Original Article

MicroRNA expression profile in the spinal cord injured rat neurogenic bladder by next-generation sequencing

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Background: An increasing amount of evidence has indicated that microRNAs (miRs) are involved in most biological conditions, including the neurogenic bladder (NB). However, to our knowledge, no studies have investigated these miR expressions in spinal cord-injured (SCI) rat NB. The goal of the study was to explore the miR expression profile in the SCI rat NB by next-generation sequencing (NGS).

Methods: Female Wistar rats underwent spinal cord transection at T9–10 and were randomly divided into the SCI-1, SCI-2 and SCI-3 groups (n=5 for each group) whose bladder tissues were collected 1, 2, and 4 weeks after transection, respectively. The normal rats were used as the normal control (NC) group. MiRs microarray assays were used to detect the differentially expressed miRs between the groups by NGS, which was then verified by quantitative real-time polymerase chain reaction (qRT-PCR). Those significantly differently expressed miRs were analyzed with Gene Ontology categories and Kyoto Encyclopedia of Genes and Genomes bioinformatical analyses.

Results: Compared with the NC group, 96, 28 and 51 miRs were downregulated in the rats' bladder in the SCI-1, SCI-2, and SCI-3 groups, respectively, and 133, 49, and 76 miRs were upregulated respectively. Specifically, miR-21-5p was the most significantly upregulated miR in all SCI groups. Also, 121 miRs (SCI-1 *vs.* SCI-2), 98 miRs (SCI-1 *vs.* SCI-3), and 26 miRs (SCI-2 *vs.* SCI-3) were of significantly different expression. Furthermore, a large set of genes implicated in essential signaling pathways were targeted by these miRs, including PI3K-Akt, MAPK, Rap1, and cGMP-PKG signaling pathways, along with the tight junction and metabolic pathways.

Conclusions: This is the first demonstration of differentially expressed miRs, which may potentially serve as new molecular targets in the SCI rat NB.

Keywords: MicroRNAs (miRs); neurogenic bladder (NB); spinal cord injury; rats; next-generation sequencing (NGS)

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Introduction

It has been estimated that 250,000 to 500,000 people worldwide are suffering from spinal cord injury and approximately 81% of these patients experience different levels of neurogenic bladder (NB) which is one of the leading causes of their morbidity (1,2). Specifically, detrusor overactivity and detrusor-sphincter dyssynergia are typical NB urodynamic findings, and are diagnosed in 95% and 68% of spinal cord-injured (SCI) patients, respectively (3).

These involuntary bladder contractions could result in urinary incontinence episodes during the storage phase, inefficient urine voiding, and high residual volume during urine voiding, potentially leading to upper urinary tract damage and substantially impacting the health-related quality of life (4). At the same time, the currently available treatment options are not satisfactory.

The exact mechanisms of NB secondary in spinal cord injury have not yet been identified. It is postulated that C fibers mediated new spinal reflex circuits and neurotrophic hormones like nerve growth factor participate in NB after spinal cord injury (5). In recent years, an increased mount of evidence has indicated that microRNAs (miRs) are essential regulators of most physiological and pathological events, which might also include the potential pathophysiology and treatment outcomes of NB. MiRs are non-coding RNAs, approximately 19–23 nucleotides (nt) in length. They participate in epigenetic post-transcriptional control of protein-coding gene expression primarily by reversible translational repression or mRNA destabilization/degradation and thereby inhibit protein synthesis (6).

Recently, Chermansky *et al.* reported that the elevated expression of miR-221 and miR-125b in detrusor overactivity in the bladder tissue of patients may predict their high risk for undergoing urinary retention following intradetrusor injection of onabotulinumtoxin-A (6). In addition, the combination of upregulated miR-98-5p and downregulated miR-139-5p in the plasm of patients' overactive bladder (OAB) was found to be a useful biomarker for OAB. However, no correlation was determined between the levels of miRs and OAB symptom score (7). Meanwhile, the above research only concentrated on finding the expression patterns of miRNA particularly in OAB patients. To our knowledge, no studies have comprehensively investigated miR expressions in the SCI rat NB.

Therefore, this study aimed to explore the miR expression profile in the SCI rat bladder by next-generation sequencing (NGS), which may yield molecular targets for NB. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) bioinformatical analyses were also performed to investigate the functions of these miRs. We present the following article in accordance with the ARRIVE reporting checklist (available at http://dx.doi.org/10.21037/tau-20-415).

Methods

Ethical approval

All experimental procedures were implemented in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (2) and approved by the Institutional Animal Care and Use Committee of Xuanwu Hospital Capital Medical University (No. 20190128).

SCI rat model

Adult female Wistar rats weighing 200–250 g (Beijing Charles River Laboratories Animal Technology Co., Ltd., Beijing, China) were used in this study, as their urethras are shorter and more conducive to bladder evacuation via abdominal compression. The animals were maintained at 20–26 ℃ and 30–70% relative humidity under a 12-h light/dark cycle with ad libitum access to food and water. The spinal cord was transected at the T9–10 in rats. Under 3% enflurane inhalation in the rats, Th10 laminectomy was performed, and the dura was sharply transected. The spinal cord was completely severed at T10, and Gelfoam (Ethicon) was placed between the cut ends to aid in hemostasis and to prevent the cut ends from healing. The muscle layer and skin were separately sutured. The rats received ampicillin sodium (100 mg/kg intramuscularly) for 5 days after operation. Bladders were emptied 3 times daily by abdominal compression until reflex voiding returned, and once a day afterward. The normal rats were used as the normal control (NC) group.

Bladder collection

Before bladder collection, 15 female Wistar rats that underwent spinal cord transection at T9–10 were randomly divided into SCI-1, SCI-2, and SCI-3 groups (n=5 for each group), and their bladder tissues were collected 1, 2, and 4 weeks after spinal cord transection, respectively. The rats were anesthetized with 3% enflurane inhalation, and a midline laparotomy was performed in the lower abdomen to expose the bladder. Each bladder was surgically removed at the level of the bladder neck and longitudinally cut into halves on ice. The bladder tissues were then stored in liquid nitrogen.

MiRs microarray procedures

Three samples of each group and 3 replicates of each tissue were used for RNA sequencing. Libraries were constructed using the NEBNext® Multiplex Small RNA Library Prep Set for Illumina (Set 2; New England BioLabs, Inc.,

Ipswich, MA, USA). Briefly, total RNA was isolated by using Trizol (Invitrogen; Thermo Fisher Scientific, Inc., USA). The quantity and integrity of RNA yield were assessed by using the Qubit®2.0 (Life Technologies, USA) and Agilent 2200 TapeStation (Agilent Technologies, USA) separately. Enriched fragments were sequenced by HiSeq 2500 Sequencing System (Illumina Inc., San Diego, CA, USA) with single-end 50 bp at Ribobio Co. Ltd (Ribobio, China).

Data processing and bioinformatic analysis

The raw reads were filtered to obtain clean reads by removing those with an adaptor sequence or those with a percentage of unknown bases more than 10%, low-quality reads, and smaller than 17 nt reads by FASTQC. The clean reads obtained were mapped to reference genome by Burrows-Wheeler Aligner (BWA) software. miRDeep2 software was used to identify the known mature miRNA based on miRBase21 (www.miRBase.org) and predict novel miRNA. Databases of Rfam12.1 (www.rfam.xfam.org) and pirnabank (www.pirnabank.ibab.ac.in) were used to identify ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and PIWI-interacting RNA (piRNA) by Basic Local Alignment Search Tool (BLAST). The miRNA expression was counted and normalized by reads per million (RPM) values [PRM = (number of reads mapping to miRNA/number of reads in clean data) $\times 10^6$]. Differential expression between samples was calculated by DESeq2 algorithm according to the criteria of $\log_2$ |fold change| >1 and P<0.05. TargetScan, miRDB, miRTarBase, and miRWalk were used to predict the genes targeted by selected miRNA. KOBAS was used for further GO and KEGG pathway analyses.

Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR analysis was performed to verify the accuracy of the microarray assays. MiR expressions were determined by the CFX Connect™ Real-Time PCR detection system (Bio-Rad Laboratories, CA, USA) in triplicate and calculated using the $2^{-\Delta\Delta Ct}$ method. U6 was used as an internal reference for miRs. Primers for qRT-PCR were provided by Bulge-Loop miRNA qRT-PCR Primer Sets (Guangzhou RiboBio Co., Ltd., Guangzhou, China). Total RNA was extracted from samples of the bladder by using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., USA). cDNAs were synthesized from 2 μg of total RNA using a mixture of Oligo-dT and random primers or specific primers with M-MLV reverse transcriptase (Promega Corporation, USA). The following thermocycling conditions were used: 95 ℃ for 1 minute, followed by 40 cycles at 95 ℃ for 10 seconds, 60 ℃ for 20 seconds, and 70 ℃ for 10 seconds.

Statistical analysis

Differential expression analysis of miRs obtained from NGS was performed by the DESeq2 (v. 1.16.1), which was an algorithm to examine differences between groups by using a generalized linear model and assuming a negative binomial distribution of RNA-Seq reads. Statistically, differences in the levels of miRs verified by qRT-PCR between groups were determined by analysis of variance (ANOVA) using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). The differentially expressed miRNAs were identified using the following thresholds: P<0.05; $\log_2$ |fold change| >1. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

Identification of differentially expressed miRs between SCI and NC groups

Compared with the NC group, 96, 28 and 51 miRs were significantly downregulated in the bladders of the SCI-1, SCI-2, and SCI-3 groups, respectively, and 133, 49 and 76 miRs were significantly upregulated in the rat bladders of SCI-1, SCI-2 and SCI-3 groups, respectively (*Tables 1-3*). Specifically, miR-21-5p was the most significantly upregulated miR in all the SCI groups. Moreover, 206 new miRs were identified in the bladder and are shown in *Table S1*.

