in different animal models of SCI, with positive outcomes in 100% of both rats and cats. However, the molecular mechanisms underlying the positive role of TMT in locomotor recovery have not been well elucidated.

Shin *et al.*[7] investigated the mechanisms underlying the positive role of TMT in locomotor recovery using microarrays. Based on the genes differentially expressed at either 1 or 3 weeks after SCI, they found that the most robust upregulation was observed in genes related to immune or inflammation responses; more strikingly, the elevated expression of many inflammation-related genes was sustained in mice subjected to TMT and TMT upregulated the expression of neuroplasticity- and angiogenesis-related genes that were downregulated after SCI.[7] Herein, using the microarray data submitted by Shin *et al.*,[7] we attempted to further investigate the molecular mechanisms underlying the positive role of TMT in locomotor recovery from a protein–protein interaction (PPI) perspective.

MATERIALS AND METHODS

Source of microarray data

The GSE52763 microarray data were downloaded from GEO database (GEO, http://www.ncbi.nlm.nih.gov/geo/). This data set was collected from lumbar spinal cord samples of three groups of mice: mice subjected to contusive injury and killed 1 week after injury ($n = 4$, I1), mice subjected to contusive injury and killed 3 weeks after injury ($n = 4$, I3) and mice subjected to contusive injury and TMT beginning at week 1 and lasting until week 3 after injury (TMT of 2-week duration) ($n = 4$, T3).

As described by Shin *et al.*[7] in their original study, rats were first subjected to a dorsal laminectomy at the ninth thoracic vertebral level (T9) to expose the spinal cord and then contusion injury was performed by mechanical impact with a force of 200 kdyn. The rats were able to walk without auxiliary support at a slow speed ($5\ m\ min^{-1}$) prior to TMT. TMT was performed in the Flat Treadmill System (Model, IW-FT; IWOO Scientific Corporation, Seoul, Korea) for rats in combination with electric stimulation. Electric shocks of 1.2 mA were applied when an animal was not walking and displaced to the end

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Received 5 May 2016; revised 6 July 2016; accepted 20 July 2016; published online 6 December 2016

of its runway, which were required several times on the first day of training, but were rarely applied after a few days. As locomotion was improved, the belt speed was gradually increased to 12 m min^{-1} during the first week and maintained at this level until the end of training. TMT was performed daily for 14 days, with each session lasting 30 min. Locomotor recovery was assessed using Basso, Beattie and Bresnahan test. Please refer to the original study[7] for details.

Identification of differentially expressed genes (DEGs) and specific DEGs

The downloaded data were first subject to background correction, quantile normalization and probe summarization by the RMA (robust multiarray average) method[8] using R/Affy package.[9] Next, differential expression analysis between I3 and I1, between T3 and I1 and between T3 and I3 was performed by *T*-test using the R/LIMMA (linear models for microarray data) package.[10] Genes with $|\log_2$FC (fold change)$| > 0.58$ and *P*-value < 0.05 were considered as DEGs. DEGs between I3 and I1 (I3 vs I1 DEGs) and DEGs between T3 and I1 (T3 vs I1 DEGs) were further compared by screening the common ones. Specific I3 vs I1 DEGs or specific T3 vs I1 DEGs were further obtained via subtracting the common DEGs, respectively. The results were visualized using Venn plots.[11]

Functional annotation and pathway enrichment analysis of specific DEGs

DAVID (Database for Annotation, Visualization and Integrated Discovery) is an online tool integrating a comprehensive set of functional annotation tools that allows researchers to systematically extract the biological meaning from large gene/protein lists.[12] The specific DEGs were submitted to DAVID for functional and pathway enrichment analysis based on Gene Ontology (GO)[13] and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway databases ($P < 0.05$).[14]

