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Identification and functional annotation of novel microRNAs in the proximal sciatic nerve after sciatic nerve transection

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The peripheral nervous system is able to regenerate after injury, and regeneration is associated with the expression of many genes and proteins. MicroRNAs are evolutionarily conserved, small, non-coding RNA molecules that regulate gene expression at the level of translation. In this paper, we focus on the identification and functional annotation of novel microRNAs in the proximal sciatic nerve after rat sciatic nerve transection. Using Solexa sequencing, computational analysis, and quantitative reverse transcription PCR verification, we identified 98 novel microRNAs expressed on days 0, 1, 4, 7, and 14 after nerve transection. Furthermore, we predicted the target genes of these microRNAs and analyzed the biological processes in which they were involved. The identified biological processes were consistent with the known time-frame of peripheral nerve injury and repair. Our data provide an important resource for further study of the role and regulation of microRNAs in peripheral nerve injury and regeneration.

microRNA, proximal sciatic nerve, rat, Solexa

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Peripheral nerve injuries are relatively common in clinical practice. The peripheral nervous system is able to regenerate after injury and it is important to understand the underlying molecular mechanisms to provide the best treatment. Thus far, these molecular mechanisms remain largely elusive, and so therapeutic and preventative strategies for peripheral nerve injury present a significant challenge.

During peripheral nerve injury and regeneration, the expression levels of many genes and proteins are changed [1–5]. MicroRNAs (miRNAs) can regulate mRNA and protein expression, and serve as key modulators of post-transcriptional gene regulation in a variety of tissues. They play an important role in the development, differentiation, proliferation, survival, and oncogenesis of cells and organisms including the nervous system. Basic and clinical studies have suggested that miRNAs are important regulators in normal physiological processes and pathology [6–9]. Many miRNAs are expressed in a tissue-specific manner [10], and thus play important roles in regulating the tissue specificity of gene expression, the function of tissue-specific genes, or both. Recent studies have also revealed the roles of miRNAs in axonal biology [11,12] as well as in the control of synaptic function and plasticity [13,14].

A single miRNA can affect, albeit modestly, the levels of thousands of proteins [15,16], and thus miRNAs form a complex regulatory network affecting the majority of genes. Although miRNA expression in central and peripheral nerve injury has been studied [17,18], there has been no miRNA research in peripheral nerve injury using large-scale sequencing technology. High-throughput technology is being applied increasingly to examine signal pathways and regulatory mechanisms. Given the importance of miRNAs, we were interested in the expression profile and regulation of miRNAs involved in peripheral nerve injury and repair. The

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discovery and identification of novel miRNAs is the first, important step. We used Solexa sequencing, a method that can successfully discover novel miRNAs with high accuracy and efficiency [19]. We validated the candidate miRNAs using bioinformatics tools and real-time reverse transcription PCR (RT-PCR). Subsequently, we predicted the target genes of the novel identified miRNAs and performed functional annotation of the target genes.

1 Materials and methods

1.1 Construction of animal models and sample preparation

Thirty adult, male Sprague-Dawley rats (180–220 g; provided by the Experimental Animal Center of Nantong University) were randomly divided into five groups (six rats per group) to undergo sciatic neurectomy. All animal experimentation was carried out in accordance with the "Guidelines for the Care and Use of Laboratory Animals" (National Institutes of Health, Bethesda, MD). All rats were anesthetized by injection of a narcotics complex (10 mg kg^{-1} xylazine, 95 mg kg^{-1} ketamine, 0.7 mg kg^{-1} acepromazine), and the sciatic nerve was exposed and lifted through an incision on the lateral aspect of the mid-thigh of the left hind-limb. A 1-cm-long segment of sciatic nerve was then resected at the site just proximal to the division of the tibial and common peroneal nerves. After the surgical incisions were closed, animals were housed in temperature- and humidity-controlled cages, maintained under a 12:12-h light-dark cycle, and were allowed free access to water and food. Rats were euthanized and 0.5 cm of the proximal sciatic nerve (PSN, proximal to the incision) was collected at 0 h, 1, 4, 7, or 14 d after transection.

