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Research Paper

cDNA microarray analysis of spinal cord injury and regeneration related genes in rat

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Abstract: The acute traumatic spinal cord injury (SCI) is a commonly seen and severe case in clinic. However, the repair and regeneration of injured spinal cord is limited. This is likely due to that different kinds of factors are involved in regeneration after SCI. In the present study, we used complementary DNA microarray consisting of 4 041 specific probes from rat to identify genes that were differentially expressed after SCI. The animals were subjected to complete transection injury of the thoracic spinal cord (T8-T9). Sham operated animals received only a laminectomy. Four and a half days later, rat spinal cord was dissected out for total RNA isolation. The fluorescent (Cy3 and Cy5) labeled probes were prepared and hybridized to the microarray. Genes that showed 2-fold difference in SCI tissue were identified. Sixty-five up-regulated genes consisted of 21 known genes, 30 known expressed sequence tags (ESTs) and 14 unknown genes. Seventy-nine down-regulated genes comprised 20 known genes, 42 known ESTs and 17 unknown genes. In 41 differentially expressed known genes, 5 up-regulated genes, i.e., tissue inhibitor of metalloproteinase 1 (Timp1), transgelin (Tagln), vimentin (Vim), Fc gamma receptor, cathepsin S (Ctss), and 3 down-regulated genes, i.e., stearyl-CoA desaturase, coagulation factor II (F2), endosulfin alpha (Ensa), were further confirmed by reverse transcription polymerase chain reaction (RT-PCR). These genes may play a role in the response to tissue damage or repair following SCI and characterization of them might be helpful to elucidate the molecular mechanisms of spinal cord injury and regeneration.

Key words: spinal cord; gene expression; cDNA microarray; RT-PCR

应用 cDNA 微阵列技术筛选大鼠脊髓损伤修复相关基因

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摘　要：脊髓损伤是一类常见的、高致残率的中枢神经系统疾病，由于多种复杂因素影响其损伤后的修复过程，损伤脊髓的再生能力非常有限。本研究采用 cDNA 微阵列技术筛选大鼠脊髓损伤后出现的差异表达基因。实验组动物在 T8-T9 进行脊髓全横断手术，对照组动物只打开椎板；4.5 d 后取脊髓进行 RNA 提取并在反转录过程中进行 Cy3/Cy5 标记，然后与预制的、带有 4 041 条特异性探针的芯片进行杂交。Cy5/Cy3 信号比值≥ 2.0 视为脊髓损伤后出现差异表达的基因。通过筛选，我们得到了 65 个上调表达基因(21 个已知基因，30 个已知 EST 和 14 个未知基因)和 79 个下调基因(20 个已知基因，42 个已知 EST 和 17 个未知基因)。进一步通过半定量 RT-PCR 对其中的 5 个上调已知基因(Timp1, Tagln, Vim, Fc gamma receptor, Ctss)和三个下调已知基因(stearyl-CoA desaturase, F2, Ensa)的表达情况进行了验证，结果显示与芯片结果一致。这些基因可能在脊髓损伤后的修复过程中起一定的作用，对其深入研究将有助于揭示脊髓损伤修复的分子机制。

关键词：脊髓；基因表达；cDNA 微阵列；反转录 PCR
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Acute traumatic spinal cord injury (SCI) is an unexpected, devastating event, the consequences of which often persist for the life of the patients and influence in diverse ways not only the patients, but also family members and society

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at large. In most countries, acute SCI occurs at an annual rate of 20~40 persons per million[1]. Although prevention programs have been initiated, there is no evidence that the incidence is declining. The initial costs of hospitalization are high, and subsequent medical care is expensive. Thus, the development of effective treatments for SCI will have great benefits. The trauma to the spinal cord can result in the destruction of neurons, nerve fibers passing through the spinal cord, supporting glial cells and blood vessels, at the site of injury. At the site of mechanical insult, approximately 30% of the neurofilament proteins were degraded within 1 h, and 70% of them were lost within 4 h after injury. At the same time, the trauma to the spinal cord gives rise to changes of many genes' expression level. These altered genes may lead to damage to the injured spinal cord or lack of regeneration in the injured spinal cord and permanent functional impairment. SCI induced by trauma is a consequence of an initial physical insult and a subsequent progressive injury process that involves various pathochemical events leading to tissue destruction. The response to SCI is known to occur in two phases. The first phase, the primary injury, is the mechanical trauma initially sustained. The second phase, termed secondary injury, is posttraumatic tissue damage. The neurological deficits caused by secondary injury occur within minutes of the injury and continue for days, weeks or longer[2]. The second phase is an autodestructive process that includes physiological, biological and metabolic changes. These changes result in axonal damage and cell loss. So, the second phase should be a target of pharmacological treatment. But despite intensive treatment efforts, the prognosis in acute SCI remains a pessimistic one. The injured spinal cord fails to regenerate due to two reasons, lack of regeneration promoters and production of regeneration inhibitors. The injured mammalian neurons can successfully regenerate in the peripheral nervous system (PNS). This regenerative capacity has been attributed to production of a variety of secreted polypeptides known as neurotrophic factors during the regeneration process[3]. In 2000, scientists found that Nogo protein may inhibit axon regeneration[4]. In addition, it has become increasingly recognized that the nervous system also initiates reactive process in response to trauma that are neuroprotective and regenerative[5]. All these responses to SCI are, in part, due to changes at the gene expression level.