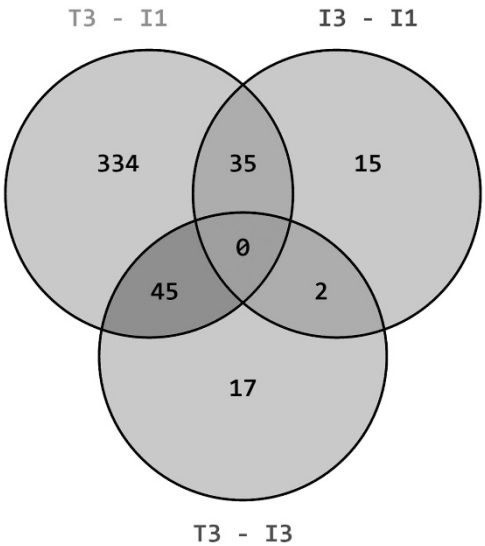

Figure 1 Comparison between T3 vs I1, I3 vs I1 and T3 vs I3 DEGs by Venn plot. T3 vs I1 DEGs represents DEGs between mice subjected to contusive injury and killed 1 week after injury (I1) and mice subjected to contusive injury and TMT beginning at week 1 and lasting until week 3 after injury (T3); I3 vs I1 DEGs represents DEGs between mice subjected to contusive injury and killed 3 weeks after injury (I3) and mice subjected to contusive injury and TMT beginning at week 1 and lasting until week 3 after injury (T3); T3 vs I3 DEGs represents DEGs between mice subjected to contusive injury and killed 3 weeks after injury ($n = 4$, I3) and mice subjected to contusive injury and TMT beginning at week 1 and lasting until week 3 after injury (T3).

Construction of PPI network

To better understand the specific DEGs from an interactive respective, we used STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) online tool to construct an interaction network of their protein products.[15] Combined score > 0.4 was set as the cutoff for PPI. The connection degree of each protein in the network was also calculated. The resulting network was visualized using the Cytoscape software (version 2.8.3, National Institute of General Medical Sciences (NIGMS), Bethesda, MD, USA).[16]

RESULTS

TMT improved locomotor recovery after thoracic SCI

As described by Shin *et al.*,[7] rats exhibited enhanced locomotor recovery and higher-quality overground locomotion as they were subjected to TMT. The locomotor improvement induced by TMT was statistically significant compared with control rats.

Screening of DEGs and specific DEGs

Finally, 16 upregulated DEGs and 36 downregulated DEGs were identified between the I3 and I1 groups (I3vs I1 DEGs), 89 upregulated and 325 downregulated between the I1 and T3 groups (T3vs I1 DEGs) and 12 upregulated and 52 downregulated between the T3 and I3 groups (T3 vs I3 DEGs), indicating that TMT has induced alteration in the expression of a substantially larger number of genes. The Venn plot revealed 35 common DEGs between T3 vs I1 DEGs and I3 vs I1 DEGs, among which 7 were upregulated and 28 were downregulated (Figure 1). Finally, 82 upregulated T3 vs I1 DEGs and 297 downregulated T3 vs I1 DEGs were found specific, and 9 upregulated I3 vs I1 DEGs and 8 downregulated I3 vs I1 DEGs were found specific (Table 1).

GO functional annotation and KEGG enrichment analysis of the specific upregulated genes and downregulated regulated genes

According to GO functional annotation, the specific upregulated DEGs were mostly enriched in biological processes related to response to organic substance (vitamin, vitamin A, lipopolysaccharide, nutrient) and endogenous/extracellular stimulus, as well as morphogenesis-related events (Table 2). According to the KEGG pathway enrichment analysis, three DEGs *IGF1*, *PRKACA* and *ITPR2* were significantly enriched in the pathway oocyte meiosis (rno04114).

Meanwhile, GO functional annotation revealed that the specific downregulated T3 vs I1 DEGs were related to positive regulation of transcription (Table 3). And some of the downregulated DEGs were enriched in two KEGG pathways: rno05200:Pathways in cancer (*FOS*, *MAX*, *BCR*, *PTGS2*, *MMP9*, *GSK3B*, *CBL* and *RUNX1T1*) and rno04660:T-cell receptor signaling pathway (*FOS*, *GSK3B*, *CBL*, *MAP3K8* and *NFAT5*), according to the pathway enrichment analysis.

