Identifying the Biomarkers of Spinal Cord Injury and the effects of Neurotrophin-3 Based on MicroRNA and mRNA Signature

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Research article

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Abstract

Background

To gain a better understanding of the molecular mechanisms of spinal cord injury and the effects of Neurotrophin-3, differentially expressed microRNAs (DEmiRNAs) and genes (DEGs) were analyzed.

Methods

The miRNA transcription profile of GSE82195 and the mRNA transcription profile of GSE82196 were downloaded from the Gene Expression Omnibus (GEO). Then, DERs were identified using limma. The noise-robust soft clustering of the intersection DERs was performed using Mfuzz package. Additionally, the integrated miRNAs–targets regulatory network was constructed using Cytoscape. Finally, the Comparative Toxicogenomics Database 2019 update was used to search the central nervous system injury related pathway.

Results

A total of 444 DERs including 382 DEGs and 62 DEmiRNAs were screened between group injury and group none whlie 576 DERs including 523 DEGs and 55 DEmiRNAs were screened between group NT-3 and group injury. Moreover, 80 intersections DERs were identified. DREs in cluster 1 were firstly significantly down-regulated in group injury and subsequently were significantly up-regulated in group NT-3. DERs in cluster 2 were firstly up-regulated in group injury and subsequently down-regulated in group NT-3. OPRL1 and GHSR were enriched in the KEGG pathway of Neuroactive ligand-receptor interaction. OPRL1 was involved in the chemical homeostasis and ion homeostasis while GHSR was related to the regulation of fatty acid metabolic process and regulation of cellular ketone metabolic process.

Conclusion

rno-miR-3072 and rno-miR-667-5p and OPRL1 and GHSR might participate in the pathogenesis of neurological injury and the neurotrophin-3 treatment.

Background

Spinal cord injury results in permanent disability due to the limited growth capacity of adult central nervous system axons[1]. Impairment of sensorimotor processing in the spinal cord, reduce mobility and upper limb function due to corticospinal tract lesions in spinal cord injury or stroke[2] is a major cause of disability in the world [3]. In the rat model, it has been found that complete transection of corticospinal pathways in the pyramids could lead to increased spasms, excessive mono- and polysynaptic spinal reflexes and impaired locomotion [4].

Neurotrophin-3 (NT-3) is a growth factor found in many body tissues including the heart, intestines, skin, nervous system and in skeletal muscles including muscle spindles [5]. It has been proven that NT-3 is required for the survival, correct connectivity and function of sensory afferents that innervate muscle spindles[6].

MicroRNAs (miRNAs) with the length from 18–25 nucleotides, is a class of non-protein coding RNAs which have been shown to be involved in a wide variety of biological processes as regulatory molecules through suppress the mRNA expression or translation. In 2018, Claudia et al. [7] conducted RNA (miRNA and mRNA) sequence (now available at GSE82195 and GSE82196) describing transcriptional changes in cervical dorsal root ganglia after bilateral pyramidotomy and forelimb intramuscular gene therapy with an adeno-associated viral vector encoding human neurotrophin-3 and Many of the dysregulated genes are involved in axon guidance and plasticity. Intramuscular neurotrophin-3 treatment normalized many of those gene changes and may be one of the mechanisms how reflexes, functional recovery and molecular markers in the spinal cord are restored.

In our study, using the same microarray data by Claudia et al., we aimed to further screen the DEmiRNAs and DEGs with linear models for microarray data (limma) package based on a threshold FDR-value < 0.05 and |log2 FC| > 0.5) and clustering analysis using Mfuzz package, miRNAs-mRNAs regulatory network followed by Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment as well as the central nervous system injury related KEGG pathway screening. It has been shown that analyses based on differential statistical tests may result in different outcomes [8]. Therefore, we believe some different results may be obtained.

