

Construction and analysis of a spinal cord injury competitive endogenous RNA network based on the expression data of long noncoding, micro- and messenger RNAs

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Abstract. Spinal cord injury (SCI) results from trauma and predominantly affects the young male population. SCI imposes major and permanent life changes, and is associated with high future mortality and disability rates. Long non-coding RNAs (lncRNAs) have recently been demonstrated to serve critical roles in a broad range of biological processes and to be expressed in various diseases, including in SCI. However, the precise mechanisms underlying the roles of lncRNAs in SCI pathogenesis remain unexplored. In the present study, the aim was to identify critical differentially expressed lncRNAs in SCI based on the competing endogenous RNA (ceRNA) hypothesis by mining data from the Gene Expression Omnibus database of the National Center for Biotechnology Information and to unveil the functions of these lncRNAs. Different approaches and tools were employed to establish a network consisting of 13 lncRNA, 93 messenger RNA and 9 microRNA nodes, with a total of 202 edges. Three node lncRNAs were identified based on the degree distribution of the nodes, and their corresponding subnetworks were subsequently constructed. Based on these subnetworks, the biological pathways and interactions of these 3 lncRNAs were detailed using FunRich software

(version 3.0). The primary results of the 3 lncRNA enrichment analyses were that they were associated with autophagy, extra-cellular communication and transcription factor networks, respectively. The phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway of XR_350851 was the classic autophagy pathway, indicating that XR_350851 may regulate autophagy in SCI. The possible role of XR_350851 in SCI revealed in the current study based on the regulatory mechanism of ceRNAs has uncovered a new repertoire of molecular factors with potential as novel biomarkers and therapeutic targets in SCI.

Introduction

Spinal cord injury (SCI) is a serious and disabling disease associated with a range of symptoms, including severe movement dysfunction, muscle weakness, and sensory changes (1). Based on pathological patterns, SCI can be classified as a primary or secondary injury. Although little is known regarding the pathocellular mechanisms of SCI, secondary damage following primary SCI are considered to involve a wide range of pathologies, including neural inflammation, demyelination, axonal degeneration, and oligodendrocyte and neuronal cell death (2,3). Great efforts have been made to improve the functional outcomes of patients with SCI; however, therapeutic advances have thus far been limited (4). Therefore, the specific objective of the present study was to identify biomarkers and therapeutic targets for SCI.

A large number of studies have implicated apoptosis, autophagy, inflammation and endoplasmic reticulum stress as important features of SCI; however, regulatory mechanisms based on crosstalk between these factors remain to be delineated (5). A strong association between autophagy and acute neurodegeneration caused by SCI has previously been reported in the literature (6). Autophagy is a highly conserved evolutionary phenomenon of intracellular degradation that maintains the homeostasis of cells by inducing rapid self-clearance or by degrading supramolecular structures (7,8). Similar to cell division, differentiation and death, autophagy dysfunction is associated with numerous diseases, including cancer, and cardiovascular and neurodegenerative diseases (9,10).

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Abbreviations: SCI, spinal cord injury; lncRNAs, long non-coding RNAs; ceRNA, competing endogenous RNA; mRNAs, messenger RNAs; miRNAs, microRNAs; GEO, Gene Expression Omnibus; NCBI, National Center for Biotechnology Information; AP-1, activator protein 1; CDC42, cell division cycle 42; SIP1, sphingosine-1-phosphate receptor 1; Arf6, ADP-ribosylation factor 6; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1

Key words: SCI, autophagy, ceRNA, lncRNA, PI3K/AKT/mTOR

Pott *et al* (11) demonstrated that inadequate autophagy in intestinal epithelial cells increases the induction of apoptosis and potentially impairs barrier integrity due to inflammatory stimuli. A study conducted by Papadakis *et al* (12) also indicated that autophagy may have a protective role in an oxygenglucose-deprivation neuronal and cerebral ischemia model. However, recent evidence (10) suggests that whether autophagy is protective or detrimental may be based on the activation status of the cell, as well as other factors. Notably, increased or decreased autophagy potentially contributes to a variety of diseases and pathological conditions (13).

Among the RNAs of different molecular ranges, the sequences of long non-coding RNAs (lncRNAs) are the least evolutionarily conserved. At the cellular level, epigenetic modulation is one of the salient roles of lncRNAs, although lncRNAs can also regulate gene expression through transcription, alternative splicing, RNA translation and organization of important structures for RNA processing. An abundance of chromatin modification complexes can be targeted by lncRNAs to reconstruct the structure and/or expression of their adjacent genes (14). Prior studies have noted the critical roles of lncRNAs in the pathogenesis of various neurological diseases, including SCI. For instance, there is evidence that the lncRNA-XIST significantly aids in the recovery from SCI by inhibiting apoptosis (15). Qiao *et al* (16) also suggested that the lncRNA MALAT1 has a neuroprotective role in spinal cord ischemic/reperfusion injury, where it acts by regulating miR-204. Furthermore, Zhou *et al* (17) confirmed that MALAT1 can inhibit acute SCI by inhibiting the inflammatory response of microglial cells.

Recent evidence has indicated that lncRNAs can regulate the expression of messenger RNAs (mRNAs) by competitively binding to microRNAs (miRNAs) and acting as competing endogenous RNAs (ceRNAs), which can systematically functionalize non-coding transcripts based on competitive sharing of miRNAs (18). It is considered that lncRNA transcripts function as competing endogenous RNAs (ceRNAs) or natural microRNA sponges that contain numerous miRNA binding sites. Salmena *et al* (19) proposed the 'ceRNA hypothesis', according to which the expression of a specific miRNA can be temporarily reduced due to ceRNA. In addition, based on the ceRNA hypothesis, scholars discovered a large-scale regulatory network in the transcriptome based on miRNA binding sites, which greatly expands the information available on human functional genetics and details a network that may serve a critical role in cancer pathology (19,20).

In the present study, a global triple network was generated based on the ceRNA hypothesis by mining data from the Gene Expression Omnibus (GEO), which is curated by the National Center for Biotechnology Information (NCBI). Based on the resulting network, target lncRNAs associated with SCI were identified. Furthermore, a subnetwork of node lncRNAs was obtained from the ceRNA network, which facilitated enrichment and identification of lncRNA pathways and functions. The flow chart for target lncRNA selection is presented in Fig. 1.

Materials and methods

Data sources. GEO (www.ncbi.nlm.nih.gov/geo) is currently the largest fully public gene expression resource data repository,

from which thousands of experiments and tens of millions of gene expression profiles can be queried and downloaded. Data examined in the present study were obtained from the NCBI GEO database. The raw mRNA expression data were downloaded from the series GSE464 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE464) and GSE5296 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5296). The lncRNA data were obtained from the aforementioned datasets by repurposing the probes in the RG_U34A, RG_U34B, RG_U34C and Mouse430_2 arrays of the Affymetrix annotation platform (www.thermofisher.com/cn/en/home/life-science/microarray-analysis/microarray-data-analysis/genechip-array-annotation-files.html) (21). Data on miRNA were obtained from GEO series GSE19890 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19890).

Raw data analysis. Subsequent tologarithmic processing, the two mRNA expression datasets were merged using Perl script (www.perl.org/), and batch effects were corrected using the Combat method from the sva and limma packages (22-24). Differential mRNA expression analysis between SCI and normal samples was performed using the limma package (bioconductor.org/packages/release/bioc/html/limma.html) (25). mRNAs with a P-value of <0.05 and a log₂fold-change (log₂FC) of >1.5 were considered to be differentially expressed. Differential lncRNA and miRNA expression analysis between SCI and normal samples was performed using the Morpheus platform (<https://software.broadinstitute.org/morpheus/>) based on the significance analysis of microarrays method, and the threshold was also set tolog₂FC>1.5 and P<0.05.