Construction of PPI network and module analysis

The PPI network of the specific upregulated T3 vs I1 DEGs includes 31 interaction pairs (Figure 2), and the top eight proteins with the highest connection degree were ATP2A1 (degree = 6),

Table 1 Specific differential genes in each group

	Up	*Down*	*Total*
T3 vs I1 DEGs	82	297	379
I3 vs I1 DEGs	9	8	17
Common	7	28	35

Abbreviation: DEG, differentially expressed gene.

Table 2 Gene Ontology (GO) analysis of the specific upregulated differentially expressed genes

GO biological process term	*Count*	P-*value*	*Differentially expressed genes*
GO:0010033—response to organic substance	9	4.11E-05	CD44, CYP1A1, LDLR, RXRA, ATP2A1, IGF1, PRKACA, CREB3L3, NR1H4
GO:0048729—tissue morphogenesis	5	3.76E-04	NOTCH2, CD44, RXRA, IGF1, PRKACA
GO:0009611—response to wounding	6	4.01E-04	NOTCH3, NOTCH2, CD44, CYP1A1, RXRA, IGF1
GO:0031099—regeneration	4	7.64E-04	NOTCH3, NOTCH2, RXRA, IGF1
GO:0030850—prostate gland development	3	1.51E-03	CD44, RXRA, IGF1
GO:0001655—urogenital system development	4	1.72E-03	CD44, RXRA, MYO1E, IGF1
GO:0042246—tissue regeneration	3	2.22E-03	NOTCH3, NOTCH2, IGF1
GO:0048565—gut development	3	2.32E-03	CYP1A1, RXRA, NR1H4
GO:0042060—wound healing	4	2.40E-03	NOTCH3, NOTCH2, CD44, IGF1
GO:0014070—response to organic cyclic substance	4	4.13E-03	CD44, CYP1A1, RXRA, IGF1

Table 3 Gene Ontology analysis of the specific downregulated differentially expressed genes

GO biological process term	*Count*	P-*value*	*Differentially expressed genes*
GO:0045944—positive regulation of transcription from RNA polymerase II promoter	14	1.97E-05	CEBPB, KLF13, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, KLF4, NFIB, MED1
GO:0045893—positive regulation of transcription, DNA dependent	15	2.74E-05	CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, KLF4, NFIB, MED1
GO:0051254—positive regulation of RNA metabolic process	15	3.02E-05	CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, KLF4, NFIB, MED1
GO:0045941—positive regulation of transcription	16	3.89E-05	CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, CCNA2, KLF4, MED1, NFIB
GO:0010628—positive regulation of gene expression	16	5.12E-05	CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, CCNA2, KLF4, MED1, NFIB
GO:0010557—positive regulation of macromolecule biosynthetic process	17	6.77E-05	UTS2, CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, CCNA2, KLF4, MED1, NFIB
GO:0006357—regulation of transcription from RNA polymerase II promoter	16	1.55E-04	CEBPB, KLF13, NFIX, SIX4, MED21, MED13L, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, NCOR1, KLF4, MED1, NFIB
GO:0009891—positive regulation of biosynthetic process	17	1.57E-04	UTS2, CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, CCNA2, KLF4, MED1, NFIB
GO:0045935—positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	16	1.67E-04	CEBPB, KLF13, ILF3, NFIX, SIX4, MED21, HOXD10, FOS, ZFP462, BCL11B, NFAT5, TCF4, CCNA2, KLF4, MED1, NFIB
GO:0006355—regulation of transcription, DNA dependent	24	1.75E-04	MEF2A, CEBPB, ARID4A, KLF13, RUNX1T1, ILF3, NFIX, SIX4, MED21, MED13L, HOXD10, MAX, FOS, ZFP462, TRPS1, GSK3B, BCL11B, NFAT5, TCF4, NCOR1, KLF4, MLLT3, MED1, NFIB

Abbreviation: GO, Gene Ontology.

MYOZ1 (degree = 6), TNNI2 (degree = 6), PRKACA (degree = 5), ACTN3 (degree = 5), CKM (degree = 4), MYH4 (degree = 5) and ITPR2 (degree = 2).