Identification of differentially expressed miRs between SCI groups

Compared with the SCI-1 group, 81 and 65 miRs were significantly downregulated in the rat bladders of the SCI-2 and SCI-3 groups, respectively, and 40 and 33 miRs were significantly upregulated in the rat bladders of the SCI-2 and SCI-3 groups respectively (*Tables 4,5*). Compared with the SCI-2 group, 5 miRs were significantly downregulated, and 21 miRs were significantly upregulated in the rat bladders of the SCI-3 groups (*Table 6*).

Table 1 Ninety-six significantly down-regulated and 133 significantly up-regulated miRNAs in SCI-1 group compared to NC group

miRNA	log_2-ratio (SCI-1/NC)	P value
miR-139-5p	–3.102182882	1.75E-56
miR-21-5p	2.221163332	1.35E-49
miR-466c-5p	3.184432599	2.95E-42
miR-125a-5p	–1.829219179	1.80E-41
miR-149-5p	–2.143417515	2.49E-37
miR-1-3p	–2.194065038	1.02E-34
miR-181a-5p	–1.623090527	3.15E-33
miR-145-5p	–2.367867781	4.84E-33
miR-411-5p	2.866397294	4.01E-32
miR-132-3p	2.337233687	1.42E-30
miR-298-5p	3.050317374	1.67E-30
miR-328a-3p	–1.91132018	7.08E-30
miR-129-5p	–2.684218688	1.19E-29
miR-133a-3p	–2.639745931	3.86E-29
miR-382-3p	3.083780543	1.81E-28
miR-495	3.246664284	1.49E-27
miR-132-5p	2.637582812	3.47E-26
miR-379-5p	2.713173134	3.87E-26
miR-212-3p	3.166034219	1.23E-25
miR-379-3p	2.891056501	1.52E-25
miR-411-3p	3.093952173	2.15E-25
miR-139-3p	–2.428682251	4.64E-25
miR-29c-5p	–2.259756176	4.76E-25
miR-1193-3p	3.095183493	7.12E-25
miR-376b-3p	3.811300461	3.84E-23
miR-212-5p	2.698225438	1.46E-22
miR-15b-3p	2.48316802	3.42E-22
miR-320-3p	–1.678142165	3.77E-22
miR-494-3p	2.782278241	4.84E-22
miR-540-3p	2.538638666	1.25E-21
miR-493-5p	2.588627944	3.42E-21
miR-434-5p	2.665719473	5.01E-21
miR-134-5p	2.399485438	5.71E-21
miR-431	2.280672634	1.87E-20

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	log_2-ratio (SCI-1/NC)	P value
miR-341	2.091476704	3.20E-20
miR-99b-5p	–1.332158911	6.20E-20
miR-133b-3p	–2.39189828	5.72E-19
miR-204-5p	–1.959297741	5.85E-19
miR-129-1-3p	–2.892021806	8.06E-19
let-7d-3p	–1.636823106	9.13E-19
miR-29b-5p	–2.447544506	9.97E-19
miR-410-3p	2.213844944	1.11E-18
miR-299a-3p	2.584887153	1.24E-18
miR-338-5p	–2.041548097	1.87E-18
miR-466b-5p	2.878992417	2.83E-18
miR-1b	–1.75345764	9.93E-18
miR-299b-3p	2.572487155	1.16E-17
miR-296-3p	3.946209334	1.40E-17
miR-127-5p	2.144357263	3.13E-17
miR-181d-5p	–1.481396568	3.28E-17
miR-370-3p	1.940524375	4.39E-17
miR-30c-2-3p	–1.46199406	5.88E-17
miR-409a-3p	2.003956093	4.11E-16
miR-130b-5p	1.90853073	4.59E-16
miR-184	2.346878698	4.61E-16
miR-543-3p	1.906948855	7.94E-16
miR-300-3p	2.067638501	8.03E-16
miR-29a-3p	–1.037912307	9.18E-16
miR-409a-5p	2.179408619	1.30E-15
miR-1249	–1.889683111	1.63E-15
miR-23b-3p	–1.555750442	2.12E-15
miR-380-3p	3.30194013	3.54E-15
miR-143-5p	–1.477749401	3.95E-15
miR-127-3p	1.460866685	6.67E-15
miR-150-5p	–1.682559818	4.99E-14
miR-541-5p	2.132434591	5.02E-14
miR-221-3p	1.369736139	5.67E-14
miR-337-5p	1.679642486	5.92E-14
miR-452-5p	2.848340092	9.00E-14

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	log_2-ratio (SCI-1/NC)	P value
miR-125b-5p	–1.083888826	1.05E-13
let-7e-3p	–1.450696119	1.16E-13
miR-30a-3p	–1.155344661	1.59E-13
miR-504	–2.292273523	1.77E-13
miR-223-5p	2.302263875	1.88E-13
miR-378b	–1.209495332	2.91E-13
miR-503-5p	1.430411192	3.19E-13
miR-708-5p	–1.719135844	3.87E-13
miR-211-5p	–1.863725451	4.51E-13
miR-31a-5p	1.460585107	4.80E-13
miR-378a-5p	–1.269112031	5.68E-13
let-7e-5p	–1.133920645	7.24E-13
miR-496-3p	2.541231703	7.40E-13
miR-378a-3p	–1.212208456	9.26E-13
miR-140-5p	1.346569902	1.70E-12
miR-330-3p	–1.575317256	2.24E-12
miR-155-5p	1.491246802	4.30E-12
miR-223-3p	1.787455625	4.88E-12
miR-322-5p	1.679814718	8.14E-12
miR-770-3p	1.980950727	2.36E-11
miR-342-3p	–1.567011952	4.32E-11
miR-676	–1.569928685	4.43E-11
miR-218a-5p	1.331073023	4.76E-11
miR-450a-5p	1.295604623	1.03E-10
miR-505-3p	–1.336336399	1.12E-10
miR-9a-5p	–1.316114776	1.25E-10
miR-423-5p	–1.037698356	1.45E-10
miR-142-5p	1.720418902	1.56E-10
miR-146b-5p	1.479442337	1.91E-10
miR-450b-5p	1.220783652	2.94E-10
miR-542-3p	1.667710786	4.17E-10
miR-331-3p	–1.23153033	5.48E-10
miR-493-3p	1.888123357	7.97E-10
miR-193a-5p	–1.306363196	8.38E-10

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	log_2-ratio (SCI-1/NC)	P value
miR-181b-5p	–1.029122766	8.74E-10
miR-26b-3p	–1.620736939	9.29E-10
miR-433-3p	1.333581542	1.31E-09
miR-31a-3p	2.320944268	1.62E-09
miR-758-3p	2.375835876	2.13E-09
miR-29c-3p	–1.334293219	2.86E-09
miR-361-3p	–1.067846562	5.43E-09
miR-511-3p	1.768513538	5.83E-09
miR-374-5p	1.057767481	7.12E-09
miR-363-3p	1.167009367	9.19E-09
miR-466b-4-3p	3.573146084	1.41E-08
miR-466b-2-3p	3.573146084	1.41E-08
miR-17-5p	1.35661569	2.26E-08
miR-434-3p	1.112430116	2.51E-08
miR-330-5p	–1.272071423	3.05E-08
miR-126a-3p	1.105891785	3.19E-08
miR-193b-3p	–1.193427185	4.13E-08
miR-485-3p	1.309238138	4.16E-08
miR-652-3p	–1.151824972	4.25E-08
miR-664-2-5p	–1.325200111	4.49E-08
miR-181c-5p	–1.344033479	5.11E-08
miR-124-3p	–2.736550476	5.53E-08
miR-296-5p	1.94671836	5.92E-08
miR-664-3p	–1.280718524	7.10E-08
miR-369-5p	1.704897781	8.05E-08
let-7i-3p	–1.144553333	1.01E-07
miR-30c-1-3p	–1.141885436	1.41E-07
miR-376a-5p	2.898910594	1.45E-07
miR-136-3p	2.276879909	2.03E-07
miR-147	4.722615042	2.07E-07
miR-324-5p	–1.474568556	2.09E-07
miR-383-5p	–1.367148471	3.87E-07
miR-369-3p	2.419026089	4.14E-07
miR-1306-5p	–1.143077487	4.41E-07