Screening and pairwise matching of lncRNAs, miRNAs and mRNAs. Pairwise matching of differentially expressed lncRNAs, miRNAs and mRNAs was performed using RNAhybrid (bibiserv.cebitec.uni-bielefeld.de/rnahybrid), LncTar (www.cuilab.cn/lncTar), TargetScan Human version 7.1 (www.targetscan.org/vert_71/) and DIANA Tools (diana.imis.athena-innovation.gr/DianaTools/index.php?r=miroT_CDS/index). The miRNA binding sites on lncRNAs were predicted using RNA hybrid (26), which is a tool used to calculate the minimum free energy hybridization of a long and short RNA. LncTar (27) is a software used for predicting putative interactions between mRNAs and lncRNAs based on free energy minimization. In addition, TargetScan (28) and DIANA (29,30) were synchronously used to predict differentially expressed pairs of miRNAs and mRNAs. TargetScan predicts the biological targets of miRNAs by searching for the presence of conserved 8mer, 7mer and 6mer sites by matching the seed region of each miRNA (31). In mammals, predictions were ranked based on the predicted efficacy of targeting as calculated using the cumulative weighted context++ scores of the sites. In the current study, the predicted mRNAs were set to a cumulative weighted context++ score of ≤0.4 in TargetScan (28). The arsenal of DIANA Tools included the target prediction algorithms of microT v4 and microT-CDS, where microT-CDS was used to predict the miRNA and mRNA pairs in the present study. In order to avoid data omission, the two online websites (TargetScan and DIANA) were used for prediction, and their results were combined.

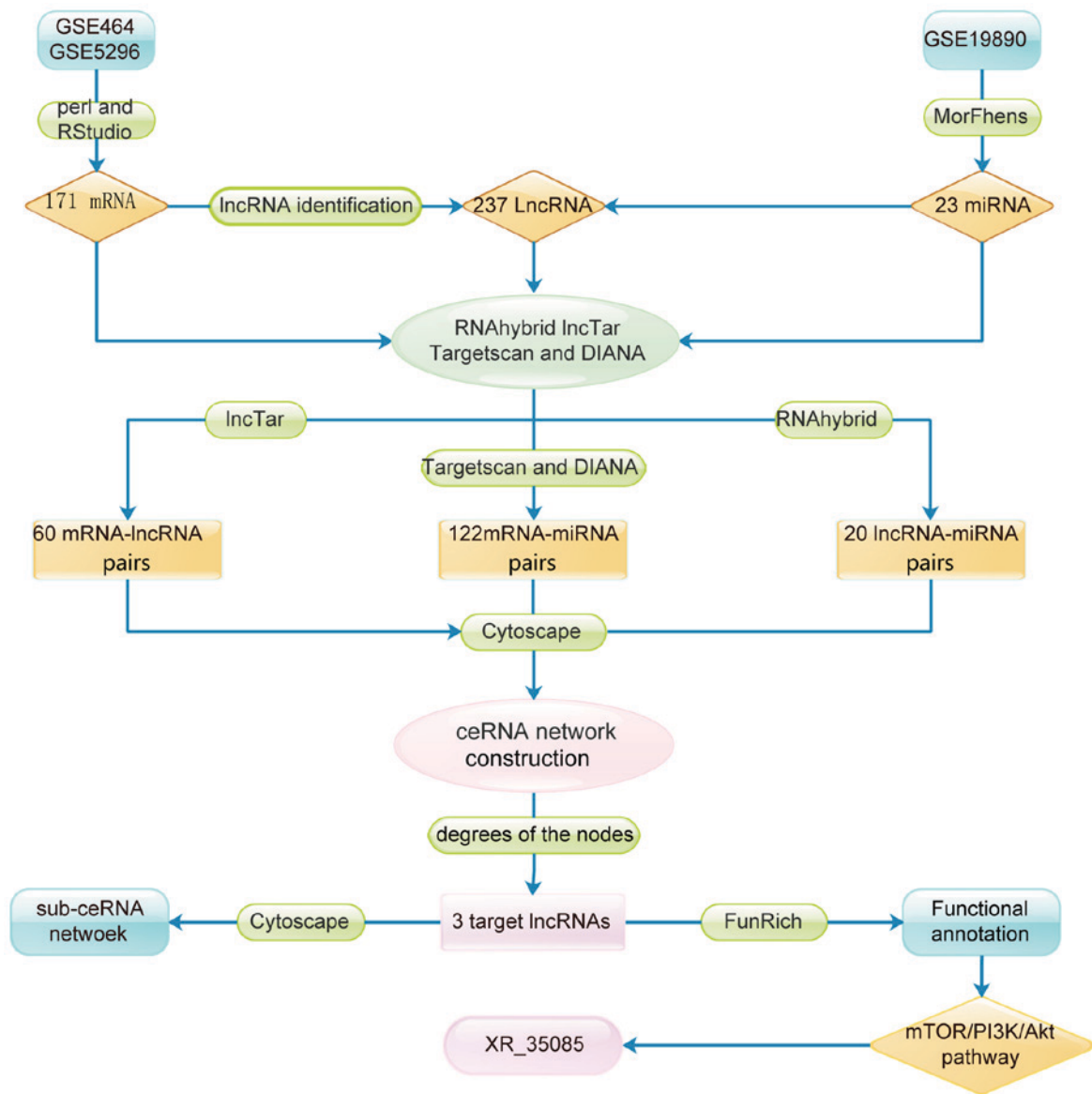


Figure 1. Flow chart for the target lncRNA selection.

Construction of the ceRNA network. For a given lncRNA-mRNA pair, which included negatively co-expressed mRNAs and lncRNAs targeted with a specified common miRNA, co-expression competing triplets were identified based on the ceRNA theory (32). Using existing miRNA target online software (RNAhybrid, lncTar, TargetScan and DIANA Tools), lncRNA-miRNA, lncRNA-mRNA and miRNA-mRNA interactions were confirmed. Using an in-house Perl script, the ceRNA associations were integrated (33). To elucidate the roles of lncRNAs, miRNAs and mRNAs within the regulatory ceRNA network, their interactive and visual mediated network was then created using Cytoscape software, version 3.5.0 (34).

Molecular function analysis of the ceRNA network. To elucidate the molecular functions within the ceRNA network, mRNAs in the network were analyzed using FunRich software (version 3.0; www.funrich.org/). The FunRich tool allows users to assign the biological process, cellular component and molecular function terms, as well as biological pathways,

protein domains, sites of expression, clinical phenotypes and transcription factors, to enriched and depleted factors (35).

Reconstruction of a sub-ceRNA network using node lncRNAs. Based on the established ceRNA network, network analysis was subsequently performed to investigate the degree distribution of the nodes (36). Three node lncRNAs were screened based on the degrees of the nodes, while miRNA and mRNA pairing with the lncRNAs was also examined. Accordingly, three sub-ceRNA networks were established with the Cytoscape software (version 3.5.0).

Functional annotation enrichment analysis. Biological pathway functional enrichment analyses were conducted using FunRich software, version 3.0. This software was used to visualize and assess pathway interactions of the lncRNA-targeted mRNAs. This tool also highlighted nodes that were enriched in specific pathways and allowed for the creation of subnetworks based on these highlighted nodes (35).