The PPI network of the specific downregulated T3 vs I1 DEGs comprises 335 interaction pairs (Figure 3), and the top eight proteins with the highest connection degree to other proteins were FOS (degree = 22), ASHLL (degree = 21), MEF2A (degree = 20), GSK3B (degree = 13), DDX6 (degree = 22), CSNK2A1 (degree = 20), ZC3H13 (degree = 14) and ESF1 (degree = 13).

DISCUSSION

Using the microarray data submitted by Shin *et al.*,[7] we focused on genes whose differential expression was specifically induced by TMT and attempted to elucidate their roles in TMT-induced locomotor recovery from a PPI perspective in the present study. It was found that a notably larger number of genes showed differential expression in mice subject to TMT compared with those that were not subjected to; furthermore, the upregulated specific DEGs in mice undergoing TMT are functionally related in response to organic substance (vitamin, vitamin A, lipopolyaccharide, nutrient) and endogenous/extracellular stimulus, as well as morphogenesis-related events, and the specific downregulated T3 vs I1 DEGs were mainly related to positive regulation of transcription.

In the present study, the upregulated specific DEGs in mice undergoing TMT are functionally related in response to organic substance (vitamin, vitamin A, lipopolyaccharide, nutrient) and endogenous/extracellular stimulus, as well as morphogenesis-related events. This is consistent with a previous finding that the expression of neuroplasticity- and angiogenesis-related genes was upregulated by TMT,[7] implying that the positive role of TMT in locomotor recovery may be related to its beneficial effect on neuronal regeneration. Among these genes, *ATP2A1* encodes an intracellular calcium pump ATPase 1, which is located in the sarcoplasmic or endoplasmic reticula of muscle cells. This protein is involved in muscular excitation and contraction, and mutation in human *ATP2A1* gene has been reported to cause Brody disease, a rare disorder characterized by impaired