To further understand the molecular regulation of complex process of injury and regeneration after SCI, many genes have been isolated and extensively studied with conventional methods. Previous studies identified several SCI-related gene expression and protein changes using *in situ* hybridization, RT-PCR, Western blotting and immunohistochemistry[6-11]. However, these techniques are slow and laborious thereby enabling only a few genes to be investigated at a time. In order to identify more genes at the same time, many new technologies have been developed in recent years. GeneChip® is one of the newest technologies. It provides insights into changes of global gene expression at a time. GeneChip® or microarrays are constructed either with thousands of immobilized cDNAs or with photochemically synthesized oligonucleotides within 1 cm^2 on a glass or a plastic slide[12]. The development of GeneChip® and related techniques has enabled researchers to profile the expression of thousands of genes simultaneously, providing an effective tool for large-scale gene expression studies. Song *et al*. analyzed changes in the mRNA abundance at 3 and 24 h after SCI in adult rats using GeneChip®[13]. Fan *et al*. identified and compared the genes that were differentially expressed in the periphery nervous systems and central nervous systems following injury by microarray techniques[14]. So microarray analysis is a powerful tool to quickly examine expression of thousands of genes at a time.

In the present study, we tried to analyze the changes in mRNA abundance at 4.5 d after traumatic injury to rat spinal cord by microarray techniques. Our results demonstrate that SCI causes a significant change in the expression of genes, the products of which are involved in signal transduction, inflammation, apoptosis, lipid metabolism, acute phase reaction, remodeling injured tissue. Changes observed by microarray analysis for representative genes were also confirmed by RT-PCR.

1 MATERIALS AND METHODS

1.1 *Animals*

Six adult littermate male Wistar rats, bought from the Experiment Animal Center of Academy of Military Medical Sciences, were used in this investigation. The animals, weighing 200~300 g, were divided randomly into two groups, injured and control. All rats were anesthetized with intraperitoneal injections of sodium pentobarbital (40 mg/kg). The skin was surgically scrubbed with ethanol and the hair at the surgical field was removed. The rats in injured group were subjected to a complete transection injury of the thoracic spinal cord (T8-T9). The control group received only a laminectomy. The animals, survived for another 4.5 d, were killed by decapitation. The whole spinal cord of each rat was dissected out for total RNA

extraction.

1.2 *Total RNA extraction*

We used RNeasy Mini Kit (Qiagen, Germany) to extract total RNA. The spinal cords were homogenized using a conventional rotor-stator homogenizer. Then total RNA was extracted according to the protocol described by the manufacturer. The purity of the isolated total RNA was analyzed by absorption at 260 nm and 280 nm with ultraviolet spectrophotometry Unicam 330. The integrity of the total RNA was assessed with TAE agarose gel electrophoresis.

1.3 *Preparation of probes*

Total RNA (30 μg) of each group was converted to cDNA probes by reverse transcription with oligodT as primer. The cDNA probes were labeled with Cy3-dCTP for control group and Cy5-dCTP for injured group, respectively. Two kinds of cDNA probes were mixed up and dissolved in the hybridization solution.

1.4 *Hybridization and washing*

The cDNA microarray including 4 041 spots was obtained from the BioStar Genechip, Inc., Shanghai. The 4 041 rat specific probes on the cDNA microarray were obtained by subtraction hybridization between rat tissues. The probes were denatured at 95ºC for 2 min and chilled on the ice quickly. The cDNA microarray (prehybridization processed) were denatured in 95ºC water for 30 s and then put into water-free ethanol. The denatured probes were added to region of chip and then sealed with a cover glass. Hybridization reaction lasted for 18 h at 42ºC. Then the cDNA microarray were washed with 0.5% washing solution 1, following with the solution containing 0.5% washing solution 1 and 2% washing solution 2 for 10 min at 60ºC and then with 5% washing solution 3 for 10 min at 60ºC. The chip was washed with 0.5% washing solution 1 again and then dried at room temperature. All hybridization solutions and washing solutions were provided by BioStar Genechip.

1.5 *Data analysis*

The cDNA micrparray used contained several probes specific for rat housekeepers (β-actin, γ-actin, GAPDH, 5 S rRNA, and so on), which served as positive controls. Several rice probes on the chip severed as negative controls and several probes severed as blank controls for background subtraction. The specific probes on the chip are products of PCR from subtraction hybridization of rat tissues, that is, these probes are specific for each tissue. The cDNA microarray was scanned with ScanArray4000 and the images were processed by GenePix 3.0 software. At first, the values of Cy3 or Cy5 were obtained after background subtraction. The values of Cy5 under 200 were replaced with 200. The number of effective genes was counted. The effective genes, valued according to both values of Cy3 and Cy5, are beyond 200 or either beyond 800 and Ri (Cy5/Cy3) is between 0.1 and 10. The average of the natural logarithm of Ri was computed, i.e. R'. The normalization index (ND) equals EXP(R'). Second, the adjusted value of Cy3, which multiply ND by Cy3, is Cy3*. The values of Cy3* under 200 were replaced with 200. At last, the value of Cy5/Cy3* of all genes were computed. The genes that ratio (Cy5/Cy3*) is beyond 2 or under 0.5 are differentially expressed in injured group and control group.