Methods

RNA sequencing data and the data preprocessing

The miRNA transcription profile of GSE82195 and the mRNA transcription profile of GSE82196 [7] (Illumina HiSeq 2500) including dorsal root ganglion (DRG) tissue samples of normal adult rat (none, n = 6), 10 weeks post-pyramidotomy, intramuscular AAV-1 GFP (injury, n = 6) and 10 weeks post-pyramidotomy, intramuscular AAV-1 prepro-neurotrophin-3 (NT-3, n = 6) were downloaded from the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/). The preprocess Core package in R language (version 1.44, https://www.bioconductor.org/packages/devel/bioc/html/preprocessCore.html) was employed to perform the background correction and the normalization [9].

Differentilly Expressed Rnas Analysis

Limma package in R language [10] (Version 3.34.0, https://bioconductor.org/packages/release/bioc/html/limma.html) to screen the differentilly expressed RNAs (DERs) including differentially expressed genes (DEGs) and differentially expressed miRNA (DEmiRNAs) between group injury and group none, as well as the DEGs and DEmiRNAs between group

NT-3 and group injury. We used the FDR-value < 0.05 and |log2 FC| > 0.5 as the cutoff criteria for DEGs and DEmiRNAs. Furthermore, the intersection DERs were also screened. The pheatmap package in R [11] (version 1.0.8, https://cran.r-project.org/package=pheatmap) was employed to conduct the bidirectional hierarchical clustering.

Clustering Analysis

With the aforementioned cut-off criteria, two clusters were obtained including cluster 1 (44 DERs, containing 10 miRNAs and 4 mRNAs) and cluster 2 (36 DERs, containing 10 miRNAs and 26 mRNAs) (Table 1). DREs in cluster 1 were firstly significantly downregulated in group injury and subsequently were significantly upregulated in group NT-3. DERs in cluster 2 were firstly upregulated in group injury and subsequently downregulated in group NT-3 (Fig. 3).

Table 1
The genes in clusters

cluster1	cluster2
Adsl	Abca4
Aurkb	Aptx
Bace2	Bdh2
Chdh	Cybrd1
Col9a1	Dmac2
Cxcl14	Drc1
Dhh	Fgd1
Ehd4	Fli1
Epha10	Frzb
Etv5	Gclc
Fam83d	Ghsr
Fam83f	Id1
Gas7	Mrnip
Gjb1	Nebl
Gucy2d	Nme3
Loxl4	Oprl1
Map4k4	Pdcd2l
Meox1	Pgf
Pdia5	Ppl
Plk2	Prkag3
Plxdc1	Rbpms2
Ppp4r1	rno-let-7d-3p
Ptger4	rno-miR-182
Radil	rno-miR-187-5p
Rasa3	rno-miR-27a-3p
Reln	rno-miR-29b-1-5p
rno-miR-145-5p	rno-miR-30e-3p

cluster1	cluster2
rno-miR-146b-5p	rno-miR-379-3p
rno-miR-1839-5p	rno-miR-455-3p
rno-miR-217-5p	rno-miR-505-5p
rno-miR-3072	rno-miR-672-3p
rno-miR-344b-1-3p	Slc22a25
rno-miR-3594-3p	Slc4a4
rno-miR-381-3p	Slc6a6
rno-miR-666-5p	Slc9a5
rno-miR-667-5p	Smpd2
Sh3rf1	
Shbg	
Shc4	

Mirna-mrna Regulatory Networks Construction

By using DERs in the clusters, the the miRNA-mRNA regulatory network was constructed. Firstly, DEmiRNA targets was predicted using starBase database (Version 2.0, http://starbase.sysu.edu.cn/). Secondly, the miRNA-mRNA pair with the negative correlation were retained. lastly, the screened miRNA-mRNA pairs were visualized using Cytoscape software with the DEmiRNAs in cluster 1 and their target DEGs in cluster 2, as well as using the DEmiRNAs in cluster 2 and their target DEGs in cluster 1 (Version 3.6.1, http://www.cyto scape.org) [13]. DAVID (version 6.8, https://david.ncifcrf.gov/) was used to perform the GO and KEGG pathway enrichment for the mRNA in the network [14].