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	log_2-ratio (SCI-1/NC)	P value
miR-665	2.72800571	6.66E-07
miR-365-3p	–1.154939824	7.90E-07
miR-1843a-3p	–1.186555516	8.82E-07
miR-466b-3p	2.014219539	1.01E-06
miR-210-3p	–1.243918488	1.04E-06
miR-133a-5p	–1.421989127	1.15E-06
miR-24-1-5p	–1.168682039	1.39E-06
miR-382-5p	1.129487387	1.93E-06
let-7b-3p	–1.115343686	2.07E-06
miR-326-3p	–1.333250513	2.08E-06
miR-346	–2.187914853	2.27E-06
miR-582-3p	1.155652793	2.62E-06
miR-29b-3p	–1.175745009	3.11E-06
miR-380-5p	2.227842964	3.16E-06
miR-146b-3p	1.133080107	3.17E-06
miR-129-2-3p	–2.216258422	3.54E-06
miR-99a-3p	–1.898295287	5.38E-06
miR-3577	–1.450639165	6.42E-06
miR-154-5p	1.303208005	6.67E-06
miR-206-3p	–1.425103365	7.05E-06
miR-338-3p	–1.139524526	7.92E-06
miR-299a-5p	1.493976295	9.67E-06
miR-7a-1-3p	1.448137132	1.26E-05
miR-3559-5p	1.157007276	1.27E-05
miR-217-5p	–1.060663628	1.86E-05
miR-34c-5p	1.165838023	1.93E-05
miR-34b-5p	1.35654223	3.37E-05
miR-708-3p	–1.298912776	3.62E-05
miR-329-3p	1.011931442	4.03E-05
miR-324-3p	–1.056232828	4.14E-05
miR-1247-3p	1.439598936	4.23E-05
miR-204-3p	–1.603099354	5.53E-05
miR-3594-3p	–2.097600786	5.83E-05
miR-3102	–1.301393539	6.19E-05

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	log_2-ratio (SCI-1/NC)	P value
miR-675-3p	5.376720886	6.25E-05
miR-298-3p	1.666250663	9.25E-05
miR-3064-5p	–1.386971792	0.0001146
miR-135b-5p	–1.018638112	0.00011753
miR-6329	1.253243538	0.00012762
miR-130b-3p	1.906869385	0.00014886
miR-3099	–2.879178527	0.00016084
miR-138-1-3p	–1.694585122	0.00017551
miR-362-5p	1.107395298	0.00018552
miR-376b-5p	1.388725246	0.00019653
miR-20b-5p	2.411053913	0.00035965
miR-673-3p	1.645167515	0.00041933
miR-203b-3p	2.187699694	0.0004772
miR-146a-3p	3.056395912	0.00052588
miR-20a-5p	1.074404903	0.00054571
miR-21-3p	1.28424385	0.00086862
miR-667-5p	2.138578479	0.00087156
miR-582-5p	1.726650312	0.00087391
miR-377-3p	4.707555166	0.00097947

GO and KEGG analyses of signaling pathways and genes targeted by these differentially expressed miRs

A large set of essential signaling pathways were targeted by these miRs, including PI3K-Akt, MAPK, Rap1, and cGMP-PKG signaling pathways, along with the tight junction, metabolic pathways, regulation of actin cytoskeleton and pathways in cancer, as shown in *Figure 1*. Meanwhile, *Figure 2* shows the genes targeted by these miRs. For example, Smad7 and Smad5 were targeted by miR-21-5p, while nfat5 was targeted by miR-146a-5p, miR-139-5p, and miR-132-3p.

Validation of miR expression by qRT-PCR

The levels of 5 differentially expressed miRs, including miR-139-5p, miR-21-5p, miR-149-5p, miR-146a-5p, and miR-134-5p, were assessed by qRT-PCR to verify the results of microarray assays. As shown in *Figure 3*, miR-139-5p was significantly downregulated in all SCI

Table 2 Twenty-eight significantly down-regulated and 49 significantly up-regulated miRNAs in SCI-2 group compared to NC group

miRNA	log_2-ratio (SCI-2/NC)	P value
miR-21-5p	1.342771	1.08E-18
miR-31a-5p	1.894352	1.32E-18
miR-139-5p	–1.67075	1.89E-17
miR-155-5p	1.834116	2.43E-16
miR-149-5p	–1.51517	2.42E-15
miR-129-5p	–1.95962	2.04E-14
miR-204-5p	–1.72873	5.57E-13
miR-223-3p	2.086425	2.68E-12
miR-429	1.296582	1.94E-11
miR-140-5p	1.388503	2.14E-11
miR-338-5p	–1.49331	2.11E-10
miR-322-5p	1.54202	2.15E-10
miR-15b-3p	1.873105	1.94E-09
miR-9a-5p	–1.15968	1.70E-08
miR-211-5p	–1.64161	1.96E-08
miR-708-3p	–1.98602	2.24E-08
miR-132-3p	1.44845	2.79E-08
miR-139-3p	–1.12477	3.02E-08
miR-212-5p	1.434302	7.44E-08
miR-221-3p	1.018423	8.46E-08
miR-200a-3p	1.206934	1.92E-07
miR-676	–1.37977	2.31E-07
miR-203a-3p	1.011467	3.33E-07
miR-223-5p	2.167995	3.66E-07
miR-17-5p	1.444012	4.51E-07
miR-362-5p	1.420823	6.40E-07
miR-129-1-3p	–1.60183	7.43E-07
miR-15b-5p	1.187088	8.77E-07
miR-31a-3p	1.962596	2.48E-06
miR-204-3p	–2.44611	2.67E-06
miR-212-3p	1.678392	3.93E-06
miR-466c-5p	1.529295	4.77E-06
miR-338-3p	–1.34871	5.35E-06
miR-339-5p	1.132377	6.99E-06
miR-370-3p	–1.31691	7.92E-06
miR-221-5p	1.025674	1.26E-05
miR-147	4.128422	1.76E-05
miR-20a-5p	1.408173	2.99E-05
miR-99a-5p	–1.0657	3.32E-05
miR-15a-5p	1.228399	6.76E-05
miR-106b-5p	1.022472	7.36E-05
miR-9a-3p	–2.00532	7.53E-05
miR-10a-3p	1.013045	8.64E-05
miR-19b-3p	1.63836	9.43E-05
miR-148a-3p	–1.03707	0.000161853
miR-543-3p	–1.24524	0.000319661
miR-466b-4-3p	2.586543	0.000373783
miR-466b-2-3p	2.586543	0.000373783
miR-132-5p	1.00103	0.000412543
miR-466b-5p	1.46694	0.000446337
miR-142-3p	1.364244	0.00051408
miR-20b-5p	2.501238	0.000667041
miR-203b-3p	2.212203	0.00093856
miR-298-5p	1.403063	0.000959402
miR-142-5p	1.045109	0.000990114

groups compared to the NC group, and miR-21-5p was significantly upregulated in all SCI groups. Furthermore, miR-149-5p was also significantly downregulated in all SCI groups. Nevertheless, miR-146a-5p was significantly upregulated by 5.48-, 2.51-, and 3.46-fold in the SCI-3 group compared to the NC, SCI-1, and SCI-2 groups, respectively, and miR-134-5p was significantly upregulated in the SCI-1 group compared to the NC, SCI-2, and SCI-3 groups.

Discussion

To the best of our knowledge, this study is the first to comprehensively investigate the miRs expression profile in SCI rat NB. The present results showed that

Table 3 Fifty-one significantly down-regulated and 76 significantly up-regulated miRNAs in SCI-3 group compared to NC group

miRNA	log_2-ratio (SCI-3/NC)	P value
miR-21-5p	2.37181238	2.11E-65
miR-450a-5p	2.62226041	1.17E-33
miR-322-5p	3.03263678	1.01E-31
miR-503-5p	2.96141352	1.05E-30
miR-450b-5p	2.65797646	7.88E-30
miR-140-5p	1.94388628	6.09E-22
miR-149-5p	–1.8875642	6.82E-22
miR-139-5p	–1.6688496	9.62E-18
miR-129-5p	–2.1236755	4.93E-17
miR-146a-5p	1.7800553	5.72E-17
miR-155-5p	2.26176078	1.39E-16
miR-542-3p	2.28731257	2.49E-15
miR-147	7.14321921	4.84E-15
miR-504	–2.3276966	1.18E-14
miR-429	2.24780895	4.41E-14
miR-338-5p	–1.8158385	1.21E-13
miR-466c-5p	2.42073356	1.53E-13
miR-223-5p	2.95912087	2.09E-13
miR-212-5p	2.18225192	3.08E-13
miR-142-3p	2.61231457	3.54E-13
let-7d-5p	–1.0453551	5.21E-13
miR-181a-5p	–1.0653858	1.34E-12
miR-466b-5p	2.69953044	1.56E-12
miR-132-3p	1.83951489	1.83E-12
miR-328a-3p	–1.2027914	2.29E-11
miR-455-3p	–1.5686893	4.56E-11
miR-200a-3p	1.76934063	4.78E-11
miR-200b-3p	1.62826789	5.17E-11
miR-139-3p	–1.4391414	9.75E-11
let-7d-3p	–1.3420112	1.73E-10
miR-363-3p	1.97278183	2.44E-10
miR-223-3p	1.98995205	2.85E-10
miR-129-1-3p	–2.2960898	1.11E-09
miR-132-5p	1.60294222	2.62E-09
miR-221-5p	1.6469837	1.39E-08
miR-130b-5p	2.24560005	1.47E-08
miR-22-3p	1.10175038	6.16E-08
miR-125a-5p	–1.0591798	1.18E-07
miR-532-5p	1.18702295	2.29E-07
miR-146b-3p	1.9820625	6.25E-07
miR-15b-3p	1.64451454	7.80E-07
miR-362-5p	1.64335274	8.72E-07
miR-142-5p	3.57927795	9.02E-07
miR-146a-3p	4.28699097	1.23E-06
miR-181a-2-3p	–1.4024575	2.42E-06
miR-676	–1.1301808	3.31E-06
miR-34c-5p	1.50066546	3.51E-06
miR-126a-5p	1.03740906	3.70E-06
miR-19b-3p	1.74623161	3.81E-06
let-7e-3p	–1.0694482	4.36E-06
miR-505-3p	–1.0887262	6.73E-06
miR-146b-5p	2.87650384	7.74E-06
miR-31a-3p	1.93258402	9.86E-06
miR-295-3p	4.83073688	2.07E-05
miR-1306-5p	–1.232282	2.72E-05
miR-20b-5p	2.97228599	2.94E-05
miR-200a-5p	1.31731223	3.69E-05
miR-17-2-3p	2.49633491	3.76E-05
miR-124-3p	–2.3386931	3.80E-05
miR-221-3p	1.02916013	4.24E-05
miR-3102	–1.7001201	4.80E-05
miR-292-5p	4.64055069	5.34E-05
miR-375-3p	–1.2552555	5.36E-05
miR-193a-5p	–1.073083	5.49E-05
miR-26b-3p	–1.2414321	7.03E-05
miR-6329	1.5884213	8.59E-05
miR-326-3p	–1.1121066	9.61E-05
miR-34b-5p	1.61261524	0.000101199