1.6 *Confirmation of differential expression by semi-quantitative RT-PCR*

The semi-quantitative PCR protocols were employed to further confirm the cDNA microarray results. We chose five up-regulated known genes and three down-regulated known genes, they were, cathepsin S (Ctss), Fc gamma receptor, vimentin (Vim), Transgelin (Tagln), tissue inhibitor of metalloproteinase 1 (Timp1) and stearyl-CoA desaturase, coagulation factor II (F2), endosulfine alpha (Ensa) respectively. At the same time, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified as an internal control. Total RNA (2 μg) from each group sample was reversely transcribed with oligo$(dT)_{15}$ primers using Superscipt II reverse transcriptase (Gibco-BRL). RT-PCR was performed in the Gene Amp PCR System 9700 (Applied Biosystems, USA). Briefly, 2 μl cDNA and gene specific primers (Table 1) were added to TaKaRa PCR Mix (TaKaRa *Taq* DNA polymerase, dNTPs, optimal buffer components; TaKaRa) and subjected to PCR amplification. PCR products were analyzed by electrophoresis on 1.5% agarose gels. Images of ethidium-bromide-stained bands were scaned with ImageMaster VDS (Amersham Pharmacia Biotech).

2 RESULTS

2.1 Results of extraction of total RNA

The total RNA isolated from spinal cords was evaluated with ultraviolet analysis and agarose gel electrophoresis. The values of OD_{260}/OD_{280} of the total RNA from injury group and control group were 1.905 and 2.087 with ultraviolet analysis, respectively. The electrophoresis result showed that the ratio of 18 S/28 S was approximately 1/2 and the bands from low to high molecular weight appeared smear (Fig. 1). The data indicated that the total RNA iso-

Table 1. Sequences of primer pairs used for semi-quantitative RT-PCR

Gene (GenBank accession #)	Primer pairs		Product size (bp)
	Bases	Sequence (5'-3')	
Timp 1(NM_053819)	135 - 163	GCTTTCTGCAACTCGGACCTGGTTA	
	488 - 513	AAGGGATGGCTGAACAGGGAAACAC	378
Tagln (NM_031549)	529 - 554	ATGGCAGCAGTGCAGAGGACTGTAA	
	833 - 858	TACCTCAAAGCTGTCCGGGCTAAGA	329
Ctss (NM_017320)	860 - 880	GTGTTCTCGTGGTTGGCTAT	
	1 179 - 1 199	GCGTCCTTGCTTACAGACTT	339
Vim (NM_031140)	715 - 740	ATGCTTCTCTGGCACGTCTTGACCT	
	1 028 - 1 053	ACTGCACCTGTCTCCGGTATTCGTT	338
Fc gamma receptor (X73371)	388 - 413	GTTTAAGGCCACAGTCAATGATAGT	
	807 - 832	GACCAAGGATACCAGGATAATAACA	444
F2 (NM_022924)	1 137 - 1 162	GATTCTTACATAGATGGGCGCATCG	
	1 464 - 1 489	AAGGGCACTGGCTTCTTTAGCTTGA	352
Ensa (NM_021842)	48 - 73	GGAGAAGCAGGATACACAGGAGAAA	
	224 - 249	CGGTAGCTGCTTGTTCTTCATCTTG	201
Stearyl-CoA desaturase (J02585)	1 624 - 1 644	AGGCAACCTTTCTGCTTTCT	
	2 001 - 2 021	TTTCCCATTCCCTTCACTCT	397
GAPDH(NM_017008)	451 - 470	TCCCTCAAGATTGTCAGCAA	
	739 - 758	AGATCCACAACGGATACATT	308

lated bears higher purity and integrity.

2.2 Differentially expressed genes between the injured and the control groups

The hybridized cDNA microarray were scaned with ScanArray4000. The obtained images were analyzed with GenePix Pro 3.0. 3 913 spots were effective genes. Our experiment revealed that the expression of 144 genes changed significantly at 4.5 d after SCI as compared with control spinal cord tissue, including up-regulation of 65 genes and down-regulation of 79 genes. The 144 differentially expressed genes included 41 known genes (Table 2), 72 known ESTs and 31 unknown genes. A scatter diagram (Fig. 2) was made and the spots near the 45º straight line represented the genes without change in expression level, while those genes far away from the 45º straight line represented the genes with differential expression.

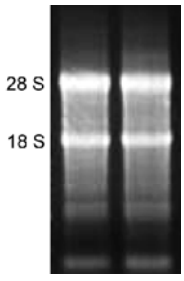

Fig. 1. Total RNA electrophoresis figure of control group and injured group. The left is the control group and the right is the injured group.