Central Nervous System Injury Related Kegg Pathway Screening

In the CTD database, 13 KEGG pathways were searched, among which Neuroactive ligand-receptor interaction (including two genes: OPRL1 and GHSR in cluster 2) was intersected with enriched KEGG pathway for the DEGs in the miRNA-mRNA regulatory network. GHSR could be the target of rno-miR-1839-5p and rno-miR-344-3p while OPRL1could be the target of rno-miR-3072 and rno-miR-667-5p (Fig. 6).

Results

DERs analysis

A total of 444 DERs including 382 DEGs and 62 DEmiRNAs were screened between group injury and group none whlie 576 DERs including 523 DEGs and 55 DEmiRNAs were screened between group NT-3 and group injury. Moreover, 80 intersections DERs were identified. We used volcano plots for the visualization and assessment of the variation (or reproducibility) of RNAs expression (Fig. 1). The bidirectional hierarchical clustering revealed that DERs could distinguish these two groups (Fig. 2).

Mirna-mrna Network Construction

By using the DEmiRNAs in cluster 2 and their target DEGs in cluster 1, the miRNA-mRNA regulatory network was constructed which contained 45 miRNA-mRNA pairs, while by using the DEmiRNAs in cluster 1 and their target DEGs in cluster 2, the miRNA-mRNA regulatory network was constructed which contained 31 miRNA-mRNA pairs (Fig. 4). Functional enrichment showed that 7 GO terms and 3 KEGG patyways were enriched for the DEGs in cluster 1 while 9 GO terms and 1 KEGG pathways were enriched for the DEGs in cluster 2 (Fig. 5)

Discussion

In order to further investigate the mechanism of neurological injury and the neurotrophin-3 treatment to improves mobility, we re-analyzed the mRNA and miRNA expression of DRG tissues from the normal adult rat, 10 weeks post-pyramidotomy, intramuscular AAV-1 GFP adult rat and 10 weeks post-pyramidotomy, intramuscular AAV-1 prepro-neurotrophin-3 adult rat and screened the two genes OPRL1 and GHSR were firstly significantly upregulated in group injury and subsequently downregulated the with the treatment of NT-3. Besides, rno-miR-1839-5p, rno-miR-3072 and rno-miR-667-5p were firstly downregulated in group injury and subsequently upregulated with the treatment of NT-3.

GHSR (growth hormone secretagogue 1 receptor) is the receptor of Ghrelin which is a brain-gut peptide hormone secreted from the stomach to stimulate food intake. Additionally, GHSR has been found to mediate the neuroprotection in rodents including the activation of UCP2 and decreasing in mitochondrial ROS production, suppression of the pro-inflammatory cytokines TNFa, IL-6 and IL-1b and augmentation of midbrain dopamine neuron electrical activity [16]. The functional enrichment showed that GHSR was related to the regulation of fatty acid metabolic process and regulation of cellular ketone metabolic process. Former study has demonstrated that the ketogenic diet improves forelimb motor function after spinal cord injury in rodents [17]. And Omega-3 polyunsaturated fatty acids could promote functional recovery in rats undergoing spinal cord injury [18]. So, GHSR could be related to neurological injury and the neurotrophin-3 treatment by regulating the cellular ketone metabolic process and fatty acid metabolic process.

OPRL1 encodes a G protein-coupled receptor for nociceptin, an endogenous opioid-related neuropeptide which is one of four opioid receptors. Opioid receptor activation could attenuate the release of inhibitory

neurotransmitters and changes in neuronal excitability [19] which indicated that OPRL1 could played a important role in the nervous system. The GO functional enrichment showed that OPRL1 was involved in the chemical homeostasis and ion homeostasis. It has been illustrated that through a return to homeostasis of chloride after spinal cord injury, exercise could help to contribute to reflex recovery [20]. Additionally, OPRL1 could be the target of rno-miR-3072 and rno-miR-667-5p. MiR-3072 has been found to be related to the decrease of damage and paralysis of lower limbs following spinal cord injury (SCI) [21]. rno-miR-667 may be associated with presence of nerve injury-induced hypersensitivity [22]. Therefore, we speculated that OPRL1 targeted by rno-miR-3072 and rno-miR-667-5p could play an important role in neurological injury and the neurotrophin-3 treatment.