1.2 RNA isolation and Solexa sequencing

Total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Solexa sequencing was performed by the Beijing Genomics Institute (Shenzhen, China). Briefly, small RNA molecules ranging from 18 to 30 nt were gel-purified and ligated with a pair of Solexa adaptors to their 5′ and 3′ ends. The ligation products were purified; reverse transcribed and amplified using adaptor primers. The amplified products were gel-purified and sequenced on an Illumina Genome Analyzer (San Diego, CA). The image files obtained by sequencing were then converted to digital-quality data. After the removal of adaptor sequences and contaminated reads, clean reads were processed for computational analysis.

1.3 Novel miRNA prediction

The cleaned small RNA sequences from Solexa sequencing were mapped to the rat genome using the Short Oligonucleotide Alignment Program (SOAP) [20]. Only those mapped perfectly to the rat genome were considered candidate miRNAs for further analysis. MIREAP [19,21] was used to analyze the hairpin structures to determine genuine miRNAs. We subsequently filtered out miRNAs with a free energy higher than –20 kcal mol^{-1}, in accordance with published standards [19,22]. Novel miRNAs were separated from those previously reported using miRBase 16.0 [23,24], then miRAlign [25] was used to identify conserved miRNA genes and MiPred [26] was used to distinguish functional non-conserved miRNA precursors (pre-miRNAs) from dysfunctional pseudo-hairpins.

1.4 Quantitative RT-PCR

The same RNA samples as those described in section 1.2 were used. All primers used are described in Appendix Table S1 in the electronic version. Ten nanograms of total RNA was reverse transcribed using a TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems) and stem-loop RT primers, according to the manufacturer's instructions. Real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems) on an Applied Biosystems Stepone Real-Time PCR System. All reactions were run in triplicate. The cycle threshold (C_t) values were analyzed by the comparative C_t ($\Delta\Delta C_t$) method. miRNA levels were normalized against those of rRNA U6. The expression levels at each time-point were compared to those at 0 h.

1.5 miRNA target prediction and functional analysis of target genes

miRNA targets in animals are usually located in the 3′ untranslated region (UTR) of mRNAs, where the silencing complex can easily interact with the initiation complex and promote attenuation of translation [27]. We downloaded the 3′ UTR sequences of all the rat genes and TargetScan source code from TargetScan (http://www.targetscan.org/). miRNA targets were predicted by complementary matches between the 2 to 8-bp seed sequences of the miRNAs and the 3′ UTR datasets. The context score for each miRNA target site was also calculated.

To annotate the biological function of these target genes further, we firstly screened novel miRNAs that demonstrated a significant change in expression level among any of the five time-points using the chi-squared test. The false discovery rate (FDR) was calculated to correct the *P*-value. Then, the filtered miRNAs (FDR<0.05) were used for expression pattern clustering using the Short Time-series Expression Miner (STEM) [28]. Expression profiles were applied to perform Gene Ontology (GO) enrichment analysis with a context score <–0.2 for the target genes. The GO Biological Processes (FDR<0.01) were attributed to several general categories by statistical analysis according to GO

website (http://www.geneontology.org/).

2 Results

2.1 Identification of small RNAs by Solexa sequencing

We collected the small RNA fractions and prepared small RNA libraries from rat PSN following sciatic nerve transection. The libraries were then sequenced using Solexa technology. After removing the reads of low quality and masking adaptor sequences, 10518910, 9825421, 9851345, 12183086, and 9775473 18 to 30-nt-long reads were obtained at 0 h, 1, 4, 7, and 14 d, respectively. The Solexa raw data are available at the Gene Expression Omnibus (GEO: GSE27653). Intriguingly, the length distribution peaked at 22 nucleotides and almost half of the clean reads were 22 nucleotides long (data not shown), consistent with the common size of miRNAs.