Table 3 (*continued*)

Table 3 (*continued*)

miRNA	log_2-ratio (SCI-3/NC)	P value
miR-298-5p	1.45716953	0.000101971
miR-466b-4-3p	2.93009212	0.000107491
miR-466b-2-3p	2.93009212	0.000107491
miR-212-3p	1.75667346	0.000112073
miR-324-3p	–1.0864263	0.000122576
miR-20a-5p	1.28625132	0.000125546
miR-1249	–1.2134585	0.000135676
miR-9a-3p	–1.7243301	0.000150736
miR-345-5p	–1.0130189	0.00018066
miR-3099	–4.9919378	0.000189656
miR-133a-3p	–1.1249025	0.000216883
miR-130b-3p	2.16623648	0.000219139
miR-106b-5p	1.09029431	0.000274774
miR-672-5p	–1.0029872	0.000296051
miR-338-3p	–1.1388944	0.000309787
miR-17-5p	1.13609523	0.000417046
miR-3577	–1.3768138	0.000428649
miR-148a-5p	1.09743129	0.00044269
miR-511-3p	1.35189925	0.000479029
miR-196a-5p	–1.1142367	0.000655131
miR-708-3p	–1.2495066	0.000776355
miR-323-3p	–1.0204913	0.000807575
miR-540-3p	–1.1112974	0.000817585
miR-296-3p	1.93252131	0.00095342

compared with the NC group, 96, 28, and 51 miRs were downregulated in the rat bladders of SCI-1, SCI-2, and SCI-3 groups, respectively, and 133, 49, and 76 miRs were upregulated in the rat bladders of SCI-1, SCI-2, and SCI-3 groups, respectively. Specifically, miR-21-5p was the most significantly upregulated miR in all SCI groups. In addition, 121 miRs (SCI-1 *vs.* SCI-2), 98 miRs (SCI-1 *vs.* SCI-3), and 26 miRs (SCI-2 *vs.* SCI-3) were of significantly different expression. Moreover, 206 new miRs were identified in the bladder. Furthermore, a large set of genes implicated in essential signaling pathways were targeted by these miRs, including PI3K-Akt, MAPK, Rap1, and cGMP-PKG

Table 4 Eighty-one significantly down-regulated and 40 significantly up-regulated miRNAs in SCI-2 group compared to SCI-1 group

miRNA	log_2-ratio (SCI-2/SCI-1)	P value
miR-133a-3p	2.608263788	2.62E-43
miR-411-5p	–2.74522037	3.11E-35
miR-134-5p	–3.01646037	2.01E-34
miR-133b-3p	2.241527254	3.13E-32
miR-540-3p	–3.4576132	1.95E-27
miR-370-3p	–3.35185426	1.19E-23
miR-434-5p	–3.26188865	4.77E-23
miR-770-3p	–3.12840943	2.15E-22
miR-379-5p	–2.65131382	1.68E-21
miR-495	–3.4738593	1.98E-21
miR-410-3p	–2.32240022	7.25E-21
miR-143-5p	1.532807273	1.56E-19
miR-485-3p	–2.33658978	2.64E-19
miR-376b-3p	–3.79903309	4.43E-19
miR-341	–2.6472862	2.49E-18
miR-145-5p	2.729755793	5.27E-18
miR-320-3p	1.359090737	1.18E-17
miR-543-3p	–3.25484939	2.27E-17
miR-127-3p	–1.9670069	1.46E-16
miR-490-3p	1.567095484	2.44E-16
miR-29b-5p	2.31974577	7.07E-16
miR-125a-5p	1.373690239	2.97E-15
miR-300-3p	–2.21735369	3.96E-15
miR-541-5p	–2.31005283	8.60E-15
miR-380-3p	–3.43639219	9.76E-14
miR-296-3p	–3.36779258	1.25E-13
miR-205	1.462348678	1.99E-13
miR-493-5p	–2.55161665	2.86E-13
miR-1b	1.191285776	3.63E-13
miR-375-3p	1.470598973	3.82E-13
miR-409a-3p	–2.22414306	4.39E-13
miR-493-3p	–2.54745748	6.05E-13
miR-382-3p	–3.11323256	9.03E-13

Table 4 (*continued*)

Table 4 (*continued*)

miRNA	log_2-ratio (SCI-2/SCI-1)	P value
miR-181a-5p	1.056129411	9.66E-13
miR-411-3p	–2.61227376	2.57E-12
miR-369-5p	–2.12220497	3.62E-12
miR-494-3p	–2.10745377	1.32E-11
miR-130b-5p	–1.26694073	1.65E-11
miR-299b-3p	–2.11524277	1.70E-11
miR-382-5p	–1.61074807	3.04E-11
miR-299a-3p	–2.01331537	6.99E-11
miR-758-3p	–2.94825106	9.10E-11
miR-218a-5p	–1.25014301	1.14E-10
miR-378a-5p	1.109953116	1.35E-10
miR-132-5p	–1.73490045	1.45E-10
miR-455-5p	–1.31786364	1.64E-10
miR-1247-3p	–2.57037848	2.97E-10
miR-379-3p	–2.26202154	3.01E-10
miR-365-3p	1.643485681	3.40E-10
miR-1-3p	1.413388204	3.92E-10
miR-1193-3p	–2.17397441	4.59E-10
miR-664-3p	1.389462332	4.90E-10
miR-127-5p	–1.78617864	7.25E-10
miR-452-5p	–2.21588601	1.13E-09
miR-139-5p	1.337379266	1.27E-09
miR-434-3p	–1.30726196	2.12E-09
miR-337-5p	–1.9546922	2.16E-09
miR-466c-5p	–1.74885079	3.20E-09
miR-330-3p	1.378312119	6.07E-09
miR-496-3p	–1.86126645	9.30E-09
miR-133a-5p	1.515887194	9.33E-09
miR-485-5p	–1.309451	1.38E-08
miR-431	–2.07956874	2.03E-08
miR-126a-3p	–1.13484835	3.27E-08
miR-1249	1.2596351	4.17E-08
miR-409a-5p	–1.7399784	6.30E-08
miR-186-5p	1.016668658	7.25E-08

Table 4 (*continued*)

Table 4 (*continued*)

miRNA	log_2-ratio (SCI-2/SCI-1)	P value
miR-1843a-3p	1.250062543	1.08E-07
miR-212-5p	–1.36063182	1.42E-07
miR-433-3p	–1.5483857	1.70E-07
miR-323-3p	–1.16570373	1.85E-07
miR-99a-3p	2.133335482	1.98E-07
let-7c-1-3p	–1.58017681	2.27E-07
let-7c-2-3p	–1.03717891	2.39E-07
let-7a-1-3p	–1.03717891	2.39E-07
let-7d-3p	1.080665154	2.61E-07
miR-26b-3p	1.232968381	2.66E-07
miR-466b-5p	–1.49395071	2.73E-07
miR-212-3p	–1.5850046	2.82E-07
miR-6331	–1.60680229	3.32E-07
miR-150-5p	1.366309319	3.50E-07
miR-487b-3p	–1.28578195	4.17E-07
miR-708-5p	1.49100303	4.23E-07
miR-665	–3.21581941	5.22E-07
miR-322-3p	–1.17394689	5.48E-07
miR-29c-5p	1.287011985	5.51E-07
miR-103-3p	1.048341116	6.97E-07
miR-673-3p	–2.71045179	1.53E-06
miR-505-3p	1.00770467	1.58E-06
miR-667-3p	–1.46537091	1.80E-06
miR-298-5p	–1.74380033	2.26E-06
miR-139-3p	1.212784082	3.34E-06
miR-346	2.681838145	4.38E-06
miR-154-5p	–1.25813496	8.95E-06
miR-369-3p	–2.1952968	9.03E-06
miR-376b-5p	–1.76232448	1.15E-05
miR-6329	–1.51973384	1.63E-05
miR-342-3p	1.532839319	1.75E-05
miR-466b-3p	–1.55817849	1.94E-05
miR-148a-3p	–1.08230586	2.31E-05
miR-1188-5p	–5.54436782	2.42E-05
miR-148a-5p	–1.14139035	3.15E-05

Table 4 (*continued*)

Table 4 (*continued*)

miRNA	log_2-ratio (SCI-2/SCI-1)	P value
miR-667-5p	–3.63119254	4.30E-05
miR-329-3p	–1.27792595	4.34E-05
miR-383-5p	1.139922737	5.92E-05
miR-3473	–1.06517431	5.97E-05
miR-136-3p	–1.84129683	8.01E-05
miR-412-5p	–1.6696497	0.00011838
miR-138-5p	1.158249483	0.00012067
miR-190a-5p	1.795778971	0.00014354
miR-673-5p	–1.44773878	0.00022481
miR-541-3p	–4.82904003	0.00022815
miR-136-5p	–1.98858235	0.00026216
miR-376a-5p	–1.54201787	0.00035526
miR-324-5p	1.513136796	0.00038516
miR-210-5p	1.351344165	0.0004134
miR-412-3p	–2.47723181	0.00051183
miR-329-5p	–1.05001232	0.00055935
miR-129-1-3p	1.190742769	0.00072475
miR-144-5p	–3.13263127	0.00084192
miR-193b-3p	1.001305355	0.00088215

signaling pathways, along with tight junction and metabolic pathways.