Besides, OPRL1 and GHSR were enriched in the KEGG pathway of Neuroactive ligand-receptor interaction which is found to associated with central nervous system injury in CTD database.

Conclusion

DEmiRNAs rno-miR-3072, DEmiRNAs rno-miR-667-5p, OPRL1 and GHSR were identified might participate in the pathogenesis of neurological injury and the neurotrophin-3 treatment. However, further research is required to validate the results.

Abbreviations

DEmiRNAs
differentially expressed microRNAs;
DEGs
differentially expressed genes;
DERs
differentilly expressed RNAs;
GEO
Gene Expression Omnibus;
DRG
Dorsal root ganglion;
GHSR
Growth hormone secretagogue 1 receptor;
NT-3
Prepro-neurotrophin-3;
GO
Gene Ontology;
KEGG
Kyoto Encyclopedia of Genes and Genomes ;

Declarations

Authors' contributions

Shanshan Yu and Shuang Qi conceived, designed, priformed the experiments and wrote the paper. Zinan Li analyzed and interpreted the data and contributed methods, materials, analysis tools or data. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

Competing interests

The author declares that they have no competing interests.

Availability of data and materials

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

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures

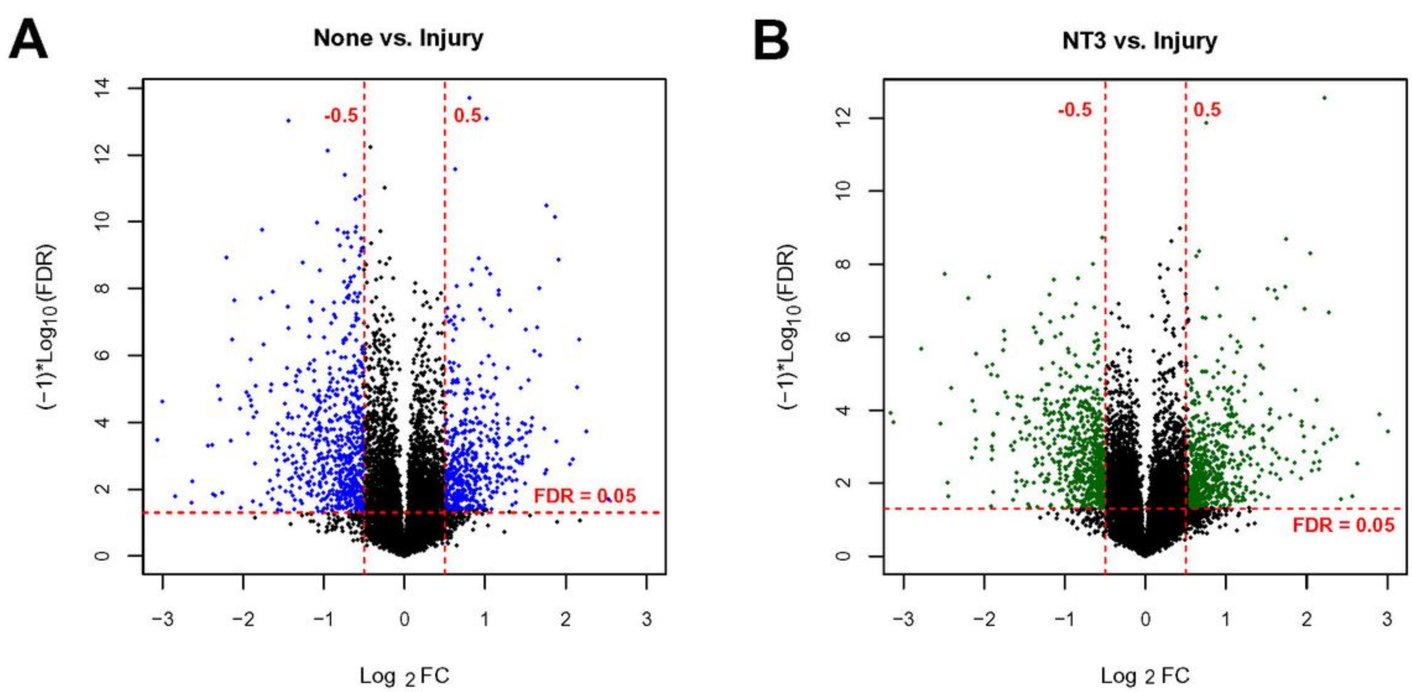

Figure 1

The volcano plots for the visualization and assessment of the RNAs expression. A, the volcano plot of DERs between group injury and group none; B. the volcano plot of DERs between group NT-3 and group injury.