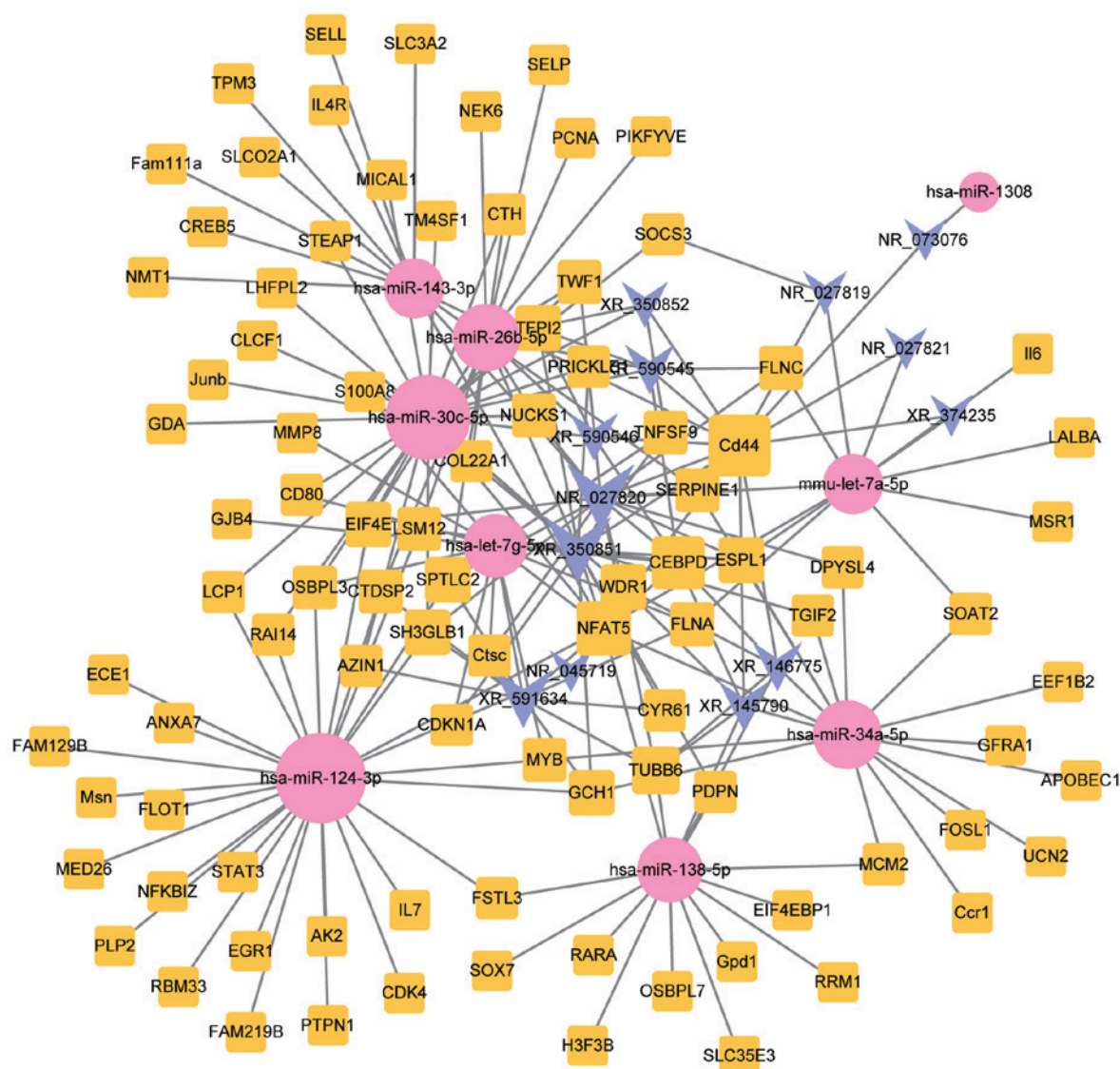


Figure 2. Competing endogenous RNA network of the lncRNA-miRNA-mRNA. The network is comprised of 13 lncRNAs, 9 miRNAs and 93 mRNAs, with a total of 202 edges. Purple inverted triangles indicate the lncRNAs, pink circles indicate the miRNAs, and orange squares indicate the mRNAs, these sizes are positively correlated with the degrees of the nodes. lncRNA, long noncoding RNA; mRNA, messenger RNA; miRNA, microRNA.

Results

Screening of differentially expressed lncRNAs, miRNAs and mRNAs. Following logarithmic processing and batch correction, the microarray data were analyzed by Morpheus and limma packages. In total, 171 mRNAs and 237 lncRNAs that were overexpressed in SCI were identified according to the threshold of $\log_2FC > 1.5$ and $P < 0.05$. In addition, 23 miRNAs that were down-regulated in SCI were selected using Morpheus software (Fig. 1).

Pairwise matching of lncRNAs, miRNAs and mRNAs. Using RNAhybrid and LncTar, 20 mature miRNA-lncRNA pairs and 60 lncRNA-mRNA pairs were respectively predicted. Furthermore, a total of 122 unique mature miRNA-mRNA pairs, predicted by both TargetScan and DIANA, were selected as reliable interaction pairs.

Construction of the lncRNA-miRNA-mRNA ceRNA network. In order to elucidate the interactions between lncRNAs, miRNAs

and mRNAs in SCI, a ceRNA network was established. As shown in Fig. 2, this network consisted of 13 lncRNA, 93 mRNA and 9 miRNA nodes, with a total of 202 edges.

Molecular annotation of lncRNAs based on the ceRNA network. To identify possible mechanisms associated with SCI, 75 molecular function modules were selected using FunRich 3.0 software. As displayed in Table I, the primary modules included calcium ion-binding, cysteine-type peptidase activity, chemokine activity, auxiliary transport protein activity, cytokine activity, hormone binding, steroid binding, protein-tyrosine kinase activity, transcription factor activity and cytoskeletal anchoring activity. The top 10 critical molecular functions of mRNAs in the ceRNA network are presented in Fig. 3. The percentage and P-values of critical molecular functions are also presented; there was a higher proportion of transcription factor activity (7.6%), auxiliary transport protein activity (4%) and calcium ion-binding (3.6%; Fig. 3).

Table I. A total of 75 molecular function modules potentially involved in spinal cord injury.

Molecular function	Fold enrichment	P-value
Calcium ion binding	3.760905	0.000706
Cysteine-type peptidase activity	6.730617	0.002923
Chemokine activity	5.841835	0.004888
Auxiliary transport protein activity	2.321344	0.011811
Cytokine activity	3.615564	0.012769
Hormone binding	77.225850	0.012949
Steroid binding	77.225850	0.012949
Protein-tyrosine kinase activity	6.115491	0.012960
Transcription factor activity	1.743523	0.013665
Cytoskeletal anchoring activity	5.958724	0.013908
Receptor activity	2.141300	0.019646
Acyltransferase activity	3.870462	0.020167
Ubiquitin-like-protein-specific protease activity	38.805030	0.025730
Translation regulator activity	3.065792	0.042320
Metallopeptidase activity	3.065792	0.042320
Complement activity	5.541734	0.050627
Ion channel activity	3.521433	0.054121
Intracellular ligand-gated ion channel activity	5.172408	0.057295
Ligand-dependent nuclear receptor activity	4.433704	0.075174
Lipid kinase activity	4.433704	0.075174
Phosphoprotein phosphatase activity	12.978060	0.075229
Guanylate cyclase activity	9.737592	0.099030
Lipid transporter activity	8.656838	0.110701
Transcription factor binding	7.792019	0.122222
Hydrolase activity	1.905825	0.124300
Lipid binding	7.084297	0.133594
Serine-type peptidase activity	2.371695	0.134282
RNA methyltransferase activity	6.494431	0.144819
Transmembrane receptor activity	2.984502	0.145688
Cytoskeletal protein binding	1.774696	0.153605
Oxidoreductase activity	1.923332	0.156614
Water channel activity	5.567317	0.166836
Kinase activity	5.567317	0.166836
Receptor signaling protein tyrosine phosphatase activity	4.871837	0.188289
Protein binding	1.701421	0.210739
Deaminase activity	4.103004	0.219441
Carboxy-lyase activity	3.897957	0.229558
Phosphorylase activity	3.897957	0.229558
Peroxidase activity	3.897957	0.229558
Transaminase activity	3.389748	0.259133
Kinase regulator activity	3.389748	0.259133
Hormone activity	3.118677	0.278218
Helicase activity	3.118677	0.278218
Protein tyrosine phosphatase activity	3.118677	0.278218
Transcription regulator activity	1.207568	0.282903
Heat shock protein activity	2.887749	0.296813
Protease inhibitor activity	1.651143	0.344255
Extracellular matrix structural constituent	1.400216	0.364123
Galactosyltransferase activity	2.052042	0.390878
Extracellular ligand-gated ion channel activity	1.901929	0.414282
Ribonucleoprotein	1.901929	0.414282
Growth factor activity	1.410999	0.417894

Table I. Continued.