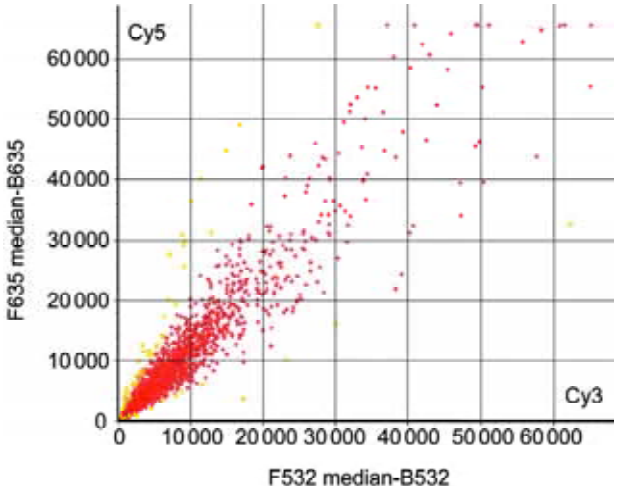

Fig. 2. A scatter diagram with X axis reflecting the intensity of hybridization signal of Cy3 and Y axis reflecting that of Cy5. Each spot stands for hybridized signal of a gene. The red spots indicate that value Y/value X are between 0.5 and 2.0 and belong to undifferentially expressed genes. The yellow spots indicate that the ratio of value Y to value X is beyond 2.0 or under 0.5 and likely to be differentially expressed genes.

Table 2. Known genes differentially expressed at 4.5 d after SCI

No.	GenBank number	Gene name	Fold change	Function
Up-regulated				
1	NM_019358	Glycoprotein 38 (Gp38)	2.011	Membrane transport
2	V01222	Preproalbumin	2.059	Albumin precursor
3	Z49761	RT1.Ma	2.123	Unknown
4	NM_031083	Phosphatidylinositol 4-kinase(Pik4cb)	2.124	Signal transduction
5	NM_012679	Clusterin (Clu)	2.181	Antiapoptotic protein
6	NM_019289	Actin-related protein complex 1b (Arpc1b)	2.197	Cytoplasm transport
7	AF154572	ERG2 protein	2.207	Control of cellular excitability
8	Y09111	tMDC IV protein	2.274	Unknown
9	NM_053538	Lysosomal-associated protein transmembrane 5 (Laptm5)	2.382	Protein degradation
10	AJ223184	DORA protein	2.425	Cell surface receptor
11	X82669	RT1.Au	2.471	MHC class molecule
12	AF065438	Mama	2.525	Unknown
13	NM_012771	Lysozyme (Lyz)	2.549	Metabolism
14	AF370882	TORID	2.660	Membrane component
15	NM_022597	Cathepsin B (Ctsb)	2.739	Inflammation factor
16	AB023781	Cathepsin Y	2.786	Inflammation factor
17	NM_017320	Cathepsin S (Ctss)	2.973	Inflammation factor
18	X73371	Fc gamma receptor	3.161	Receptor
19	NM_031140	Vimentin (Vim)	3.306	Cytoskeletal elements
20	NM_031549	Transgelin(Smooth muscle 22 protein) (Tagln)	3.403	Actin cross-linking protein
21	NM_053819	Tissue inhibitor of metalloproteinase1 (Timp1)	4.575	Tissue reconstruction
Down-regulated				
1	J02585	Stearyl-CoA desaturase	0.197	Lipid metabolism
2	NM_053389	Survival of motor neuron protein interacting protein1	0.358	Splicing pre-mRNA
3	NM_022924	Coagulation factor II (F2)	0.390	Apoptosis
4	NM_021842	Endosulfine alpha (Ensa)	0.411	Inhibitor of voltage-gated Ca^{2+} channels
5	NM_012681	Transthyretin (prealbumin,amyloidosis type I) (Ttr)	0.425	Acute-phase response
6	NM_053917	Inositol polyphosphate-4-phosphatase type II	0.447	Signal transduction
7	NM_012598	Lipoprotein lipase (Lpl)	0.456	Lipid metabolism
8	NM_022629	Gamma-butyrobetaine hydroxylase (Bbox)	0.458	Lipid metabolism
9	U94856	Paraoxonase	0.465	Antioxidant
10	NM_032083	Chimerin (chimaerin)1 (Chn1)	0.465	Cell differentiation
11	M31109	UDP-glucuronosyltransferase	0.467	Metabolism
12	NM_012559	Fibrinogen, gamma polypeptide (Fgg)	0.475	Cellular and matrix interactions
13	NM_017096	C-reactive protein (Crp)	0.478	Inflammatory response
14	NM_017268	3-hydroxy-3-methylglutaryl-Coenzyme A synthase1 (HmgcN-14-7)	0.479	Lipid metabolism
15	AF084933	ClassII MHC RT1.D(n) beta chain precursor (RT1.D(n))	0.480	MHC molecule
16	NM_017300	Bile acid-CoenzymeA dehydrogenase:amino acid *n*-acyltransferase (Baat)	0.482	Lipid metabolism
17	NM_053348	Fetuin beta (Fetub)	0.486	Antagonist of cytokines
18	NM_053356	Procollagen type I alpha2 (Col1a2)	0.490	Cell adhesion
19	NM_017342	Surfactant protein C (SP-C)	0.492	Lipid mixing
20	NM_024382	Leuserpin-2 (Serpind1)	0.495	Inhibitor of apoptosis

2.3 Results of RT-PCR

The agarose gel electrophoresis of RT-PCR products also showed significantly increasing expression of Timp 1, Vim, Tagln, Ctss, and Fc gamma receptor, decreasing expression of stearyl-CoA desaturase, F_2 and Ensa at 4.5 d after SCI, compared with control group (Fig. 3).