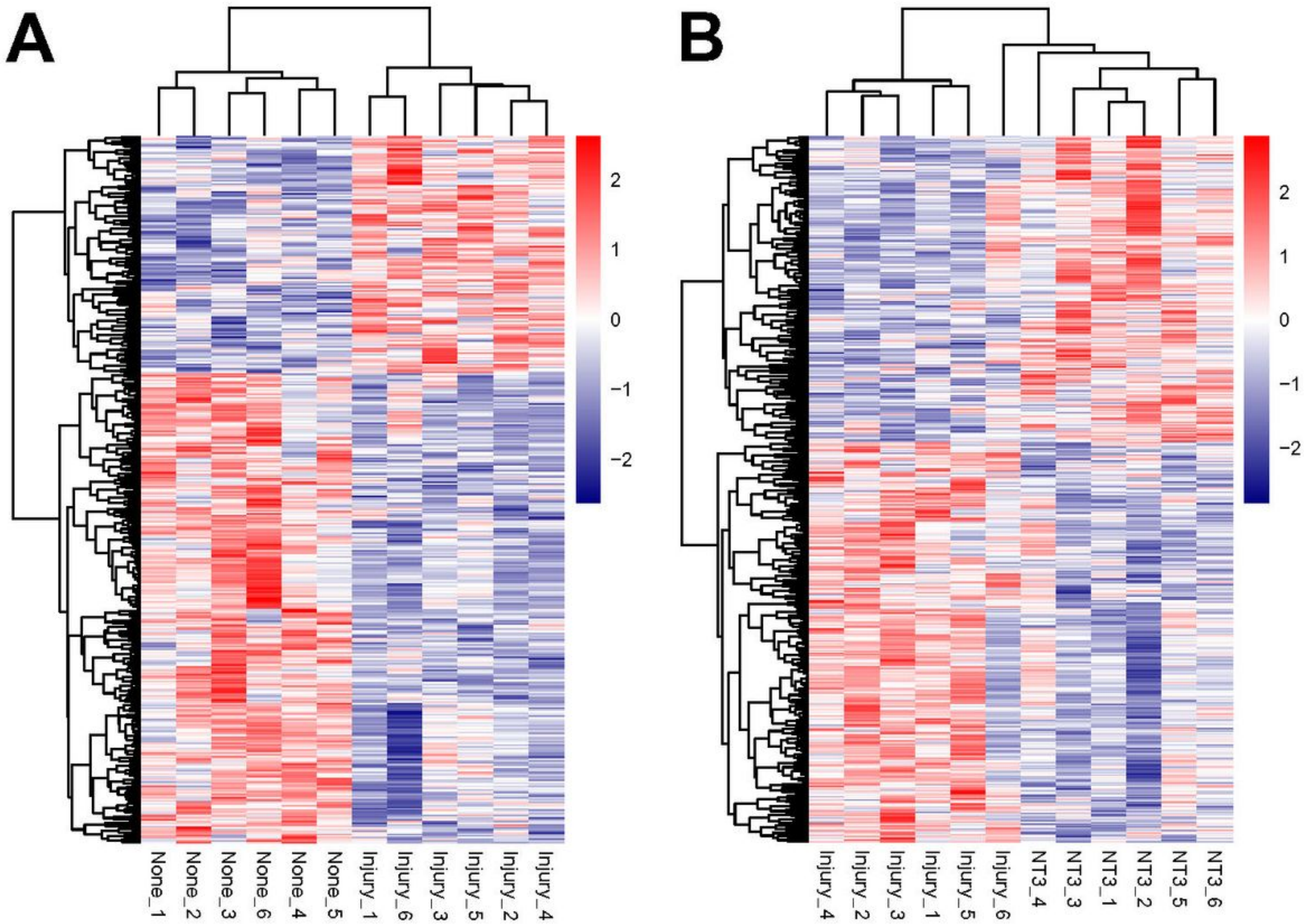

Figure 2

The two-way clustering of differentially expressed RNAs (DErs); A for the DERs between group injury and group none; B for the DERs between group NT-3 and group injury; horizontal axis, the samples; vertical