Molecular function	Fold enrichment	P-value
Ligase activity	1.385805	0.426817
Isomerase activity	1.732906	0.444099
Phospholipase activity	1.732906	0.444099
Transporter activity	1.073903	0.470688
DNA binding	1.063906	0.474045
Chaperone activity	1.231838	0.487156
Cell adhesion molecule activity	1.086772	0.490374
Transmembrane receptor protein tyrosine kinase activity	1.392575	0.518527
Peptidase activity	1.344563	0.530951
Structural constituent of cytoskeleton	1.132939	0.531709
Defense/immunity protein activity	1.278448	0.548991
Motor activity	0.987193	0.643607
RNA binding	0.846085	0.700779
Receptor signaling complex scaffold activity	0.721871	0.790031
Receptor binding	0.604590	0.814912
Voltage-gated ion channel activity	0.599939	0.817325
Transferase activity	0.499956	0.870160
Protein serine/threonine kinase activity	0.515677	0.903888
Catalytic activity	0.582086	0.916616
Ubiquitin-specific protease activity	0.411724	0.957707
Structural molecule activity	0.289945	0.970716
G-protein coupled receptor activity	0.105974	0.999944
Molecular function unknown	0.557085	0.999999

Target lncRNA-miRNA-mRNA subnetwork. To illuminate the competitive mechanisms and potential biological functions of lncRNAs in SCI, the degree distribution of nodes in the ceRNA network were investigated using Cytoscape software. The horizontal axis of Fig. 4 represents the degree of RNA in the ceRNA network. As presented in Fig. 4 and Table II, hsa-miR-124-3p had the highest degree of node, with a node value of 29. In Fig. 5, the subnetworks of XR_350851, NR_027820, and XR_591634, respectively, are presented, showing how the mRNA directly or indirectly is associated with the lncRNA.

Enrichment analysis of lncRNA-targeted mRNAs. Biological pathway annotation analysis of significantly differentially expressed mRNAs in the ceRNA subnetwork revealed relevant pathways and molecular interactions with associated genes. Based on FunRich software enrichment analyses, 263, 139 and 174 biological pathways were identified to be the components associate with the genes in the XR_350851, NR_027820 and XR_591634 subnetworks, respectively. As shown in Fig. 6, the critical biological pathways in the XR_350851 subnetwork included the activator protein 1 (AP-1) transcription factor network, integrin-linked kinase signaling, cell division cycle 42 (CDC42) signaling events, sphingosine-1-phosphate receptor 1 (S1P1) pathway, ADP-ribosylation factor 6 (Arf6) trafficking events, epidermal growth factor receptor 1 downstream signaling, class I phosphoinositide 3-kinase (PI3K) signaling events mediated by protein kinase B (Akt), insulin pathway,

Table II. Differentially expressed genes of the competing endogenous RNAs (node degree ≥ 5).

Number	RNA name	Degree
1	hsa-miR-124-3p	29
2	hsa-miR-30c-5p	26
3	hsa-miR-34a-5p	17
4	NR_027820	16
5	hsa-miR-26b-5p	16
6	XR_350851	15
7	hsa-miR-138-5p	15
8	hsa-let-7g-5p	14
9	Cd44	13
10	hsa-miR-143-3p	12
11	mmu-let-7a-5p	12
12	XR_591634	11
13	NFAT5	9
14	CEBPD	8
15	XR_590545	6
16	XR_145790	6
17	XR_146775	6
18	XR_590546	5
19	TUBB6	5

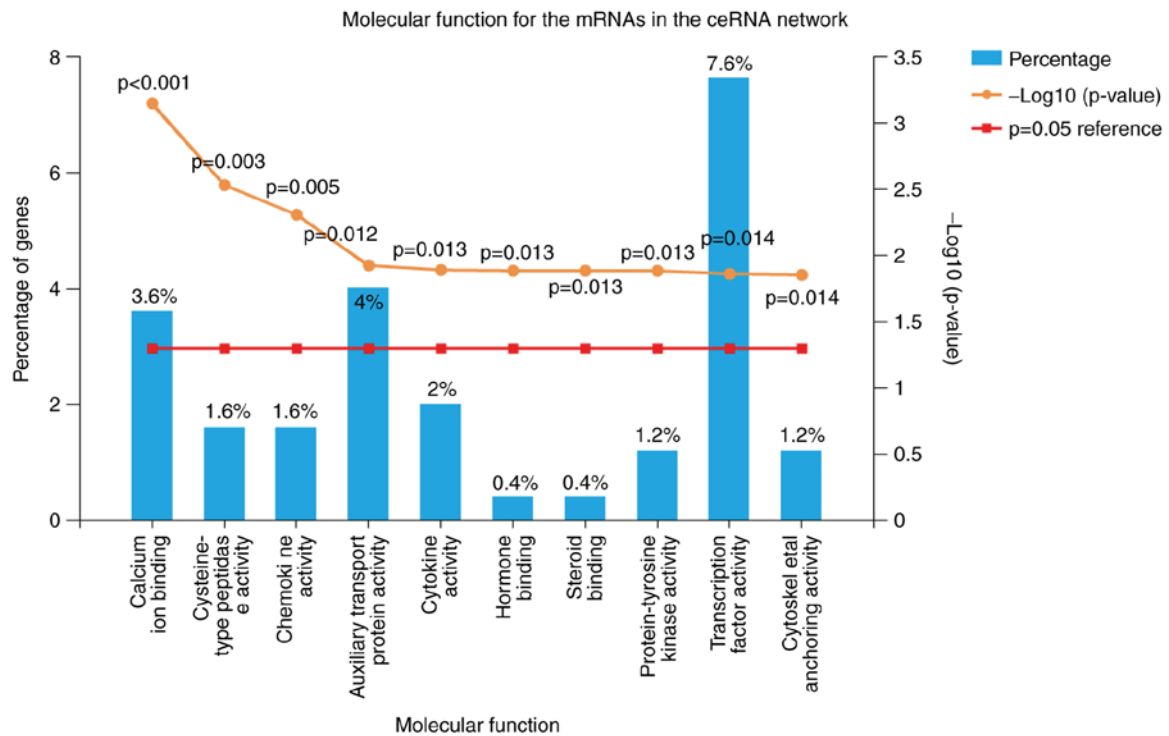


Figure 3. Top 10 critical molecular functions of the mRNAs in the ceRNA network. mRNA, messenger RNA; ceRNA, competing endogenous RNA.