Next, all the Solexa reads were aligned against the rat genome using SOAP. We found that 8213858 (78.09%), 7770977 (79.09%), 7852278 (79.71%), 9850603 (80.85%), and 7684960 (78.61%) reads matched perfectly to the rat genome at each respective time-point (Appendix Table S2 in the electronic version).

Subsequently, the rat small RNAs were categorized according to their biogenesis and annotation. rRNAs, tRNAs, small nuclear RNAs, and small nucleolar RNAs (Appendix Table S2 in the electronic version) were disregarded, which left 259, 261, 262, 266, and 259 known miRNAs and 55, 56, 56, 56, and 52 miRNAs* (the other strand of duplex, known as the passenger strand or miRNA* strand) at the five time-points, respectively. The known miRNAs and their read count are shown in Appendix Table S3 in the electronic version.

2.2 Computational selection of genuine and novel miRNAs

By mapping the cleaned small RNA sequences from Solexa sequencing to the rat genome sequences using SOAP, scanning canonical stem-loop hairpin structures using MIREAP, removing the 13 loci with free energy greater than –20 kcal mol^{-1} (Appendix Table S4 in the electronic version) and filtering out the previously reported miRNAs using miRBase 16.0, Solexa sequencing generated 174 loci that were considered candidate novel miRNA genes. We then used miRAlign to identify miRNA genes that were paralogs or orthologs to known miRNAs, detecting seven conserved miRNA genes (Appendix Table S7 in the electronic version) among the 174.

Although the hairpin structure is a necessary feature for the computational classification of genuine pre-miRNAs, many random inverted repeats (pseudo-hairpins) in eukaryotic genomes can also fold into dysfunctional hairpins [26,29]. To overcome this problem, we adopted an *ab initio* prediction method, MiPred, to distinguish pre-miRNAs from other similar segments in the rat genome [26]. It has been reported that MiPred performs as well as, or significantly better, in terms of sensitivity and specificity, than existing classifiers at distinguishing non-conserved functional pre-miRNAs from genomic pseudo-hairpins and non-pre-miRNAs (most classes of non-coding RNAs and mRNAs) [29]. Among the 174 pre-miRNA-like hairpins, 76 were classified as pseudo-pre-miRNAs (Appendix Table S5 in the electronic version) and 98 as novel and genuine miRNAs (Table 1, Appendix Tables S6 and S7, Appendix Figures S1 and S2 in the electronic version). Among the 98 novel miRNAs, the length ranged from 19 to 24 nucleotides, with a peak at 22 nucleotides that accounted for 41.8% of the miRNAs (Figure 1A). In addition, 46 novel miRNAs were identified at 0 h, 43 at 1 d, 48 at 4 d, 69 at 7 d, and 44 at 14 d (Figure 1B and C).

2.3 Validation of novel miRNAs by quantitative RT-PCR

To verify the accuracy and efficiency of Solexa sequencing, the same RNA preparation used for the Solexa sequencing was subjected to quantitative RT-PCR [30]. The expression of 20 miRNAs that were expressed in rat PSN at all time-

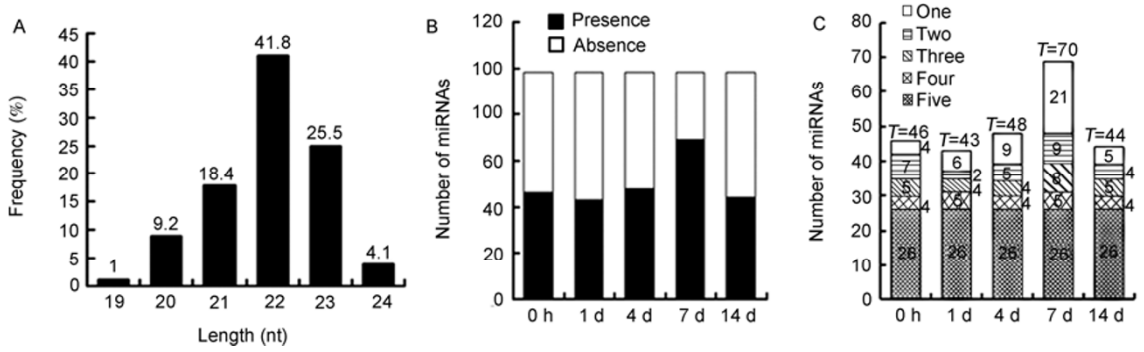

Figure 1 Characterization of the novel miRNAs. A, Length distribution of the novel miRNAs. B, The number of miRNAs present and absent at each time-point. C, The number of miRNAs at each time-point. One, two, three, four, and five represent the number of time-points at which a particular miRNA was expressed. *T*, total number of miRNAs expressed at each time-point.