axis, samples; color key, the logFC of DERs.

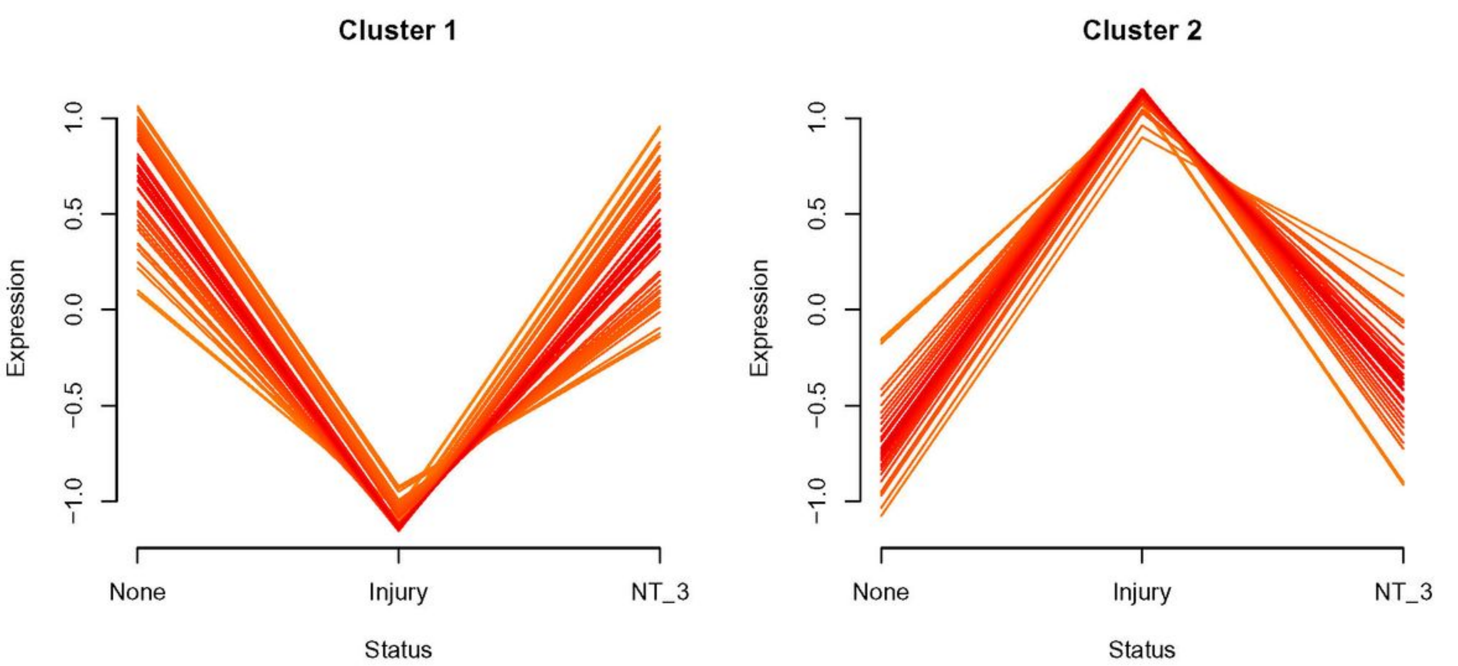

Figure 3

The gene expression changes in two clusters. The color varying from orange to red represents that the trends of genes become more suitable to the changes of the cluster.