These essential signaling pathways have been previously implicated in bladder dysfunction. miR - 139 - 5p may inhibit epithelial-mesenchymal transition (EMT) and fibrosis by targeting the lysophosphatidic acid receptor 4 via the PI3K-Akt signaling pathway (8). Also, the activation of the PI3K-Akt signaling pathway may play a pivotal part in bladder ischemia, which might be a mediating variable in the development of detrusor overactivity or fibrosis (9). Furthermore, collagen expression and bladder hypertrophy were regulated by nerve growth factor through the Akt and MAPK pathways (10). In addition, activation of the cGMP-PKG signaling pathway may result in bladder relaxation or reduce phasic contractions in rat bladder strips (11,12). Whether the signaling pathways targeted by these miRs can exhibit these functions mentioned above *in vivo* will be explored in further studies.

Among these miRNAs, miR-139-5p was the most

Table 5 Sixty-five significantly down-regulated and 33 significantly up-regulated miRNAs in SCI-3 group compared to SCI-1 group

miRNA	log_2-ratio (SCI-3/SCI-1)	P value
miR-146a-5p	1.878042516	8.93E-37
miR-540-3p	–3.751734752	3.73E-27
miR-543-3p	–2.958431994	5.83E-27
miR-134-5p	–2.624393149	1.57E-19
miR-494-3p	–2.973587278	3.83E-19
miR-370-3p	–2.664379953	5.38E-17
miR-493-5p	–2.492148624	3.97E-15
miR-770-3p	–2.780437641	5.27E-15
miR-485-3p	–2.311086657	5.48E-15
miR-1193-3p	–2.704554006	8.41E-15
miR-495	–2.743261007	1.31E-14
miR-382-3p	–2.513966714	1.33E-14
miR-541-5p	–2.328672663	6.51E-14
miR-493-3p	–2.435884804	3.51E-13
miR-337-5p	–2.312570335	3.85E-13
miR-341	–2.434201752	5.56E-12
miR-376b-3p	–3.111137592	8.89E-12
miR-379-3p	–2.551934532	1.65E-11
miR-411-3p	–2.323997165	2.01E-11
miR-1-3p	1.659870986	2.07E-11
miR-410-3p	–2.054195029	2.39E-11
miR-147	2.286330615	4.25E-11
miR-127-3p	–1.978595216	5.04E-11
miR-434-5p	–2.322200603	9.57E-11
miR-411-5p	–2.291934623	1.21E-10
miR-409a-3p	–2.018814703	1.56E-10
miR-450b-5p	1.327335478	3.50E-10
miR-379-5p	–2.078289512	4.19E-10
miR-503-5p	1.407318699	5.38E-10
miR-380-3p	–2.735493487	1.11E-09
miR-299b-3p	–2.132435754	1.41E-09
miR-296-3p	–2.112403772	1.63E-09
miR-378a-3p	1.274040685	2.90E-09
miR-431	–2.279766651	3.67E-09

Table 5 (*continued*)

Table 5 (*continued*)

miRNA	log_2-ratio (SCI-3/SCI-1)	P value
miR-299a-3p	–2.028869086	3.76E-09
miR-298-5p	–1.678094702	4.48E-09
miR-139-5p	1.338557747	4.94E-09
miR-667-3p	–2.121081559	5.85E-09
miR-433-3p	–2.032443133	6.54E-09
miR-378b	1.330449134	6.58E-09
miR-450a-5p	1.213624392	9.28E-09
miR-30a-5p	1.009740011	1.41E-08
miR-673-5p	–2.582751768	1.48E-08
miR-200b-3p	1.281104992	1.85E-08
miR-369-3p	–3.511118964	2.17E-08
miR-150-5p	1.584894696	2.77E-08
miR-199a-3p	–1.008269662	3.79E-08
miR-200a-5p	1.37962997	4.43E-08
miR-29c-5p	1.286799126	1.02E-07
miR-142-3p	1.494463185	1.66E-07
miR-409a-5p	–1.857335578	1.79E-07
miR-452-5p	–1.78484171	3.14E-07
miR-758-3p	–2.049501653	3.20E-07
miR-143-5p	1.211529311	3.25E-07
miR-295-3p	4.385869068	3.26E-07
miR-382-5p	–1.717644065	4.18E-07
miR-133a-3p	1.444110968	4.65E-07
miR-31a-5p	–1.069559874	5.19E-07
miR-665	–3.289848451	5.46E-07
miR-369-5p	–1.84198704	6.33E-07
miR-211-5p	1.487439804	7.04E-07
miR-132-5p	–1.154477693	8.31E-07
miR-154-5p	–1.727332132	1.23E-06
miR-296-5p	–1.66198682	1.49E-06
miR-434-3p	–1.722258416	1.62E-06
miR-323-3p	–1.508148562	1.69E-06

Table 5 (*continued*)

miRNA	log_2-ratio (SCI-3/SCI-1)	P value
miR-29b-5p	1.566473951	3.92E-06
miR-214-3p	–1.03183723	4.58E-06
miR-322-5p	1.228178334	5.88E-06
miR-1247-3p	–1.723138821	6.53E-06
miR-429	1.203161356	7.13E-06
miR-320-3p	1.006670327	9.97E-06
miR-490-3p	1.313994447	1.12E-05
miR-224-5p	–1.195347596	1.39E-05
miR-300-3p	–1.740405801	1.58E-05
miR-496-3p	–1.716315672	1.72E-05
miR-292-5p	3.161923228	2.09E-05
miR-127-5p	–1.498705818	2.52E-05
miR-217-5p	1.067552536	2.84E-05
miR-3068-5p	1.103827166	3.16E-05
miR-133b-3p	1.260733165	4.41E-05
miR-145-5p	2.10010759	4.44E-05
miR-212-3p	–1.559406326	6.83E-05
miR-142-5p	1.693814748	8.30E-05
miR-329-3p	–1.516830797	9.78E-05
miR-1247-5p	–1.156715953	9.89E-05
miR-376a-5p	–1.849609395	0.000106477
miR-487b-3p	–1.416933909	0.000183408
miR-667-5p	–2.942414133	0.000184457
miR-673-3p	–2.263333428	0.000245072
miR-342-3p	1.008002812	0.000290457
miR-485-5p	–1.287206338	0.000305075
miR-138-5p	1.088542695	0.000349689
miR-376b-5p	–1.734005747	0.000448709
miR-134-3p	–2.765506432	0.000510032
miR-541-3p	–4.093660794	0.000697524
miR-494-5p	–2.583117839	0.000707698
miR-7a-5p	1.096870146	0.000976127

Table 6 Five significantly down-regulated and 21 significantly up-regulated miRNAs in SCI-3 group compared to SCI-2 group

miRNA	log_2-ratio (SCI-3/SCI-2)	P value
miR-146a-5p	1.430251032	5.24E-17
miR-503-5p	2.311893164	7.21E-16
miR-450a-5p	1.769066039	4.18E-13
miR-147	3.020864346	4.29E-13
miR-21-5p	1.035558921	4.19E-12
miR-450b-5p	1.979394682	1.61E-10
miR-375-3p	−1.83606718	1.39E-09
miR-142-5p	2.537262571	1.26E-08
miR-31a-5p	−1.38115753	5.06E-08
miR-322-5p	1.495334178	5.89E-08
miR-542-3p	1.387065444	1.23E-06
miR-200a-5p	1.437411924	1.65E-06
miR-363-3p	1.433656424	8.33E-06
miR-295-3p	4.835196043	2.03E-05
miR-133a-3p	−1.17375178	2.21E-05
miR-146b-3p	1.718251812	2.93E-05
miR-292-5p	3.632736184	6.28E-05
miR-211-5p	1.401188165	7.22E-05
miR-6329	1.792658779	7.88E-05
miR-130b-5p	1.515981749	0.000101517
miR-148a-5p	1.259651293	0.000129725
miR-451-5p	2.563264678	0.000214476
miR-210-5p	−1.8865576	0.000354357
miR-466b-5p	1.246519093	0.000387425
miR-146b-5p	2.000586182	0.000834152
miR-3102	−1.51339849	0.000890494

significantly downregulated miR in the SCI-1 group. Though its level was inclined to follow an increasing trend within 2 weeks after spinal cord injury and thereafter remained stable, its level was also significantly lower than that of the NC group. Specifically, compared with NC group, the expression of miR-139-5p was downregulated by 4.22-fold in the SCI-1 group, while its level was upregulated by 1.98- and 1.94-fold in the SCI-2 and SCI-3 groups compared to the SCI-1 group, with no statistical difference between the SCI-2 and SCI-3 groups. These results were confirmed by the qRT-PCR findings.