Table 1 The read count of the novel miRNAs at each time-point

miRNAs	Read count					miRNAs	Read count				
	0 h	1 d	4 d	7 d	14 d		0 h	1 d	4 d	7 d	14 d
mmu-miR-3075	16	19	32	91	25	rno-miR-sc38				7	
mmu-miR-3099/1	22	60	37	47	65	rno-miR-sc39				5	
mmu-miR-3102-5p.2	47	72	56	41	42	rno-miR-sc40				4	
mmu-miR-1247*	7	68	157	111	79	rno-miR-sc41				4	
rno-miR-sc1	5	10	9	8	8	rno-miR-sc42				4	
rno-miR-sc2	12	24	7	24	8	rno-miR-sc43				9	
rno-miR-sc3-1	9	12	11	37	5	rno-miR-sc44				6	
rno-miR-sc3-2	9	12	11	37	5	rno-miR-sc45				4	
rno-miR-sc4	14	16	20	6	16	rno-miR-sc46				4	
rno-miR-sc5	224	304	428	771	317	rno-miR-sc47				6	
rno-miR-sc6	16	18	16	25	15	rno-miR-sc48				10	
rno-miR-sc7	69	42	32	31	17	rno-miR-sc49				13	
rno-miR-sc8	12	18	22	7	16	rno-miR-sc50				4	
rno-miR-sc9	14	15	6	12	6	rno-miR-sc51-1				4	
rno-miR-sc10-1	17	32	46	76	16	rno-miR-sc51-2				4	
rno-miR-sc10-2	17	32	46	76	16	rno-miR-sc52				4	
rno-miR-sc10-3	17	32	46	76	16	rno-miR-sc53				7	
rno-miR-sc10-4	17	32	46	76	16	rno-miR-sc54					5
rno-miR-sc10-5	17	32	46	76	16	rno-miR-sc55					6
rno-miR-sc10-6	17	32	46	76	16	rno-miR-sc56					6
rno-miR-sc11	11	25	7	19	7	rno-miR-sc57					6
rno-miR-sc12	30	20	10	22	6	rno-miR-sc58					11
rno-miR-sc13	302	273	357	467	225	rno-miR-sc59	5	10	5		
rno-miR-sc14	104	49	107	83	174	rno-miR-sc60	8	6	9	6	
rno-miR-sc15	47	42	62	80	49	rno-miR-sc61		8	28	20	13
rno-miR-sc16	5	7	16	39	9	rno-miR-sc62		9	11	8	7
rno-miR-sc17	10					rno-miR-sc63			6	8	6
rno-miR-sc18	7					rno-miR-sc64			11	13	13
rno-miR-sc19	5					rno-miR-466e			5	9	5
rno-miR-sc20	5					rno-miR-sc65				10	5
rno-miR-sc21		6				rno-miR-sc66	7			10	
rno-miR-sc22		9				rno-miR-sc67	7			6	
rno-miR-sc23		7				rno-miR-sc68	8			6	
rno-miR-sc24		9				rno-miR-sc69	6			6	
rno-miR-sc25		6				rno-miR-sc70	9			6	
rno-miR-sc26		21				rno-miR-sc71	8			5	
rno-miR-sc27			6			rno-miR-sc72	6			9	
rno-miR-sc28			8			rno-miR-sc73		7	6		
rno-miR-sc29			6			rno-miR-sc74		8	9	5	
rno-miR-sc30			14			rno-miR-sc75		5		15	
rno-miR-sc31			7			rno-miR-sc76			8		8
rno-miR-sc32			8			rno-miR-sc77			15		14
rno-miR-sc33			6			rno-miR-sc78	10	7		12	
rno-miR-sc34			5			rno-miR-sc79	8	13		18	
rno-miR-sc35			5			rno-miR-sc80	5	7	8	17	
rno-miR-3072*				6		rno-miR-sc81	7	6		7	10
rno-miR-sc36				6		rno-miR-sc82	7			8	6
rno-miR-sc37				6		rno-miR-sc83	7	5		14	9
rno-miR-3082b				6		rno-miR-sc84	9		24	19	21