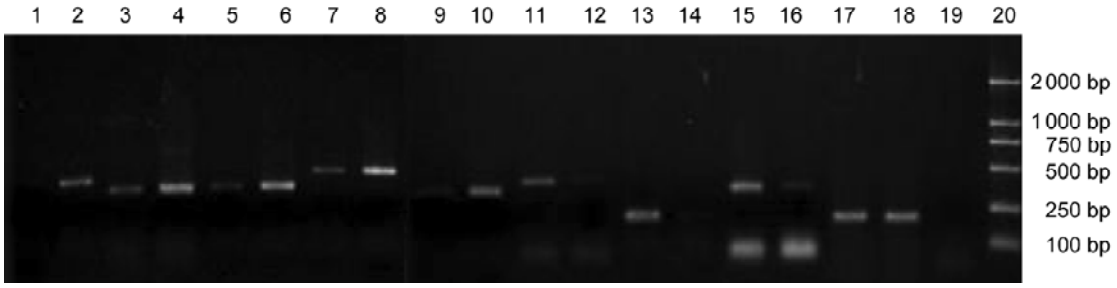

Fig. 3. Electrophoresis figure of RT-PCR. 1, 3, 5, 7, 9,11,13,15,17 are samples of control group and 2, 4, 6, 8, 10,12,14,16,18 are samples of injured group. 1 and 2 are Timp 1; 3 and 4 are Tagln; 5 and 6 are Vim; 7 and 8 are Fc gamma receptor; 9 and 10 are Ctss; 11 and 12 are stearyl-CoA desaturase; 13 and 14 are Ensa; 15 and 16 are F2; 17 and 18 are GAPDH; 19 is negative control; 20 is DL2000 marker.

3 DISCUSSION

We used RNeasy Mini Kit to isolate total RNA from the rat spinal cord tissue. The isolated total RNA from injured and control groups were of higher quality in purity and integrity, which assured the success of cDNA microarray. In this cDNA microarray, normalization index (ND=1.0907) is between 0.4 and 2.5 and the average ratios of signal density from injured group or control group to background are not less than 3. These data indicate that our experiment is in conformance with the successful standard of cDNA microarray. The RT-PCR results of eight selected genes further confirmed that cDNA microarray results are credible.

Using GeneChip® protoclos, Song *et al*. had reported the changes of mRNA abundance at 3 and 24 h after SCI in adult rats. Nesic *et al*. had reported that SCI, 1 h after trauma, induced change in mRNA levels of 165 genes and ESTs[15]. Zhang *et al*. investigated gene expression profiles at 14 d after a complete spinal transection at the 11th thoracic level in adult rats using cDNA microarray. Among 7 523 genes and ESTs examined, 444 transcripts, including 218 genes and 226 ESTs, were identified to be either up-regulated (373 of 444) or down-regulated (71 of 444) greater than 2.0-fold in the spinal cord, which were categorized into seven classes which included cell division-related protein, channels and receptors, cytoskeletal elements, extracellular matrix proteins, metalloproteinases and inhibitors, growth-associated molecules, metabolism, intracellular transducers and transcription factors, as well as others[16]. By interrogating Affymetrix U34A rat genome GeneChip microarrays, Velardo *et al*. defined the transcriptional expression patterns in midcervical contusion lesion sites between 1 and 90 d postinjury of athymic nude (AN) and Sprague- Dawley (SD) strains. Stringent statistical analyses detected significant changes in 3 638 probe sets, with 80 genes differing between the AN and SD groups[17]. Tachibana *et al*. analyzed changes in the mRNA abundance at 24 h after SCI in adult rats using cDNA microarray. However, their data were related to within one day after SCI. The injured spinal cord evaluated during 8 weeks by the inclined plane test showed that the motor disturbances peaked within 24 h after injury and were not completely recovered after 56 d. However, there was a striking enhancement phase at motor function recovery, that was, from the first day to the seventh day after SCI [18]. So, it is likely to find some genes related to regeneration after SCI at this phase.

It was accepted that the injury reactions mainly occurred in the first 2 d after SCI. And beginning from 3 d after injury, the regeneration became actively, predominant, and arrived the top at 4~5 d. So in the present study we chose 4.5 d postinjury to screen differentially expressed genes that highly related to the repair and regeneration process of the injured spinal cord. There are different animal models used to investigate SCI. Many scientists use physically transected spinal cord for studying spinal cord regeneration and the high reproduction and easy standardization are features of this model.