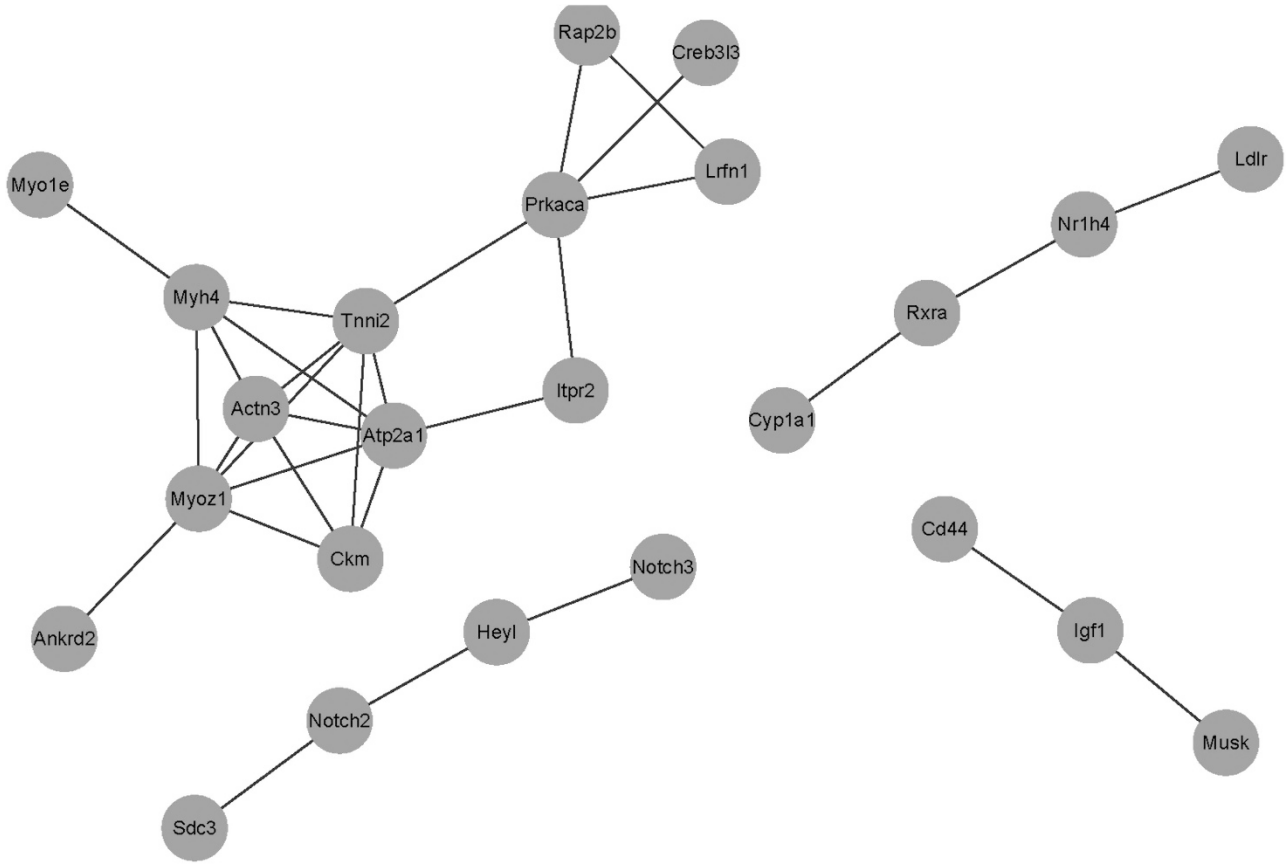

Figure 2 The PPI network of the specific upregulated T3 vs I1 DEGs. T3 vs I1 DEGs represents DEGs between mice subjected to contusive injury and killed 1 week after injury (I1) and mice subjected to contusive injury and TMT beginning at week 1 and lasting until week 3 after injury (T3). A full color version of this figure is available at the *Spinal Cord* journal online.

skeletal muscle relaxation.[17] This gene was expressed in neural stem cells.[18] Its upregulation in mice subject to TMT indicates that it may have a positive role in locomotor recovery following TMT. Additionally, we also observed the upregulated expression in another two genes *PRKACA* and *ITPR2. PRKACA* encodes the catalytic subunit α of a cAMP-dependent protein kinase A that can activate substrate via phosphorylation at serine or threonine residue.[19] Meyer *et al.*[20] have reported a noted increase in its expression within the anterior horn of murine lumbar spinal cord and presented that this gene is closely associated with motor neuron disease. *ITPR2* encodes inositol 1,4,5-trisphosphate receptor, type 2, a calcium channel expressed in the endoplasmic reticulum,[21] which is one of the main regulators of intracellular calcium concentrations in neurons.[22] The overexpression of this gene was said to be responsible for the apoptosis of motor neurons.[23,24] The upregulation of these two proteins may suggest that the apoptosis of motor neurons continues in mice with TMT, which seemingly conforms to the finding that the inflammation was not weakened in mice subjected to TMT.[7]

Among the specific downregulated TMT-induced genes, *FOS* encodes C-FOS transcription factor protein that can form heterodimer activator protein 1 with C-JUN (a JUN family of transcription factors)[25,26] and activator protein 1 regulates gene expression in a wide range of biological events.[27] *C-FOS* has been suggested as a marker for neuronal activity.[28,29] Previous studies have consistently reported the increase in these two proteins in the spinal dorsal horn shortly (around a week) following SCI.[30,31] However, the dynamics and site of its long-term expression remain controversial,[32–34] indicating a complex role of this protein after SCI. Here mice subject to TMT showed a decrease in C-FOS level 3 weeks after SCI, supporting the view that this immediate early protein has been downregulated after 2 weeks of exercise; although differential expression was not observed in C-JUN. Another downregulated gene *GSK3B* encodes GSK3β, one of the two glycogen synthase kinase-3 isoforms which inhibits axon growth.[35] However, the role of this kinase in neural development may be complex. Inhibition of GSK3β can promote the regeneration of CNS axons in SCI animal model[36] while robust inhibition of this enzyme may impair axon regeneration.[37] Furthermore, Parkitna *et al.*[38] have reported that intrathecal injection of GSK3β inhibitor can restore the analgesic effect of morphine in morphine-tolerant rats, thus GSK3β inhibition is supposed to alleviate neuropathic pain.[39] Here GSK3β downregulation was observed in mice subject to TMT, implying that TMT can reduce GSK3β expression, which may not only benefit neuronal regeneration but also ameliorate the neuropathic pain. In the present study, both C-FOS and GSK3β were significantly enriched in the two pathways: pathways in cancer and T-cell receptor signaling pathway. This suggests that TMT may benefit locomotor recovery via reducing the expression of some key genes involved in these two pathways.