points following sciatic nerve transection was confirmed by semi-quantitative RT-PCR (Figure 2A). We therefore concluded that these were authentic miRNAs.

The expression levels of the newly identified rat miRNAs were also analyzed by quantitative real-time RT-PCR. We selected six miRNAs that showed a clear band without

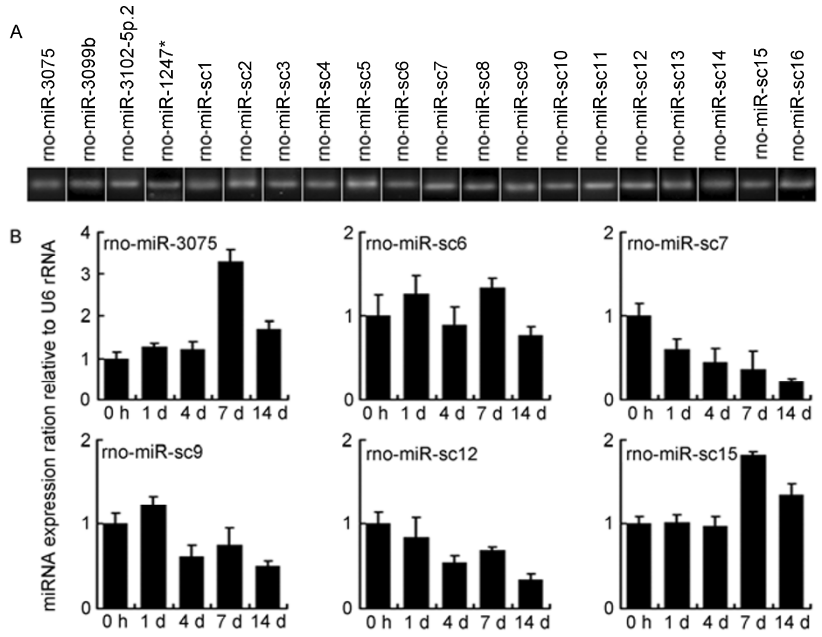

Figure 2 A, Semi-quantitative RT-PCR demonstrating the specific expression of the indicated novel miRNAs. B, Real time quantitative RT-PCR showing expression levels of six novel miRNAs at each time-point relative to the level of U6 rRNA.

background signal for further analysis, and demonstrated that the relative changes in miRNA expression determined by Solexa sequencing or real-time PCR were generally consistent with each other (Figure 2B).

2.4 miRNA target prediction

miRNAs function by regulating target genes, so we next predicted the target genes of our novel miRNAs. Typical methods for recognizing miRNA binding sites rely on searching for complementary matches between an miRNA sequence and phylogenetically conserved portions in the 3′ UTR of target mRNAs. Many online prediction programs that can be used to predict target genes of reported miRNAs cannot be applied to novel miRNAs. Here, we followed a canonical approach of searching for complementary matches between the "seed" region of an miRNA and the 3′ UTRs. According to TargetScan [31], we downloaded the 3′ UTR sequences of all rat genes and predicted the target genes of our novel miRNAs using Basic Local Alignment Search Tool (BLAST). There were 124416 target sites and 9662 target genes for the novel miRNAs that we identified (data not shown).

2.5 Functional annotation

We determined that 83 novel miRNAs demonstrated significant change in expression levels among the five time-points. Then, their expression patterns were clustered, and five expression profiles revealing high expression of the novel miRNAs at the different time-points were selected for GO biological processes analysis (Appendix Table S8 in the electronic version).