Concerted expression of thousands of genes in a controlled manner is essential for spinal cord function. Traumatic injury to the spinal cord causes the altered expression of many genes. The results of the present study found altered mRNA abundance (>2.0) of 144 genes at 4.5 d

after SCI, compared with sham control. The 144 changed genes include 79 up-regulated genes and 65 down-regulated genes. The up-regulated genes consist of 20 known genes, 42 known ESTs and 17 unknown genes. The down-regulated genes comprise 21 known genes, 30 known ESTs and 14 unknown genes. Some of 41 known genes (Vim, Timp 1, Coagulation factor II, Clusterin) were previously reported. However, many changes observed presently are first-time reports. The changed genes are involved in two types of molecules. One is promoter and the other is inhibitor for regeneration after SCI. The regeneration after SCI is mediated by the two types of molecules together.

The spinal regeneration is highly dependent on intracellular calcium concentration[19]. Alpha-endosulphine, a protein that belongs to the cAMP-regulated-phosphoprotein family, has been detected in a wide variety of tissues, concentrated particularly in the central nervous system. Under physiological conditions, alpha-endosulphine mainly acts to block voltage-gated Ca^{2+}-channels[20]. The level of phosphatidylinositol 4-kinases (PiK4cb) is up-regulated and that of inositol polyphosphate 4-phosphatase (Inpp4b) is down-regulated at 4.5 d after SCI PiK4cb catalyze the first step in the synthesis of an important information transmittance regulator — phosphatidylinositol 4,5-bisphosphate[21]. Inpp4b catalyzes the hydrolysis of the 4-position phosphate of inositol 3,4-bisphosphate [Ins(3,4)P2], inositol 1,3,4-trisphosphate [Ins(1,3,4)P3], and to a greater degree phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P2][22]. The first two substrates are intermediates in the breakdown of inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] to inositol, the former being important for Ca^{2+} release via calcium channel. The common function of PiK4cb and Inpp4b is to promote Ca^{2+} concentration in the cytoplasm. Meanwhile, the phospholipid substrate of Inpp4b, PtdIns(3,4)P2, has been shown to increase following stimulation of cells by growth factors that activate phospholipid 3-kinase (PI3-K)[23]. [Ins(3,4)P2] has been shown to directly bind and activate the *Akt* protooncogene, which is involved in cell proliferation.

Alpha-chimaerin (Table 2) occurs as alpha 1- and alternatively spliced Src homology 2 (SH2) domain-containing alpha2-isoforms. Alpha2-chimaerin mRNA was highly expressed in the rat embryonic nervous system, especially in early postmitotic neurons. Alpha2-chimaerin protein localized to neuronal perikaryons, dendrites, and axons. The overall pattern of alpha2-chimaerin mRNA expression resembles that of cyclin-dependent kinase regulator p35, which participates in neuronal differentiation and with which chimaerin interacts. The transfection experiments of N1E-115 neuroblastoma cells demonstrated that alpha2-chimaerin transfectants generated neurites and alpha1-chimaerin transfectants displayed numerous microspikes and containing F-actin clusters, but generated few neurites[24].

Vimentin (Table 2) is one of the members of intermediate filaments. It exists in mesenchyme cells, chondrocyte and endothelial cells, particularly in fibroblast and differentiating cells. Many experiments showed that there was a large increase in vimentin (+) cells at the lesion site[25,26]. Dervan and Roberts observed that the newly formed canal was greatly enlarged and was dominated by ependymocytes that are vimentin immunopositive after the spinal cord transection of eel Anguilla whose injured spinal cord can quickly regrow, reconnect and recover function. Vimentin may play an important role in regeneration of spinal cord[27].

Cysteine lysosomal proteases are essential for turnover of intracellular and extracellular proteins. These enzymes are strongly implicated in normal and pathological processes involving tissue remodeling[28]. Cathepsin B, cathepsin S and cathepsin Y are the members of cysteine lysosomal proteases which belong to papain superfamily. Cathepsin B can degrade myelin basic protein[29] and cathepsin Y can produce bradykinin potentiating peptide[30]. Cathepsin S can degrade a number of extracellular matrix molecules at neutral pH since it remains most of its enzymatic activity at neutral pH. Inflammatory mediators can stimulate secretion of cathepsin S from the microglia and macro-phages.

The survival of motor neuron (SMN) protein is expressed in all tissues of mammalian organisms, but particularly high levels are expressed in motor neurons. SMN and SMN interacting protein 1 (SIP1) are found both in the nucleus and cytoplasm of somatic cells. The interaction of SMN and SIP1 plays a crucial role in the biogenesis of spliceosomal small nuclear ribonucleoproteins (snRNPs) which function in pre-mRNA splicing[31]. The impaired production of snRNPs may contribute to motoneuron degeneration.

Clusterin is a glycoprotein that has been implicated in a number of cellular processes, such as lipid transfer, cell-cell interaction, apoptosis and inhibition of the terminal complement complex formation. Analysis of the promoter region of the TRPM-2 gene demonstrated that the putative control region contains several potential regulatory elements that may regulate the complex tissue-specific control of a gene which must be constitutively expressed in some tissues but repressed in others until induced during active cell death[32].