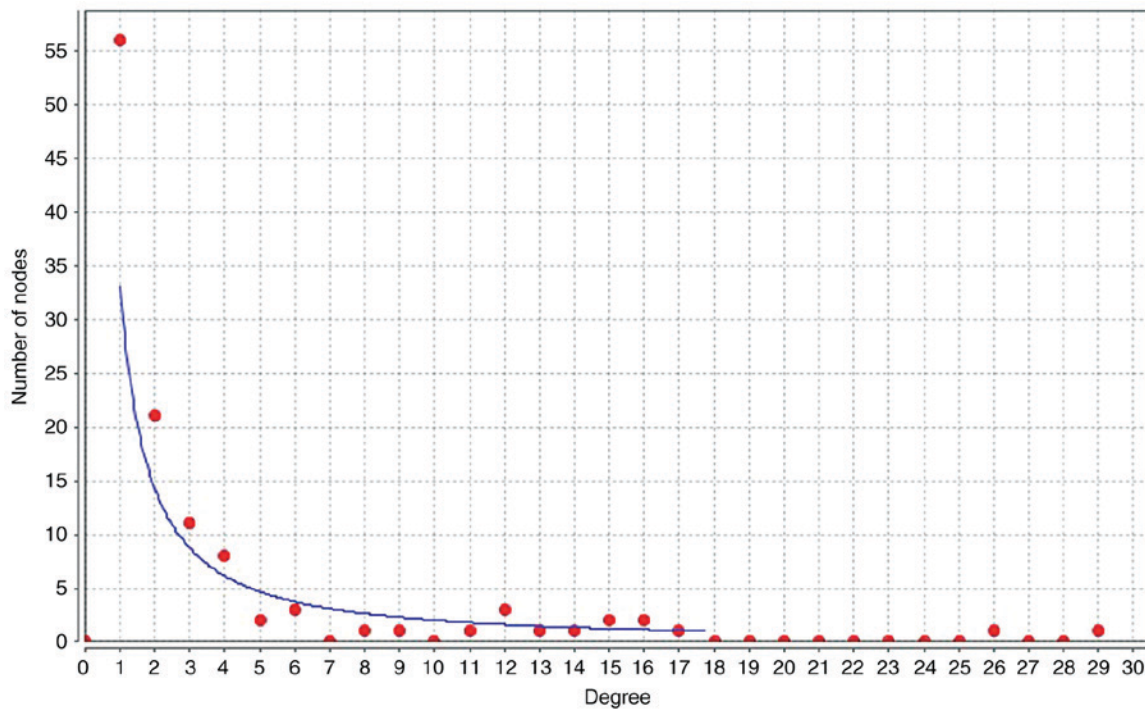


Figure 4. Degree distribution of nodes in the competing endogenous RNA network.

mammalian target of rapamycin (mTOR) signaling pathway and Arf6 downstream pathway. In addition, it was observed that the significant biological pathways identified for the subnetwork of NR_027820 were the following: Cell-extracellular matrix interactions, platelet degranulation, response to elevated platelet cytosolic Ca^{2+} , cell junction organization, ceramide biosynthesis, hemostasis, cell-cell communication, release of

eukaryotic translation initiation factor 4E, platelet activation, signaling and aggregation, and glycoprotein Ib-IX-V activation signaling (Fig. 7). Furthermore, the top 10 biological pathways for the XR_591634 subnetwork were as follows: C-MYB transcription factor network, AP-1 transcription factor network, integrin-linked kinase signaling, CDC42 signaling events, non-canonical Wnt signaling pathway, regulation of

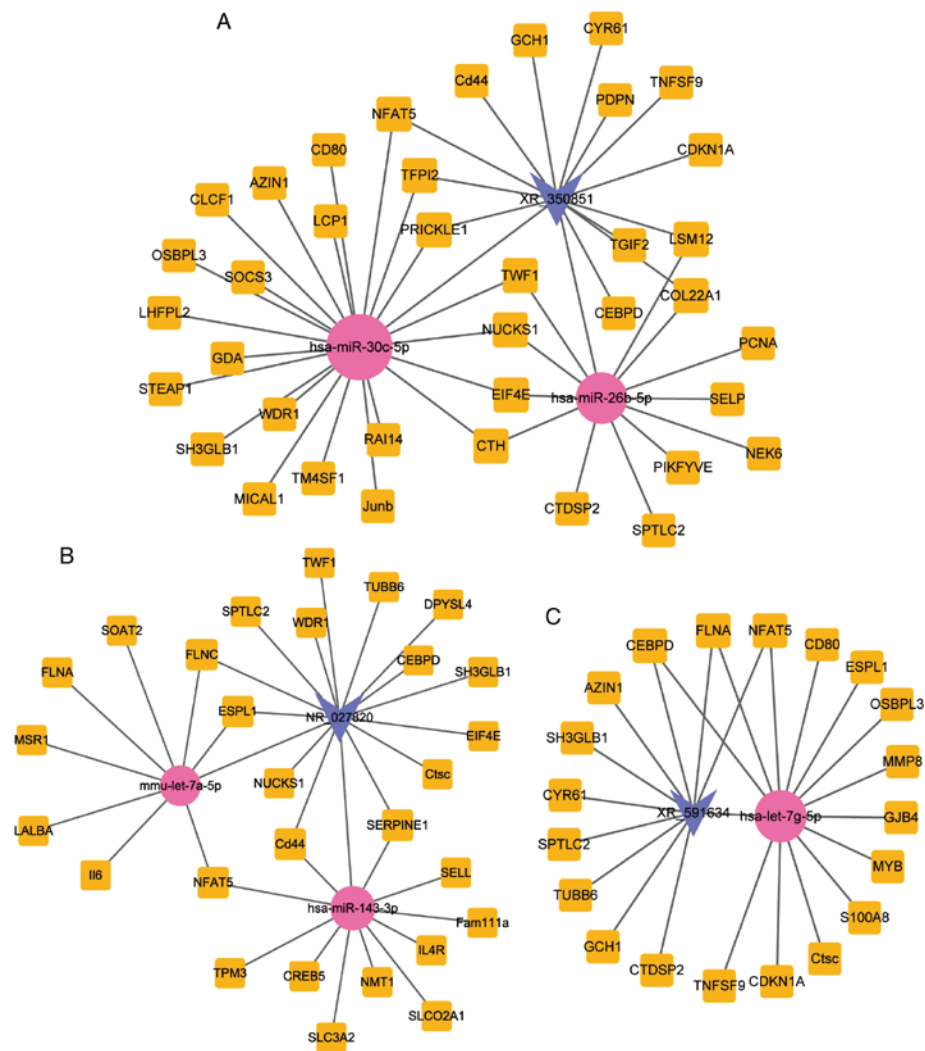


Figure 5. Sub-ceRNA networks of three node lncRNAs, including (A) XR_350851, (B) NR_027820 and (C) XR_591634 are displayed. Purple inverted triangles indicate the lncRNAs, pink circles indicate the miRNAs, and orange squares indicate the mRNAs, while the sizes of these shapes are positively correlated with the degrees of the nodes. ceRNA, competing endogenous RNA; lncRNA, long noncoding RNA; mRNA, messenger RNA; miRNA, microRNA.

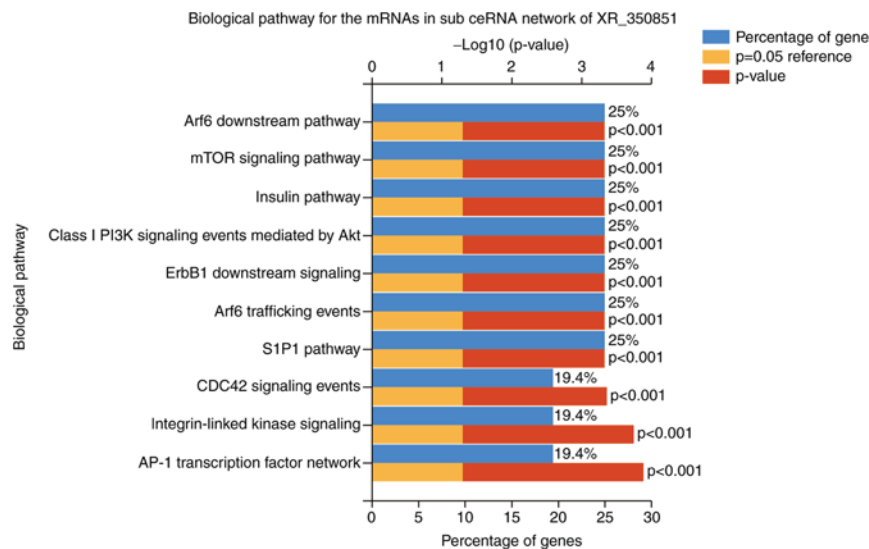


Figure 6. Top 10 biological pathways of the mRNAs in the sub-ceRNA network of the long noncoding RNA XR_350851. The top identified pathways included the AP-1 transcription factor network, integrin-linked kinase signaling, CDC42 signaling events, S1P1 pathway, Arf6 trafficking events, ErbB1 downstream signaling, class I PI3K signaling events mediated by Akt, insulin pathway, mTOR signaling pathway and Arf6 downstream pathway. mRNA, messenger RNA; ceRNA, competing endogenous RNA; AP-1, activator protein 1; CDC42, cell division cycle 42; S1P1, sphingosine-1-phosphate receptor 1; Arf6, ADP-ribosylation factor 6; ErbB1, epidermal growth factor receptor 1; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B.