Because a single miRNA has the potential to regulate hundreds of distinct target genes [32], we raised the threshold value of the context score of the target genes to increase accuracy. According to the cumulative distribution of the context score (Figure 3), and to provide a suitable number of target genes for analysis, we used a context score <–0.2 as the selection threshold. The 1078, 1364, 1492, 4633, and 2549 target genes included in the five expression profiles (Table 2) were used for GO biological process analysis. We classified the GO biological processes (FDR<0.01) into several general categories, including response to stimulus, death, developmental process, cellular component organization or biogenesis, cellular process, metabolic process, bio-

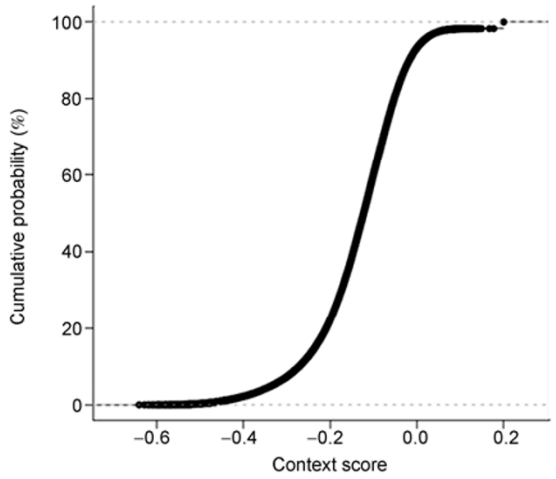

Figure 3 Cumulative distribution of novel miRNA target genes based on context score.

Table 2 Statistical analysis of the biological processes in which the novel miRNA target genes were involved

	0 h	1 d	4 d	7 d	14 d
miRNAs	5	8	9	31	8
Target genes	1078	1364	1492	4633	2549
GO Biological Process	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Response to stimulus	0	5	5	23	4
Death	0	2	3	6	3
Developmental process	2	12	22	60	44
Cellular component organization or biogenesis	0	3	5	19	9
Cellular process	1	5	5	29	8
Metabolic process	0	4	22	48	29
Biological regulation	0	7	15	41	16
Multicellular organismal process	0	0	4	10	6
Others	1	7	15	56	21
Total	4	45	96	292	140

logical regulation, and multicellular organismal process (Appendix Tables S9–S13 in the electronic version). The results (Table 2) demonstrated only four biological processes (FDR<0.01), two developmental processes, one cellular process and one localization, that were regulated by the novel miRNAs highly expressed at 0 h. On 1 d following sciatic nerve transection, biological processes in the almost all the categories were activated. On 4 d, genes involved in developmental processes, metabolic processes, biological regulation, and multicellular organismal processes continued to be significantly enhanced. On 7 d, all biological processes were active. By day 14, most of the biological processes had been downregulated compared with those on day 7, but metabolic processes, developmental processes, and cellular component organization or biogenesis remained at a high level.

3 Discussion

When the class of RNA regulatory genes known as miRNAs was discovered, it introduced a whole new layer of gene regulation in eukaryotes [33]. Recently, increasing attention has been focused on miRNAs, which is encouraging in the attempt to explain the molecular mechanisms of gene regulation, pathological processes, and drug action. To date, 1048 miRNAs in humans and 672 miRNAs in mice have been reported, while only 408 miRNAs have been reported in rats. This suggests that many miRNAs in rats remain undiscovered. miRNAs are particularly plentiful in neurons, and together with the fact that a given miRNA usually regulates the expression of hundreds of target mRNAs, neuronal miRNA pathways provide an extremely powerful mechanism to adjust the protein content of neuronal compartments dynamically without the need for new gene transcription [34]. To better understand the biological processes and regulatory mechanisms of the signaling pathways activated during peripheral nerve injury and regeneration, we searched for interactions and regulatory networks between miRNAs and mRNAs in the rat PSN following sciatic nerve transection.