Gamma-butyrobetaine hydroxylase (Bbox), lipoprotein

lipase (LPL), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (Hmgcs1), bile acid-coenzyme A dehydrogenase: amino acid *n*-acyltransferase (Baat) and stearyl-CoA desaturase play important roles in the lipid metabolism. Bbox catalyzes conversion of gamma-butyrobetaine to carnitine. The carnitine system plays a key role in beta-oxidation of long-chain fatty acids by permitting their transport into the mitochondrial matrix. LPL is a key enzyme involved in the metabolism of lipoproteins. Spinal cord of the rat contains more lipoprotein lipase than other brain regions. LPL-mediated hydrolysis of exogenous triacylglycerol is an important source of free fatty acids for the Schwann cell[33]. Hmgcs1 is responsible for 3-hydrox-3-methylglutaryl CoA biosynthesis in the cell. The 3-hydrox-3-methylglutaryl CoA is an important mediator in synthesis of cholesterol and ketone bodies. Baat catalyzes the conjugation of bile acids with glycine or taurine. The level of Baat deceased at 6 and 12 h in the regenerating liver after partial hepatectomy[34]. The Baat may play a role in acute-phase reactions. The clinic evidence showed that the ratio of Baat quantity in tumorous tissue to nontumorous is positive relative to prognosis of hepatocellular carcinoma patients after partial hepatectomy. Stearyl-CoA desaturase is expressed by oligodendrocytes as well as Schwann cells. It is involved in the synthesis and regulation of long-chain unsaturated fatty acids essential for myelination[35].

In addition to the above discussed genes, there are some genes, such as RT1.D(n), RT1.Ma and RT1.Au genes encoding MHC class I and MHC class II molecules are also differentially expressed 4.5 d after SCI. The function of these genes in the regeneration after SCI is not clear and they maybe play roles in the cell-cell recognition and communication. So, we should strive to explore the function of these altered expression genes and identify some targets for clinic treatment. At the same time, we should realize that molecular mechanism of regeneration after SCI is very complex. There will be a farther way to go in this region.

REFERENCES

1 Shingu H, Ohama M, Ikata T, Katoh S, Akatsu T. A nationwide epidemiological survey of spinal cord injuries in Japan from January 1990 to December 1992. Paraplegia 1995; 33(4): 183-188.

2 Bowtell DD. Options available — from start to finish — for obtaining expression data by microarray. Nat Genet 1999; 21(1 Suppl): 25-32.

3 Funakoshi H, Risling M, Carlstedt T, Lendahl U, Timmusk T, Metsis M, Yamamoto Y, Ibanez CF. Targeted expression of a multifunctional chimeric neurotrophin in the lesioned sciatic nerve accelerates regeneration of sensory and motor axons. Proc Natl Acad Sci USA 1998; 95(9): 5269-5274.

4 GrandPre T, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000; 403(6768): 439-444.

5 Namiki J, Tator CH. Cell proliferation and nestin expression in the ependyma of the adult rat spinal cord after injury. J Neuropathol Exp Neurol 1999; 58(5): 489-498.

6 Hamada Y, Ikata T, Katoh S, Nakauchi K, Niwa M, Kawai Y, Fukuzawa K. Involvement of an intercellular adhesion molecule 1-dependent pathway in the pathogenesis of secondary changes after spinal cord injury in rats. J Neurochem 1996; 66(4): 1525-1531.

7 Bartholdi D, Schwab ME. Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: an *in situ* hybridization study. Eur J Neurosci 1997; 9(7): 1422-1438.

8 Grossman SD, Wolfe BB, Yasuda RP, Wrathall JR. Alterations in AMPA receptor subunit expression after experimental spinal cord contusion injury. J Neurosci 1999; 19(14): 5711-5720.

9 Hayashi M, Ueyama T, Nemoto K, Tamaki T, Senba E. Sequential mRNA expression for immediate early genes, cytokines, and neurotrophins in spinal cord injury. J Neurotrauma 2000; 17(3): 203-218.

10 Lee YL, Shih K, Bao P, Ghirnikar RS, Eng LF. Cytokine chemokine expression in contused rat spinal cord. Neurochem Int 2000; 36(4-5): 417-425.

11 Mautes AE, Noble LJ. Co-induction of HSP70 and heme oxygenase-1 in macrophages and glia after spinal cord contusion in the rat. Brain Res 2000; 883: 233-237.

12 Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, Brown EL. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 1996; 14(13): 1675-1680.

13 Song G, Cechvala C, Resnick DK, Dempsey RJ, Rao VL. GeneChip analysis after acute spinal cord injury in rat. J Neurochem 2001; 79(4): 804-815.

14 Fan M, Mi R, Yew DT, Chan WY. Analysis of gene expression following sciatic nerve crush and spinal cord hemisection in the mouse by microarray expression profiling. Cell Mol Neurobiol 2001; 21(5): 497-508.

15 Nesic O, Svrakic NM, Xu GY, McAdoo D, Westlund KN, Hulsebosch CE, Ye Z, Galante A, Soteropoulos P, Tolias P, Young W, Hart RP, Perez-Polo JR. DNA microarray analysis of the contused spinal cord: effect of NMDA receptor inhibition. J Neurosci Res 2002; 68(4): 406-423.