Taken together, the mechanisms underlying TMT's positive role in locomotor recovery may be complex. TMT seems to benefit neuronal regeneration by regulating related genes, especially *ATP2A1*, *C-FOS* and *GSK3B*; the proteins encoded by these genes were speculated to

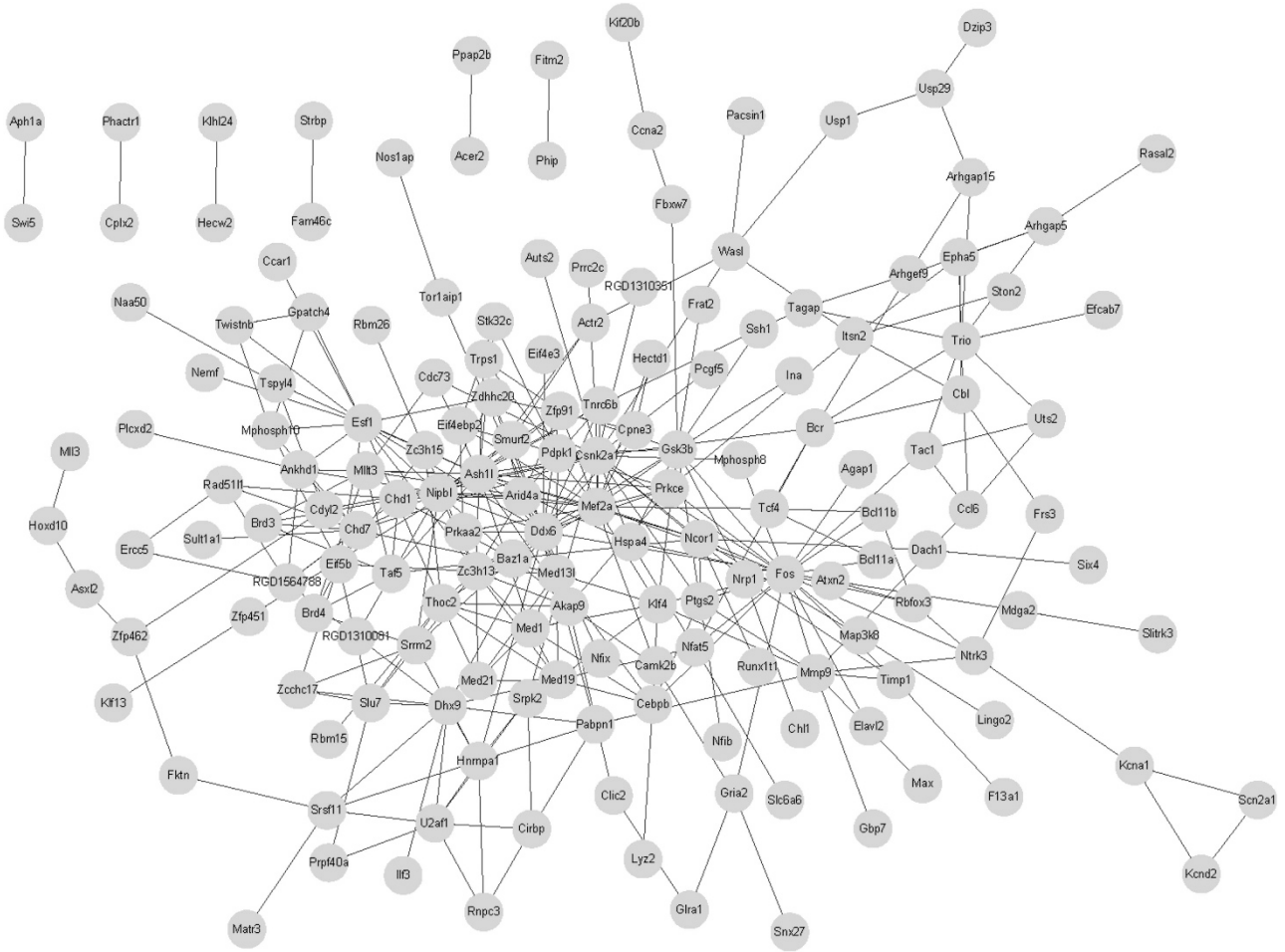

Figure 3 The PPI network of the specific downregulated T3 vs I1 DEGs. T3 vs I1 DEGs represents DEGs between mice subjected to contusive injury and killed 1 week after injury (I1) and mice subjected to contusive injury and TMT beginning at week 1 and lasting until week 3 after injury (T3). A full color version of this figure is available at the *Spinal Cord* journal online.

interact with many other specific proteins according to the PPI analysis, indicating their critical roles in locomotor recovery following TMT. Nevertheless, TMT seems not to change some adverse events following SCI in mice, such as apoptosis of motor neurons. However, our findings need to be validated by further experimental proofs and thus should be taken prudently at present.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Oliveri RS, Bello S, Biering-Sørensen F. Mesenchymal stem cells improve locomotor recovery in traumatic spinal cord injury: systematic review with meta-analyses of rat models. *Neurobiol Dis* 2014; **62**: 338–353.
2 Forssberg H, Grillner S, Halbertsma J. The locomotion of the low spinal cat I. Coordination within a hindlimb. *Acta physiol Scand* 1980; **108**: 269–281.
3 Belanger M, Drew T, Provencher J, Rossignol S. A comparison of treadmill locomotion in adult cats before and after spinal transection. *J Neurophysiol* 1996; **76**: 471–491.