Following sciatic nerve transection, the body underwent a stress response during the first two days, and prepared for nerve regeneration during the following two days. On days 5–7, the injured nerve began to grow, metabolism was very active and the new expression and regulation of proteins and genes were particularly common. We selected five time-points according to this scheme and analyzed the relative expression of novel miRNAs at these time-points in relation to the scheme. The greatest expression change was observed in the rat PSN 7 d after sciatic nerve transection, which is consistent with the above model.

We predicted the target genes of our novel miRNAs and analyzed their biological processes. Initially (at 0 h), the PSN demonstrated biological processes consistent with maintenance in the non-injury state. Most of the biological processes regulated by the identified highly expressed novel miRNAs were not statistically significant (FDR>0.01). However, following sciatic nerve transection, the PSN received and responded to stimulus signals. The death-associated biological processes were activated. Other biological processes that possibly provide a regeneration signal also showed up-regulation. Then, various metabolic processes increased to prepare for subsequent regeneration, and simultaneously, various developmental processes became functional. Correspondingly, metabolic processes, biological regulation, cellular component organization or biogenesis, cellular processes, and multicellular organismal processes were very active. On day 7, the injured nerve began to grow, accompanied with the highest activation of biological progress. By day 14, the PSN reverted towards stability, and most biological processes showed a downward trend, but the regeneration was still in progress, thus metabolic processes, developmental processes, and cellular component organization or biogenesis remained high.

It has been hypothesized that the molecular mechanisms

underlying regeneration-associated alterations in gene expression may recapitulate developmental processes [35], and to a certain extent, the development and homeostasis of organ systems depend on minas [36,37]. miRNAs are important players in many regulatory processes and are thus critical in the understanding of disease etiology. The identification and functional analysis of novel miRNAs in the rat PSN described here will prove important in understanding the biological processes following human peripheral nerve injury. In addition, our study provides an important resource for the future analysis of function and regulation of miRNAs. Experimental determination of their target genes and identification of the gene networks that trigger peripheral nerve repair and regeneration after injury remain a challenge for understanding and promoting peripheral nerve regeneration, counteracting unwanted consequences and assessing the utility of miRNAs as molecular targets for therapeutic intervention.