16 Zhang KH, Xiao HS, Lu PH, Shi J, Li GD, Wang YT, Han S, Zhang FX, Lu YJ, Zhang X, Xu XM. Differential gene expres-

sion after complete spinal cord transection in adult rats: an analysis focused on a subchronic post-injury stage. Neuroscience 2004; 128(2): 375-388.

17 Velardo MJ, Burger C, Williams PR, Baker HV, Lopez MC, Mareci TH, White TE, Muzyczka N, Reier PJ. Patterns of gene expression reveal a temporally orchestrated wound healing response in the injured spinal cord. J Neurosci 2004; 24(39): 8562-8576.

18 Taoka Y, Okajima K, Uchiba M, Murakami K, Harada N, Johno M, Naruo M. Activated protein C reduces the severity of compression-induced spinal cord injury in rats by inhibiting activation of leukocytes. J Neurosci 1998; 18(4): 1393-1398.

19 Unlu A, Hariharan N, Iskandar BJ. Spinal cord regeneration induced by a voltage-gated calcium channel agonist. Neurol Res 2002; 24(7): 639-642.

20 Virsolvy A, Smith P, Bertrand G, Gros L, Heron L, Salazar G, Puech R, Bataille D. Block of Ca^{2+}-channels by alpha-endosulphine inhibits insulin release. Br J Pharmacol 2002; 135 (7): 1810-1818.

21 Zhao X, Varnai P, Tuymetova G, Balla A, Toth ZE, Oker-Blom C, Roder J, Jeromin A, Balla T. Interaction of neuronal calcium sensor-1 (NCS-1) with phosphatidylinositol 4-kinase beta stimulates lipid kinase activity and affects membrane trafficking in COS-7 cells. J Biol Chem 2001; 276(43): 40183-40189.

22 Norris FA, Auethavekiat V, Majerus PW. The isolation and characterization of cDNA encoding human and rat brain inositol polyphosphate 4-phosphatase. J Biol Chem 1995; 270(27): 16128-16133.

23 Franke TF, Kaplan DR, Cantley LC, Toker A. Direct regulation of the *Akt* proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science 1997; 275(5300): 665-668.

24 Hall C, Michael GJ, Cann N, Ferrari G, Teo M, Jacobs T, Monfries C, Lim L. Alpha2-chimaerin, a Cdc42/Rac1 regulator, is selectively expressed in the rat embryonic nervous system and is involved in neuritogenesis in N1E-115 neuroblastoma cells. J Neurosci 2001; 21(14): 5191-5202.

25 Raginov IS, Chelyshev IuA, Shagidullin TF. Interaction of sensory neurons and satellite cells during stimulation of the nerve regeneration. Morfologiia 2002; 122(4): 37-39.

26 Wang X, Messing A, David S. Axonal and nonneuronal cell responses to spinal cord injury in mice lacking glial fibrillary acidic protein. Exp Neurol 1997; 148(2): 568-576.

27 Dervan AG, Roberts BL. Reaction of spinal cord central canal cells to cord transection and their contribution to cord regeneration. J Comp Neurol 2003; 458(3): 293-306.

28 Petanceska S, Canoll P, Devi LA. Expression of rat cathepsin S in phagocytic cells. J Biol Chem 1996; 271(8): 4403-4409.

29 Banik NL, Matzelle D, Terry E, Gantt-Wilford G, Hogan EL. Inhibition of proteolysis by a cyclooxygenase inhibitor, indomethacin. Neurochem Res 2000; 25(11): 1509-1515.

30 Sakamoto E, Sakao Y, Taniguchi Y, Yamafuji K. Cathepsin Y (a novel thiol enzyme) produces kinin potentiating peptide from the component protein of rat plasma. Immunopharmacol 1999; 45(1-3): 207-214.

31 Fischer U, Liu Q, Dreyfuss G. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell 1997; 90 (6): 1023-1029.

32 Wong P, Taillefer D, Lakins J, Pineault J, Chader G, Tenniswood M. Molecular characterization of human TRPM-2/clusterin, a gene associated with sperm maturation, apoptosis and neurodegeneration. Eur J Biochem 1994; 221(3): 917-925.

33 Paradis E, Clement S, Julien P, Ven Murthy MR. Lipoprotein lipase affects the survival and differentiation of neural cells exposed to very low density lipoprotein. J Biol Chem 2003; 278 (11): 9698-9705.

34 Furutani M, Arii S, Higashitsuji H, Mise M, Fukumoto M, Takano S, Nakayama H, Imamura M, Fujita J. Reduced expression of kan-1 (encoding putative bile acid-CoA-amino acid *N*-acyltransferase) mRNA in livers of rats after partial hepatectomy and during sepsis. Biochem J 1995; 311: 203-208.

35 Furutani M, Arii S, Higashitsuji H, Mise M, Niwano M, Harada T, Nakayama H, Fukumoto M, Imamura M, Fujita J. kan-1 (bile acid CoA:amino acid *N*-acyltransferase) messenger RNA as a novel predictive indicator for prognosis of hepatocellular carcinoma patients after partial hepatectomy. Hepatology 1996; 24 (6): 1441-1445.