4 Leblond H, L'Espérance M, Orsal D, Rossignol S. Treadmill locomotion in the intact and spinal mouse. *J Neurosci* 2003; **23**: 11411–11419.
5 Barbeau H, Rossignol S. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res* 1987; **412**: 84–95.
6 Battistuzzo CR, Callister RJ, Callister R, Galea MP. A systematic review of exercise training to promote locomotor recovery in animal models of spinal cord injury. *J Neurotrauma* 2012; **29**: 1600–1613.
7 Shin HY, Kim H, Kwon MJ, Hwang DH, Lee K, Kim BG. Molecular and cellular changes in the lumbar spinal cord following thoracic injury: regulation by treadmill locomotor training. *PLoS ONE* 2014; **9**: e88215.
8 Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U *et al*. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003; **4**: 249–264.
9 Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy–analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 2004; **20**: 307–315.
10 Diboun I, Wernisch L, Orengo CA, Koltzenburg M. Microarray analysis after RNA amplification can detect pronounced differences in gene expression using LIMMA. *BMC Genomics* 2006; **7**: 252.
11 Oliveros JC. An interactive tool for comparing lists with Venn Diagrams. VENNY, 2007. http://bioinfogp.cnb.csic.es/tools/venny/index.html.
12 Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2008; **4**: 44–57.
13 Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM *et al*. Gene Ontology: tool for the unification of biology. *Nat Genet* 2000; **25**: 25–29.
14 Aoki-Kinoshita KF, Kanehisa M. Gene annotation and pathway mapping in KEGG. *Methods Mol Biol*. 2007; **396**: 71–91.
15 Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A *et al*. STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 2013; **41**: D808–D815.