Recently, Fırat *et al.* also reported that miR - 139 - 5p was significantly downregulated in OAB patient plasma (7). Rho-associated coiled-coil-containing protein kinase 2 (ROCK2) was identified as a direct target of miR-139-5p and was effective in the ROCK2/myosin-light chain (MLC) and cholinergic pathway. It was proven that contractions of the bladder detrusor in humans are primarily mediated by M_3 receptors and depend on inhibiting MLC phosphatase by the activation of ROCK, leading to increased sensitivity to Ca^{2+} (13). Previous data have suggested that the inhibition of ROCK could ameliorate or reverse detrusor overactivity (14,15). By considering the previous data showing that upregulated RhoA/ROCK signaling is one of the factors that contribute to the development of detrusor overactivity, we speculate that a reduced level of miR - 139 - 5p may upregulate the expression of ROCK2, resulting in or aggravating detrusor overactivity. Moreover, miR - 139 - 5p was found to inhibit EMT and fibrosis by targeting lysophosphatidic acid receptor 4 via the PI3K-Akt signaling pathway (8).

In addition, miR-21-5p was the most significantly upregulated miR in all SCI groups. Indeed, miR-21-5p has been reported to act as an oncogene through inhibiting cellular apoptosis by targeting tumor suppressor genes (16). However, it should be noted that the overexpression of miR-21-5p abnormally activates transforming growth factor-β1 (TGF-β1) and Hedgehog signaling pathways, promoting tumor invasion by the induction of EMT. It is widely accepted that, TGF-β1 signaling pathway plays a pivotal role in EMT and fibrogenesis. Recently, the upregulated expression of miR-21-5p was reported to be involved in renal, myocardial, pulmonary, and peritendinous fibrosis and may serve as an alternative target to directly inhibit this fibrosis (17-20). Further research indicates that miR-21-5p overexpression may enhance TGF-β1-induced EMT by inhibiting Smad7 (21). Moreover, proliferation, migration, and pro-fibrotic activities of fibroblasts were found to be promoted by miR-21-5p through reducing Smad7 expression (18). More specifically, the increase of intracellular miR-21-5p induced fibroblasts differentiation into myofibroblasts and the overexpression of extracellular matrix (ECM) and fibrogenic markers. Moreover, tissue inhibitor of metalloproteinases (TIMPs) which was implicated in collagen synthesis and accumulation during fibrosis were also targeted by miR-21-5p (22).

Importantly, previous study suggests that miR-21-5p is upregulated by TGF-β1 via activation of Smad3 rather than

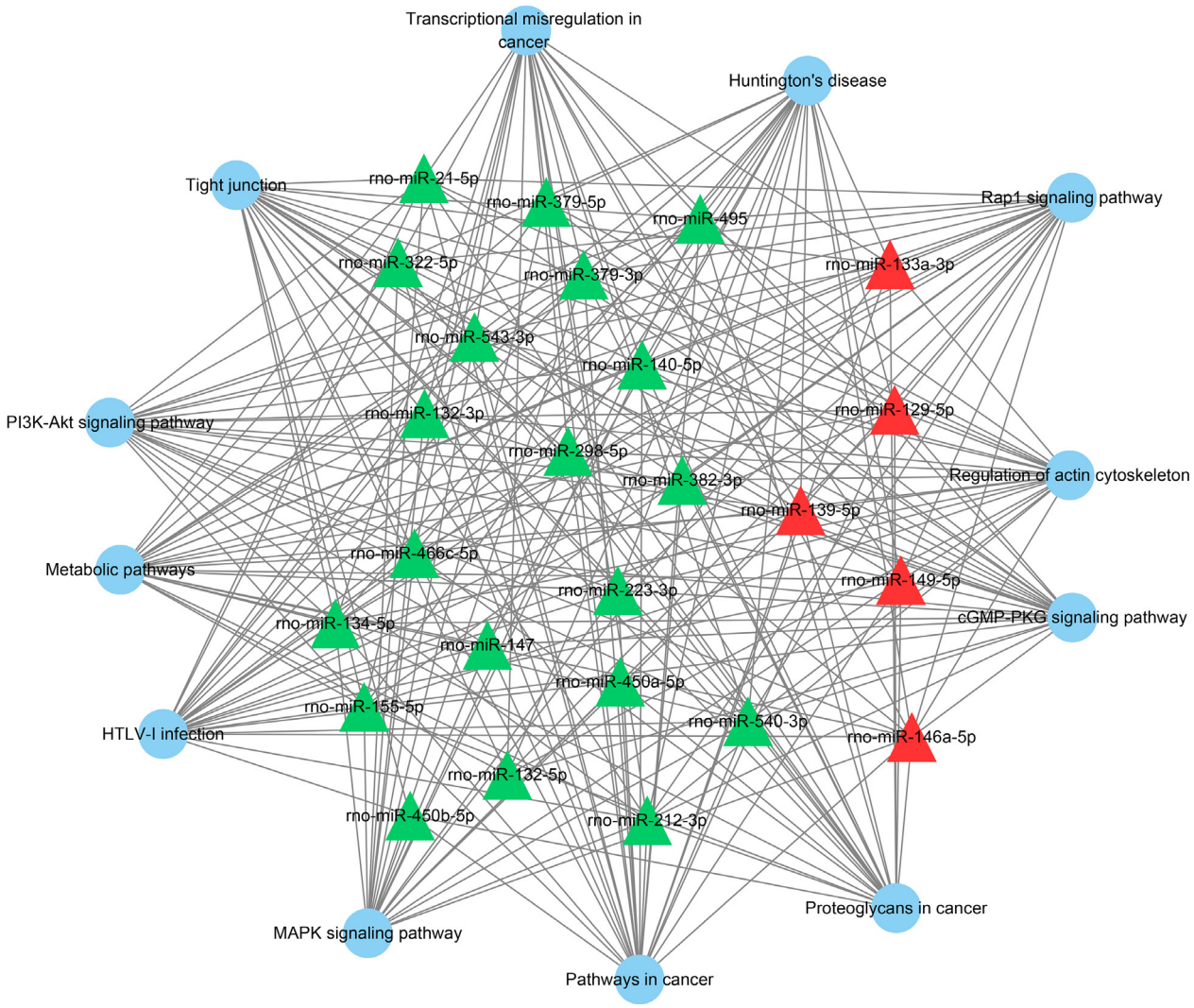

Figure 1 Signaling pathways targeted by miR-139-5p, miR-21-5p, miR-149-5p, miR-146a-5p, miR-134-5p, miR-132-3p, and miR-132-5p. Red color indicates the upregulated miRs in the SCI-1 group compared to the NC group, while green indicates those that were downregulated.

Smad2 (23). In a normal state, Smad-3 activation can induce the expression of Smad7, which forms a negative feedback mechanism (24). Nevertheless, in pathological situations, the expression of Smad7 was found to be suppressed, and the negative feedback damaged, which may due to the upregulated expression of miR-21-5p. In contrast, the conditional knockout of Smad2 could enhance miR-21-5p expression (23). Further research is needed to explore the mechanisms underlying the interactions between miR-21-5p and the TGF-β1 signaling pathway. Also, bladder fibrosis after spinal cord injury may bear responsibility for the high intravesical pressures, low bladder compliance, bladder wall stiffness and vesicoureteral reflux (25). Currently, however, there is no effective method for preventing bladder fibrosis. Therefore, it is also of great significance to investigate the functional role of miR-21-5p in bladder fibrosis after spinal cord injury.

The differentially expressed miRs between SCI groups were also investigated in this study and showed distinct patterns of expression over time. Specifically, we found that miR-146a-5p was upregulated by 5.48-, 2.51-, and 3.46-fold in the SCI-3 group compared to the NC, SCI-1, and SCI-2 groups respectively, while miR-146a-5p was upregulated by 2.19-fold in the SCI-1 group compared to