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1 Hama A T, Unnerstall J R, Siegan J B, *et al*. Modulation of NMDA receptor expression in the rat spinal cord by peripheral nerve injury and adrenal medullary grafting. Brain Res, 1995, 687: 103–113
2 Zhang D, Liu M, Ding F, *et al*. Expression of myostatin RNA transcript and protein in gastrocnemius muscle of rats after sciatic nerve resection. J Muscle Res Cell Motil, 2006, 27: 37–44
3 Angelov D N, Walther M, Streppel M, *et al*. Tenascin-R is antiadhesive for activated microglia that induce downregulation of the protein after peripheral nerve injury: a new role in neuronal protection. J Neurosci, 1998, 18: 6218–6229
4 Chen K H, Yang C H, Cheng J T, *et al*. Altered neuronatin expression in the rat dorsal root ganglion after sciatic nerve transection. J Biomed Sci, 2010, 17: 41–47
5 Costigan M, Mannion R J, Kendall G, *et al*. Heat shock protein 27: developmental regulation and expression after peripheral nerve injury. J Neurosci, 1998, 18: 5891–5900
6 Kloosterman W P, Plasterk R H. The diverse functions of microRNAs in animal development and disease. Dev Cell, 2006, 11: 441–450
7 Ambros V. The functions of animal microRNAs. Nature, 2004, 431: 350–355
8 Hwang H W, Mendell J T. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer, 2007, 96: R40–R44
9 Jovanovic M, Hengartner M O. miRNAs and apoptosis: RNAs to die for. Oncogene, 2006, 25: 6176–6187
10 Sood P, Krek A, Zavolan M, *et al*. Cell-type-specific signatures of microRNAs on target mRNA expression. Proc Natl Acad Sci USA, 2006, 103: 2746–2751
11 Kim J, Krichevsky A, Grad Y, *et al*. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proc Natl Acad Sci USA, 2004, 101: 360–365
12 Natera-Naranjo O, Aschrafi A, Gioio A E, *et al*. Identification and quantitative analyses of microRNAs located in the distal axons of sympathetic neurons. RNA, 2010, 16: 1516–1529
13 Fiore R, Schratt G. MicroRNAs in vertebrate synapse development. Sci World J, 2007, 7: 167–177
14 Schratt G. microRNAs at the synapse. Nat Rev Neurosci, 2009, 10: 842–849
15 Baek D, Villen J, Shin C, *et al*. The impact of microRNAs on protein output. Nature, 2008, 455: 64–71
16 Selbach M, Schwanhausser B, Thierfelder N, *et al*. Widespread changes in protein synthesis induced by microRNAs. Nature, 2008, 455: 58–63
17 Liu N K, Wang X F, Lu Q B, *et al*. Altered microRNA expression following traumatic spinal cord injury. Exp Neurol, 2009, 219: 424–429
18 Nakanishi K, Nakasa T, Tanaka N, *et al*. Responses of microRNAs 124a and 223 following spinal cord injury in mice. Spinal Cord, 2010, 48: 192–196
19 Chen X, Li Q, Wang J, *et al*. Identification and characterization of novel amphioxus microRNAs by Solexa sequencing. Genome Biol, 2009, 10: R78
20 Li R, Li Y, Kristiansen K, *et al*. SOAP: short oligonucleotide alignment program. Bioinformatics, 2008, 24: 713–714
21 Liu S, Li D, Li Q, *et al*. MicroRNAs of *Bombyx mori* identified by Solexa sequencing. BMC Genomics, 2010, 11: 148
22 Wang X, Zhang J, Li F, *et al*. MicroRNA identification based on sequence and structure alignment. Bioinformatics, 2005, 21: 3610–3614
23 Griffiths-Jones S, Grocock R J, van Dongen S, *et al*. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res, 2006, 34: D140–D144
24 Griffiths-Jones S, Saini H K, van Dongen S, *et al*. miRBase: tools for microRNA genomics. Nucleic Acids Res, 2008, 36: D154–D158
25 Wang X, Zhang J, Li F, *et al*. MicroRNA identification based on sequence and structure alignment. Bioinformatics, 2005, 21: 3610–3614
26 Jiang P, Wu H, Wang W, *et al*. MiPred: classification of real and pseudo microRNA precursors using random forest prediction model with combined features. Nucleic Acids Res, 2007, 35: W339–W344
27 Pillai R S. MicroRNA function: multiple mechanisms for a tiny RNA? RNA, 2005, 11: 1753–1761
28 Ernst J, Bar-Joseph Z. STEM: a tool for the analysis of short time series gene expression data. BMC Bioinformatics, 2006, 7: 191
29 Ng K L, Mishra S K. *De novo* SVM classification of precursor microRNAs from genomic pseudo hairpins using global and intrinsic folding measures. Bioinformatics, 2007, 23: 1321–1330
30 Chen C, Ridzon D A, Broomer A J, *et al*. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res, 2005, 33: e179
31 Lewis B P, Shih I H, Jones-Rhoades M W, *et al*. Prediction of mammalian microRNA targets. Cell, 2003, 115: 787–798
32 Lewis B P, Burge C B, Bartel D P. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell, 2005, 120: 15–20
33 Bartel D P. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004, 116: 281–297
34 Schratt G. Fine-tuning neural gene expression with microRNAs. Curr Opin Neurobiol, 2009, 19: 213–219
35 Vogelaar C F, Hoekman M F, Brakkee J H, *et al*. Developmental regulation of homeobox gene expression in dorsal root ganglion neurons is not recapitulated during regeneration of the crushed sciatic nerve. Neuroscience, 2004, 125: 645–650
36 Yekta S, Shih I H, Bartel D P. MicroRNA-directed cleavage of HOXB8 mRNA. Science, 2004, 304: 594–596
37 Stefani G, Slack F J. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol, 2008, 9: 219–230