16 Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003; **13**: 2498–2504.
17 Odermatt A, Taschner PE, Khanna VK, Busch HF, Karpati G, Jablecki CK *et al.* Mutations in the gene–encoding SERCA1, the fast–twitch skeletal muscle sarcoplasmic reticulum Ca2+ ATPase, are associated with Brody disease. *Nat Genet* 1996; **14**: 191–194.
18 Cai J, Cheng A, Luo Y, Lu C, Mattson MP, Rao MS *et al.* Membrane properties of rat embryonic multipotent neural stem cells. *J Neurochem* 2004; **88**: 212–226.
19 Banday AR, Azim S, Hussain MA, Nehar S, Tabish M. Computational prediction and characterisation of ubiquitously expressed new splice variant of Prkaca gene in mouse. *Cell Biol Int* 2013; **37**: 687–693.
20 Meyer MA. Identification of 17 highly expressed genes within mouse lumbar spinal cord anterior horn region from an in-situ hybridization atlas of 3430 genes: implications for motor neuron disease. *Neurol Int* 2014; **6**: 5367.
21 Yamamoto-Hino M, Sugiyama T, Hikichi K, Mattei M, Hasegawa K, Sekine S *et al.* Cloning and characterization of human type 2 and type 3 inositol 1, 4, 5-trisphosphate receptors. *Receptors Channels* 1993; **2**: 9–22.
22 Mikoshiba K. Inositol 1, 4, 5-trisphosphate (IP3) receptors and their role in neuronal cell function. *J Neurochem* 2006; **97**: 1627–1633.
23 Van Den Bosch L, Verhoeven K, De Smedt H, Wuytack F, Missiaen L, Robberecht W. Calcium handling proteins in isolated spinal motoneurons. *Life Sci* 1999; **65**: 1597–1606.
24 Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* 2001; **2**: 806–819.
25 Kerppola T, Curran T. Selective DNA bending by a variety of bZIP proteins. *Mol Cell Biol* 1993; **13**: 5479–5489.
26 Karin M, Liu Z-g, Zandi E. AP-1 function and regulation. *Curr Opin Cell Biol* 1997; **9**: 240–246.
27 Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 2002; **4**: E131–E136.
28 Dragunow M, Faull R. The use of c-fos as a metabolic marker in neuronal pathway tracing. *J Neurosci Methods* 1989; **29**: 261–265.
29 Bullitt E. Expression of C-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. *J Comp Neurol* 1990; **296**: 517–530.
30 Munglani R, Hunt SP. Proto-oncogenes: basic concepts and stimulation induced changes in the spinal cord. *Prog Brain Res* 1995; **104**: 283–298.
31 Morgan JI, Curran T. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annu Rev Neurosci* 1991; **14**: 421–451.
32 Hudspith M, Harrisson S, Smith G, Bountra C, Elliot P, Birch P *et al.* Effect of post-injury NMDA antagonist treatment on long-term Fos expression and hyperalgesia in a model of chronic neuropathic pain. *Brain Res* 1999; **822**: 220–227.
33 Catheline G, Le Guen S, Honoré P, Besson J-M. Are there long-term changes in the basal or evoked Fos expression in the dorsal horn of the spinal cord of the mononeuropathic rat? *Pain* 1999; **80**: 347–357.
34 Kajander KC, Madsen AM, Iadarola MJ, Draisci G, Wakisaka S. Fos-like immunoreactivity increases in the lumbar spinal cord following a chronic constriction injury to the sciatic nerve of rat. *Neurosci Lett* 1996; **206**: 9–12.
35 Seira O, Gavín R, Gil V, Llorens F, Rangel A, Soriano E *et al.* Neurites regrowth of cortical neurons by GSK3β inhibition independently of Nogo receptor 1. *J Neurochem* 2010; **113**: 1644–1658.
36 Dill J, Wang H, Zhou F, Li S. Inactivation of glycogen synthase kinase 3 promotes axonal growth and recovery in the CNS. *J Neurosci* 2008; **28**: 8914–8928.
37 Alabed YZ, Pool M, Tone SO, Sutherland C, Fournier AE. GSK3β regulates myelin-dependent axon outgrowth inhibition through CRMP4. *J Neurosci* 2010; **30**: 5635–5643.
38 Parkitna JR, Obara I, Wawrzczak-Bargiela A, Makuch W, Przewlocka B, Przewlocki R. Effects of glycogen synthase kinase 3β and cyclin-dependent kinase 5 inhibitors on morphine-induced analgesia and tolerance in rats. *J Pharmacol Exp Ther* 2006; **319**: 832–839.
39 Yang M, Li J, So K, Chen J, Cheng W, Wu J *et al.* Efficacy and safety of lithium carbonate treatment of chronic spinal cord injuries: a double-blind, randomized, placebo-controlled clinical trial. *Spinal Cord* 2012; **50**: 141–146.