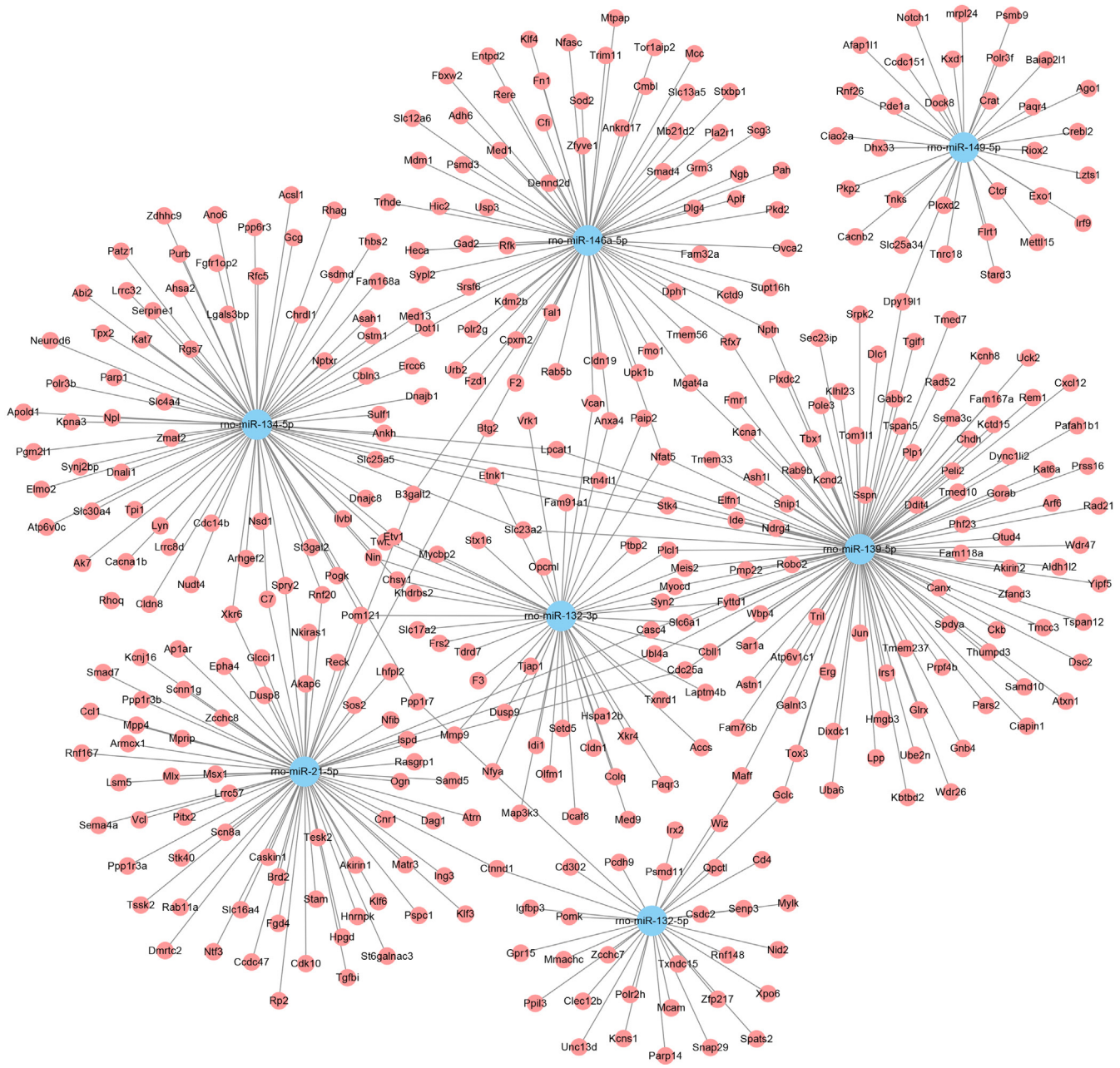

Figure 2 Genes targeted by miR-139-5p, miR-21-5p, miR-149-5p, miR-146a-5p, miR-134-5p, miR-132-3p, and miR-132-5p. Red color indicates the upregulated miRs in the SCI-1 group compared to the NC group, while green indicates those that were downregulated.

the NC group. In addition, miR-134-5p was significantly upregulated in the SCI-1 group compared to the NC, SCI-2, and SCI-3 groups as revealed by NGS and qRT-PCR. Other studies have found that miR-146a-5p could attenuate hepatic fibrosis by negatively regulating the PTPRA-SRC signaling pathway or inhibiting the profibrogenic effects of TGF-β1 and lipopolysaccharide (26,27). Furthermore, transcription factor twist1 directly targeted by miR-134-5p was also implicated in EMT and fibroblast activation and tissue fibrosis in a TGF-β/Smad3-dependent manner (28).

Suprasacral spinal cord injury can abruptly disrupt intraspinal pathways and result in the "spinal shock" phase, during which the bladder is often atonic and areflexic and typically present with overflow incontinence (29).

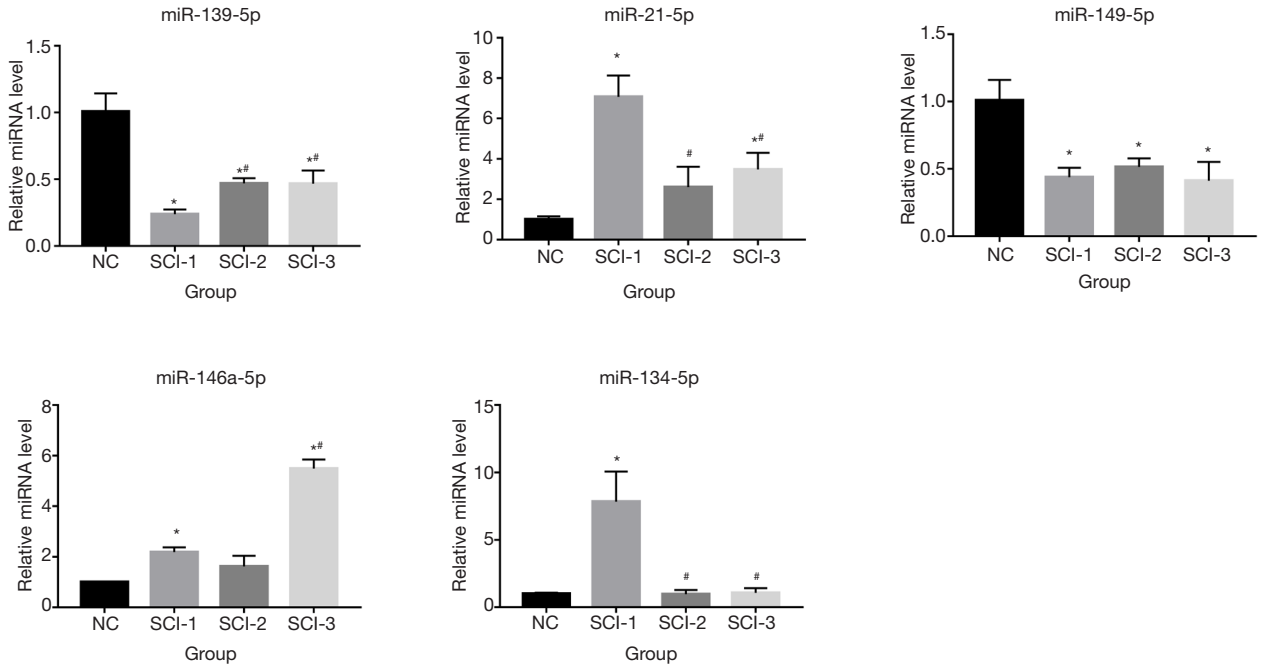

Figure 3 The level of miR-139-5p, miR-21-5p, miR-149-5p, miR-146a-5p, and miR-134-5p between different groups by qRT-PCR. Data are presented as the mean ± SD. *, P<0.05 *vs.* NC group; #, P<0.05 *vs.* SCI-1 group. NC, normal control; SCI-1, spinal cord-injured-1; SCI-2, spinal cord-injured-2; SCI-3, spinal cord-injured-3.

However, the relative concentration of collagen in rat bladders was reported to be significantly decreased in the first 10 days after spinal cord injury (30), which may be in agreement with the expression of miR-134-5p to a certain extent. Therefore, it is reasonable to presume that miR-134-5p might play a role in it. After spinal shock, hypermechanosensitive C-fiber bladder wall afferents were activated gradually and urodynamic findings were mainly characterized by detrusor overactivity or detrusor-sphincter dyssynergia. miR-146a-5p may be involved in this stage of NB due to that it was significantly upregulated in the SCI-3 group compared to the SCI-1 group. In summary, it will be very interesting and meaningful to investigate the relationship between the dynamic change of these differentially expressed miRs and the different stages of NB.

In this study, we investigated the differentially expressed miRs between groups by NGS and qRT-PCR. Furthermore, 206 new miRs were identified in the bladder, and a large set of genes implicated in essential signaling pathways targeted by these miRs were identified. Nevertheless, this study also has some limitations. Firstly, the interactions between miRs and mRNA were not explored. Thus, further experimental studies are needed to verify the proposed interactions and their roles in NB in the future. Secondly, bladder tissues collected 8 weeks or more after spinal cord transection may be required to investigate those miRs that might function at that time. For example, Wang *et al.* reported that there was no significant difference in the expression of bladder miR-1949 between rats without spinal cord injury and those collected at 3 months following spinal cord injury, while it was significantly increased after the third month (31). Thirdly, more samples or human bladder tissue should be analyzed to confirm our results.

Conclusions

Several miRs were differentially expressed in the SCI rat NB, and may potentially serve as new molecular targets for NB.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All experimental procedures were implemented in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Xuanwu Hospital Capital Medical University (No. 20190128).