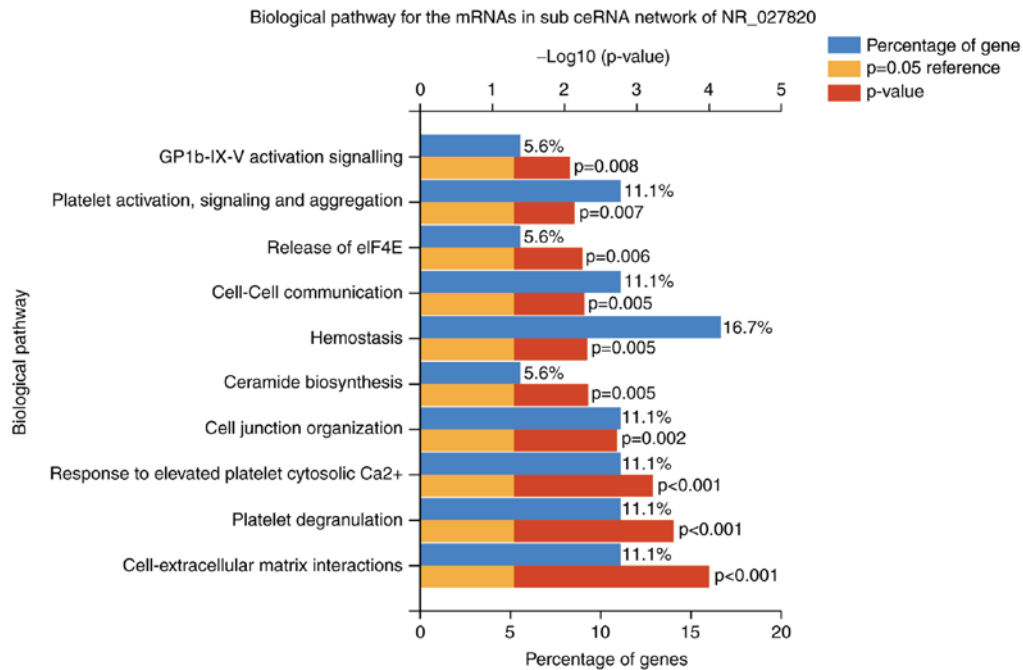


Figure 7. Top 10 biological pathways of the mRNAs in the sub-ceRNA network of the long noncoding RNA NR_027820. These pathways included the cell-extracellular matrix interactions, platelet degranulation, response to elevated platelet cytosolic Ca²⁺, cell junction organization, ceramide biosynthesis, hemostasis, cell-cell communication, release of eIF4E, platelet activation, signaling and aggregation, GPIb-IX-V activation signaling. mRNA, messenger RNA; ceRNA, competing endogenous RNA; eIF4E, eukaryotic translation initiation factor 4E; GPIb-IX-V, glycoprotein Ib-IX-V.

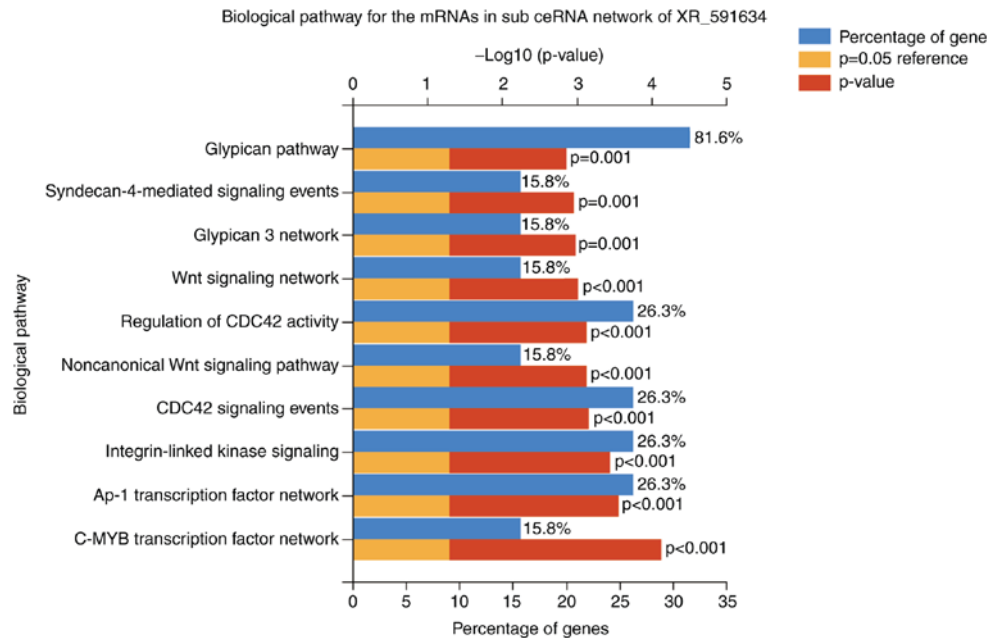


Figure 8. Top 10 biological pathways of the mRNAs in the sub-ceRNA network of the long noncoding RNA XR_591634. The pathways included the C-MYB transcription factor network, AP-1 transcription factor network, integrin-linked kinase signaling, CDC42 signaling events, noncanonical Wnt signaling pathway, regulation of CDC42 activity, Wnt signaling network, glypican 3 network, syndecan-4-mediated signaling events and glypican pathway. mRNA, messenger RNA; ceRNA, competing endogenous RNA; AP-1, activator protein 1; CDC42, cell division cycle 42.

CDC42 activity, Wnt signaling network, glypican 3 network, syndecan-4-mediated signaling events, and Glypican pathway (Fig. 8).

Comparing the results of these three subnetworks, XR_350851 was observed to be associated with the classic autophagy pathway, namely the PI3K/Akt/mTOR signaling pathway. The interactions of the

three lncRNA-targeted mRNAs were also enriched using FunRich software (Figs. 9-11).

Discussion

Approximately 130,000 people, predominantly young adults, suffer from SCI paralysis annually worldwide, which has

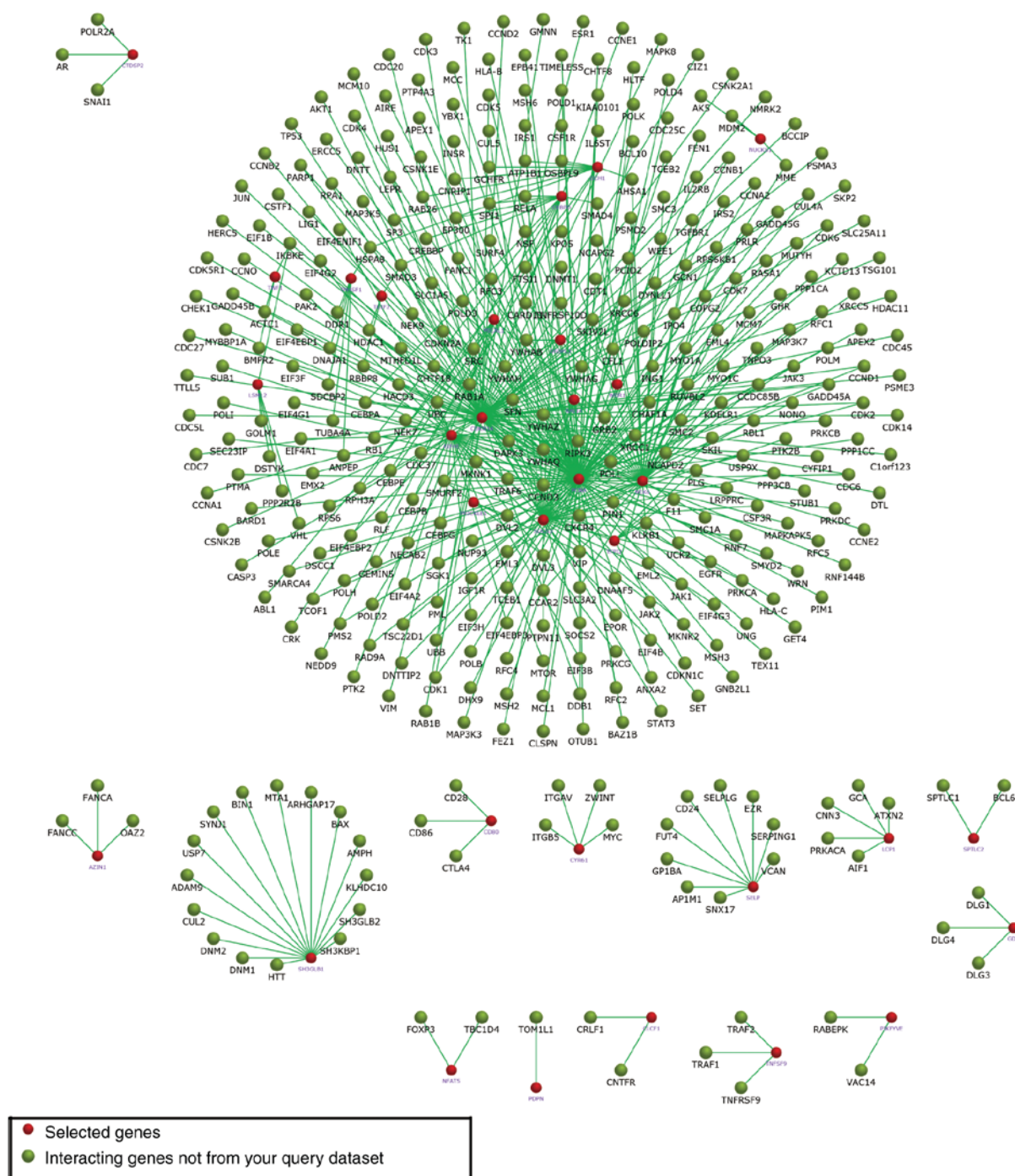


Figure 9. Protein-protein interaction network of XR_350851-targeted messenger RNAs. Red circles indicate the selected genes, while green circles indicate nodes from outside the selected dataset that exhibited interactions.

a serious socioeconomic impact (37). The complex pathophysiology of SCI, which includes primary and secondary mechanisms, involves a complicated cascade of molecules and hampers the generation of effective therapies (38). To identify target lncRNAs that have potential as novel predictors in clinical diagnoses and treatments, a network based on the ceRNA theory was generated by mining lncRNA, miRNA and mRNA data from NCBI GEO database. The resulting lncRNA-miRNA-mRNA regulatory network consisted of 13 lncRNA, 93 mRNA and 9 miRNA nodes, with a total of 202 edges.

Molecular function and biological pathway analyses were used to assign the biological functions of differentially

expressed genes. The module analysis identified 75 molecular function modules associated with SCI. The identified molecular functions included calcium ion-binding, cysteine-type peptidase activity, chemokine activity, auxiliary transport protein activity, cytokine activity, hormone binding, steroid binding, protein-tyrosine kinase activity, transcription factor activity and cytoskeletal anchoring activity. In the current study, it was observed that the node degrees of three lncRNAs, namely XR_350851, NR_027820 and XR_591634, were significantly higher compared with those of other lncRNAs. These three lncRNAs also exhibited an increased number of lncRNA-miRNA and miRNA-mRNA pairs. Therefore, it is

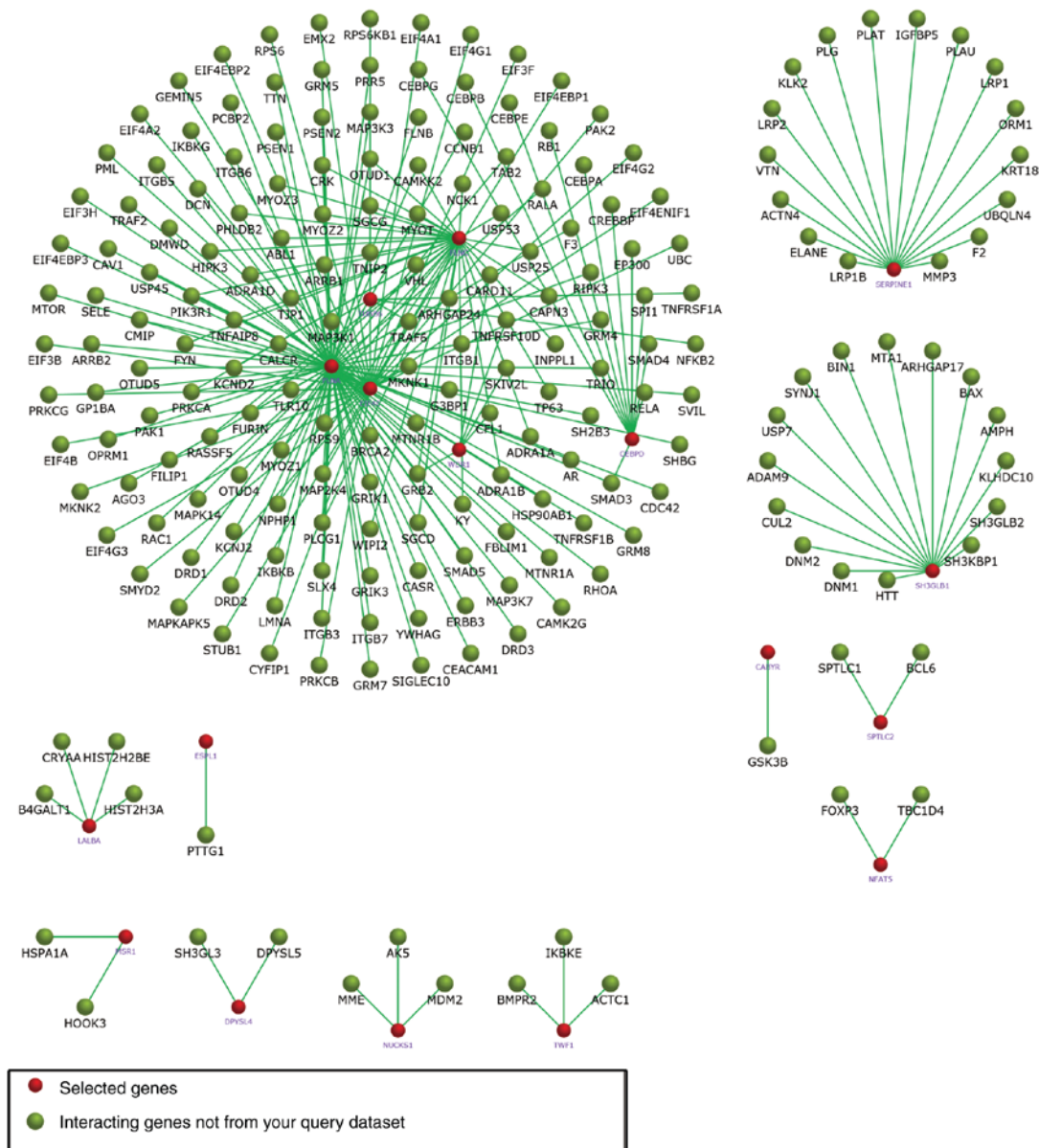


Figure 10. Protein-protein interaction network of NR_027820-targeted messenger RNAs. Red circles indicate the selected genes, while green circles indicate nodes from outside the selected dataset that exhibited interactions.

suggested that these lncRNAs (XR_350851, NR_027820 and XR_591634) may have profound implications in SCI and may be potential target lncRNAs. Subsequently, sub-ceRNA networks of the three aforementioned node lncRNAs were reconstructed in the current study.