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Table S1 Two hundred and six new miRs identified in the bladder

miRNA_id	Mature sequence
chr9_13944	augagcgcuccuauccacaagcu
chr7_10857	cacagucuaguggccuuggaagc
chr9_13550	uggcauuccuggagcuccugga
chr5_8282	cacagcggagcugggcacuggcgu
chr18_22514	cacagcggagcugggcacuggcgu
chr3_5314	acaagcuuaaagguuggggacu
chr15_19802	ucacggaguccaggcuagccuug
chr5_8901	uuggaaggacuugugaaggugu
chr7_11359	aucucgguggaaccucca
chrX_24487	aucucgguggaaccucca
chr3_5244	aucucgguggaaccucca
chr17_21861	uugucuguguguauguccaugugu
chr13_18372	uucccggccaaugcacca
chr10_14593	uaggauuugcugaaggagg
chr16_20779	ugggcgcuccgauugugguuc
chr17_21589	cuuggcaccugguaagcacuca
chr1_1510	caggaggggcgggcggggg
chr6_9331	caggaggggcgggcggggg
chr11_16694	aagccaacucuccagaucuga
chr6_10216	ucagacuucugcucacccacga
chrX_23975	ucaucaccucuuguguccugcagc
chr6_9812	aggaguuggggauuuagcu
chr17_21639	ucggcgccccacacugagcuu
chr1_603	cauaaguguagagagucuguagu
chr3_5685	cauguuccacucacucucaga
chr2_3705	uuggccagguggugugucugaca
chr1_361	caccugguccucaaucucuaga
chr1_340	uugggaacggggugucucuggga
chr16_20784	cggaccaugugaaccagagugc
chr17_21860	acacacauaaacacacacgca
chrX_24300	acuacccacuuccaucuccacagc
chr12_17242	acuugcauguacacuuuccuga
chr12_17244	acuugcauguacacuuuccuga
chr2_4354	uccgacucucugagcucugccagg
chrX_24426	cuggauuggcuggcccc
chr10_15831	ugcagacucccucuggcugaugug
chr2_3993	uccuacuagugugaacagcgcau
chr10_16212	caucucccaccuugucucccgca
chr12_17361	ugaccaugccucuuucccaag
chr4_6449	ucuccgccaccuccaccgcagc
chr5_8531	aaagccuccgagucacuggau
chr7_11153	ggugacaguaagcuguaguugc
chr3_5493	auguguuccugacuauccugg
chr8_12585	cuccgcgccugucaaaccccucau
chr20_23557	cggaggagguggcugccgcggc
chr7_11744	agcugaucacucucccuacagg
chr4_6026	aagccccuggaaacacucuucc
chrX_24141	uagucugucucuucucuguca
chr12_17462	ucccuguucgggcgccacu
chr2_4466	uucggcugcauuucucgguugu
chr5_8280	uugacucucugggcccagcag
chr1_1381	ucacucugccuuucucccucaga
chr1_1382	ucacucugccuuucucccucaga
chr7_10721	cagcuggcucugcuuuccuuu
chr18_22438	aagccccucucaucucccaac
chr1_2525	aguggcuaugagggcaccuggu
chr1_2511	ucacgggcacaggaagcugau
chr1_2513	ucacgggcacaggaagcugau
chr17_21586	uuaggacucuggucaucuuugg
chr10_14618	ugccccgccuuccucccucagg
chr7_11083	gggggucuagaggugcauggu
chr14_18974	ucugcgcgcagcguuugcucucu
chr1_328	auggagggagacaggagagugu
chr1_437	uagggucuguucuguguccucc
chr10_15933	ugaggggccucagaccgagcuuu
chr8_12972	uugguaccuguuuccuguu
chrX_24393	ugggggcaggccggaucuagugg
chr1_916	cucuggcaccgguguaugggacu
chr10_15921	ggucuggccuggugggcccugc
chr16_20914	uccucuguaagagcagugcagu
chr18_22500	gugguguguuaguuacuuuuc
chr9_13419	cagcccccuucucugcucuugu
chrX_24406	ugacuuuccagagaacccuuga
chr11_16570	ccagguccacucugcugagcacu
chr6_9744	ugguagacuauggaacguagg
chr12_17313	caagcuccccuacagccgccagc
chr11_16624	uuccuucuguauugugugugcu
chr20_23743	cuacugagccacauucccagcc
chr12_17745	cagaaccugcucccgacaauga
chr7_11689	agcugugcugugcuauguu
chrX_24231	aagacuguggugauggcacau
chr5_8046	acacaggagggagcaugcauuu
chr5_8194	uugugucccaccaugcccaguu
chr19_23144	ucucucuuccuccucacugugg
chr18_22131	cuuguguuucucuucuccuucccagu
chr14_19590	uuagagccagacugccuggguuu
chr5_7648	ugaugucacugucacaugaggu
chr5_7647	ugaugucacugucacaugaggu
chr8_13325	aucugacuugaggcugcugcucu
chr15_20213	aacggucugugguuccu
chr5_9184	uucucuugugcccccucuugc
chr5_8577	uggguccagauguggucaagcu
chr5_8578	uggguccagauguggucaagcu
chr5_8169	guguacugugaccuguccuagg
chr7_11752	ucugacucuugccucccacaga
chr5_8555	ugagugcuaggaaccaaacucu
chr3_5775	aggaguuggggauuuagcu
chr14_19318	ugauguguuuauauccaguugg
chr17_21864	uacacaugcacacacacgcga
chr19_23022	ugacccugugcccugcccccac
chr4_6719	caacacugcacuggaagaugga
chr11_16638	cuaaggcaggcagacuucagu
chr3_4543	ucaagaguucugaagucacugg
chr4_7462	ucauuccaccuucccucccacagg
chr4_7120	agacaugugccggaagacugca
chr1_1573	ucccccucccccccacauacagg
chr7_11930	uugacuugcauucccuucucaga
chr4_6414	uggggucuuugacauggaugugu
chr12_17840	cagaacccacagcucccucaguu
chr1_2083	acugggaccuggacagggacuuuu
chr18_22011	gauguuagguuuuacugcugu
chr12_17307	ugucuccaucugcuucccacagg
chr1_862	uucggggugaggcggagucagc
chr4_7310	caaggacuuugaugcuggaga
chr5_9040	auacggagcuagagagaugacu
chr8_12723	uccacucuuaauccuagcacu
chr10_15695	uugggccucagccaaggacugg
chr9_13459	uguaugucuguauguacaugug
chr1_1400	uguaugucuguauguacaugug
chrX_24150	agggguuggagauuuagcuc
chr2_3446	agggguuggagauuuagcuc
chr2_3542	uugccugguuuagucucugcu
chr1_1619	ugacggacaggacggagggag
chr8_13260	uauccccuuugagucucccaca
chr9_14226	uauguccugucucuuccuccagu
chr5_8174	agagacagagagcgcuagcugcg
chr7_11241	ugacccgccccaccccgcagg
chr1_100	cauugugaauggggaaugagg
chr7_11944	ucagccucacacuccuccgggu
chr20_23652	cacuagacaugcucacucugu
chr16_20541	ucugguucugucuuccugcaga
chr17_21152	acucugggugucugugaacaagu
chr10_15514	ccuccggaucccgggaccccgga
chr3_5566	uaaagggggccaggcucugagc
chr3_5556	uaaagggggccaggcucugagc
chr1_2654	gacugggccuucuacuuccucu
chr17_21565	agcagggagagcuacagagcuuugu
chr19_22791	ucacacgagggcugcucugagc
chr2_3946	uguaguuguuauuguuguugcu
chr3_5923	aacugggagaaggaugggacacu
chr7_10924	uggcucacugaccucuacuuug
chr13_18744	cacugagcagcagacaugucuga
chr5_8132	aggccuggucaggcaguucuguc
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chr5_8778	ugagguaguuugugcuguc
chr12_17541	cacuucagaggagagauggcucgg
chr2_3411	uugcagauggccugagcgugau
chr17_21857	auguaaguguguauguauaugu
chr20_23190	ggacguccagacgcaacucucg
chr1_1401	ugguacaguguaacuacagugu
chr1_1406	ugguacaguguaacuacagugu
chr1_1403	ugguacaguguaacuacagugu
chr1_1402	ugguacaguguaacuacagugu
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chr9_13614	agggcuaguuaacaguuuaggu
chr18_22253	acuggcuugcucuucaaggguuu
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chr10_16058	aggacugagggcgugagucucuu
chr6_10344	ucuccugagcccuguacugugg
chr12_17299	ucucuccuguuacccccuguag
chr1_2582	uuccucacuggcccacucccuag
chr13_18518	uuucucuuuaucccaucccugg
chr6_9847	uucucagucuugucucccguccug
chr8_12680	gaagucugcugaggagcuccagc
chr12_17880	uuggguguugugccuucugcagg
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chr3_5989	ccauagccccuacuuucugcagg
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chr14_18752	ugcgggaaggguagcaugaguacc
chr1_1047	uuggccucggcucuccucccagg
chr13_18458	uuuggaaccuccucccuagggc
chr1_1914	ucccucuuccauguccuccagu
chr6_9478	cagucuaucugucugucugucu
chr3_5408	ugcucuauggcugcggcuccag
chr7_10953	cgccuucuugugucccugcaga
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chr9_13413	ugugaguucacucuggcguga
chr17_21572	uuacccuagccucugccccucu
chr5_9125	ccuggcaaggaagguucucugu
chr8_13188	cucgcuccggcuccgccuugac
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chr10_15867	caccacagugugguuuggacgugg
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chr15_20240	uauucucucggccucccucagg
chr14_19708	ccucugcuggcuuagggugaga
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chr3_5852	cagccuggaccuacuuccuguu
chr7_10822	aggucuucccaucuuuugcuag
chr6_9471	augaugacgacgacgacgauga
chr9_14144	ucgguugguuccugugucuag
chr6_9316	cgagcuaucuccuaaacucugg
chr10_14521	uucugccugggauuuccuugu
chr5_8036	ugaggauuacgaagaggauggu
chr6_10253	uauaccuacccuagcacugugug
chr9_14039	uguagucuuucugcccacggcu
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chr6_10184	agaagccuaagucugcucugacu
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chr11_16818	uucugugacagagucucccucu