Biological pathway analysis then identified that 263, 139 and 174 pathways were respectively enriched in the XR_350851, NR_027820 and XR_591634 subnetworks. The predominant biological pathways in the XR_350851 subnetwork were the AP-1 transcription factor network, S1P1 pathway, class I PI3K signaling events mediated by Akt, insulin pathway and mTOR signaling pathway. The significant biological pathways in the NR_027820 subnetwork included platelet degranulation, cell junction organization, ceramide biosynthesis, hemostasis and platelet activation. In addition, the AP-1 transcription factor network, integrin-linked kinase signaling, regulation of CDC42 activity, Wnt signaling network and glycan pathway were the critical biological pathways in the XR_591634 subnetwork.

A previous study by Tsuboyama *et al* (39) determined that starvation conditions or mTOR inhibition promoted VPS34-dependent ribophagic flux in cells. In addition, a previous study reported that rapamycin may aid in the restoration of motor function and act in a neuroprotective manner by suppressing the mTOR pathway (40). As a central regulator of autophagy and a serine/threonine kinase, mTOR is involved in cancer, and in cardiovascular and neurological diseases. In addition, mTOR is the catalytic subunit of two distinct signaling complexes, including mTOR complex 1 (mTORC1) and mTORC2 (41,42), which are significantly involved in the control of cell proliferation. It is noteworthy that the growth factor/PI3K/Akt signaling pathway is the upstream modulator of mTORC1 (43). Another important finding was that mTORC2-ribosome binding can be improved by insulin stimulation of the PI3K signaling pathway (44). There is increasing evidence that inhibition of the autophagy-associated mTOR pathway subsequent to SCI may have a neuroprotective effect (39). Furthermore, Bai *et al* (45) revealed that stimulating

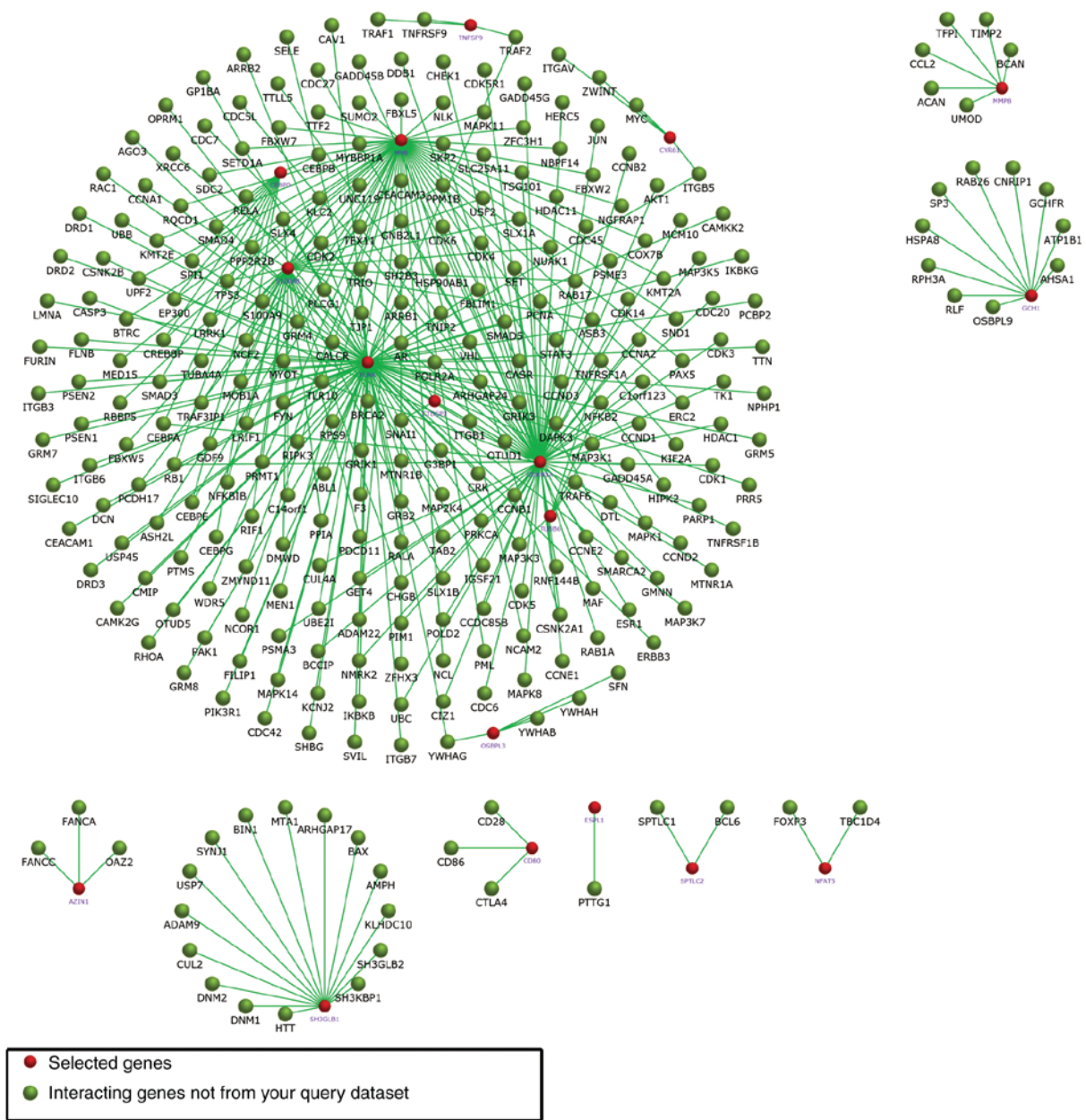


Figure 11. Protein-protein interaction network of XR_591634-targeted messenger RNAs. Red circles indicate the selected genes, while green circles indicate nodes from outside the selected dataset that exhibited interactions.

the AMPK/mTOR signaling pathway of autophagy may facilitate functional recovery from SCI. The PI3K/Akt/mTOR pathway has previously been identified as a classic autophagy pathway (46). In addition, the PI3K/Akt/mTOR pathway is important for XR_350851 associated mRNA. Therefore, XR_350851 may be used as a target gene for neuroprotection following SCI. However, whether the lncRNA XR_350851 regulates autophagy in SCI has yet to be determined. Therefore, it is suggested that future studies should focus on the role of XR_350851 in SCI.

In conclusion, using mined data and based on the ceRNA theory, an SCI-associated lncRNA-miRNA-mRNA network was constructed in the current study. According to the distribution of the nodes in the ceRNA network, three lncRNAs (XR_350851, NR_027820 and XR_591634) were identified, and their sub-ceRNA networks constructed. Furthermore, the functions of these three node lncRNAs were predicted

by enriching the pathways of their associated mRNAs in the sub-ceRNA networks. The present study provided further insight into the involvement of lncRNAs in the mechanism of SCI, and the identified lncRNAs may serve as potential novel biomarkers and therapeutic targets for SCI. Finally, it is speculated that the lncRNA XR_350851 is closely associated with autophagy. However, further research is required to determine the biological role of XR_350851 in SCI.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZQ conceived and designed the present study. LW, JL and BW collected, extracted and analyzed the data. LW wrote the manuscript. LW and ZQ reviewed the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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