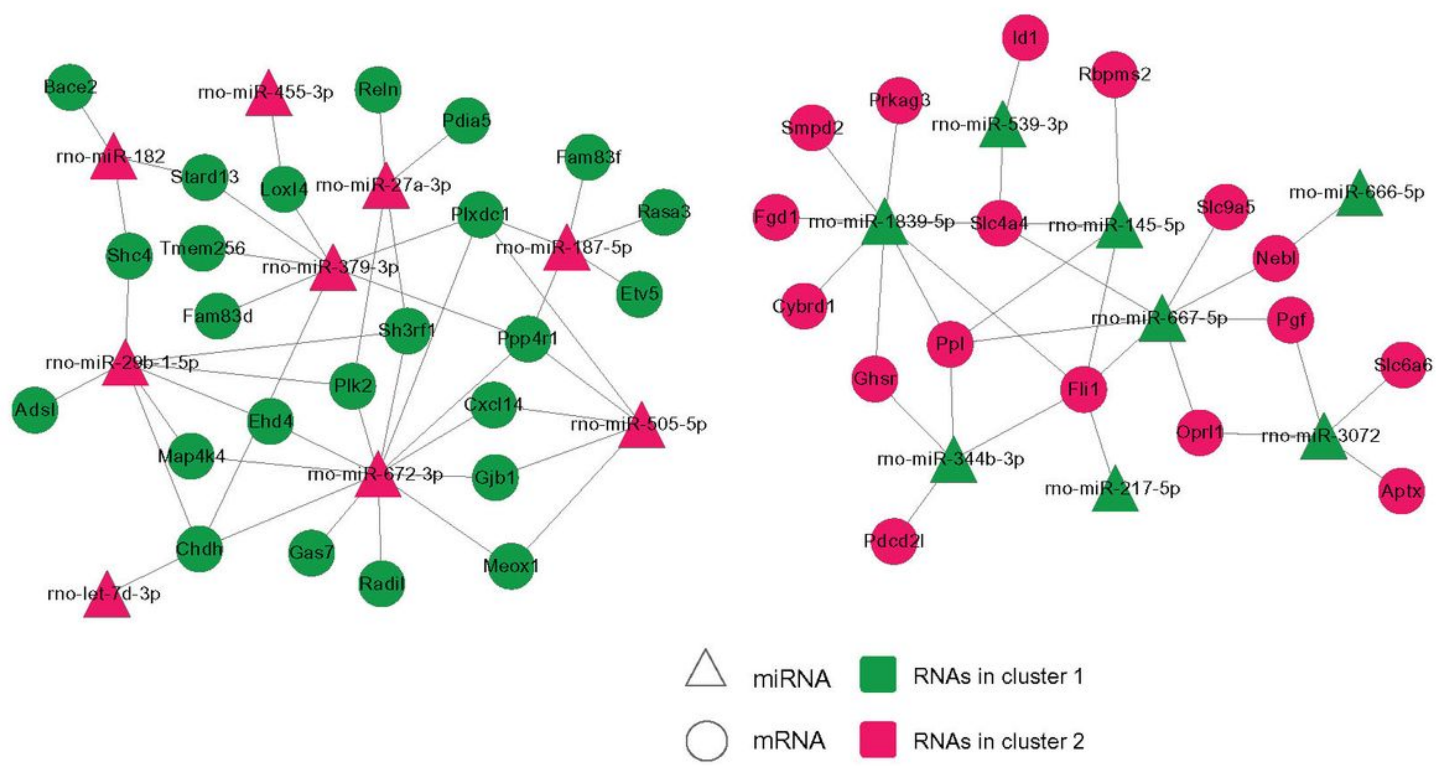

Figure 4

The integrated DEmiRNAs–Targets regulatory network; triangle nodes represent DEmiRNAs; cycle nodes represent DEGs; Green represent DERs in cluster 1; Red represent DERs in cluster 2.

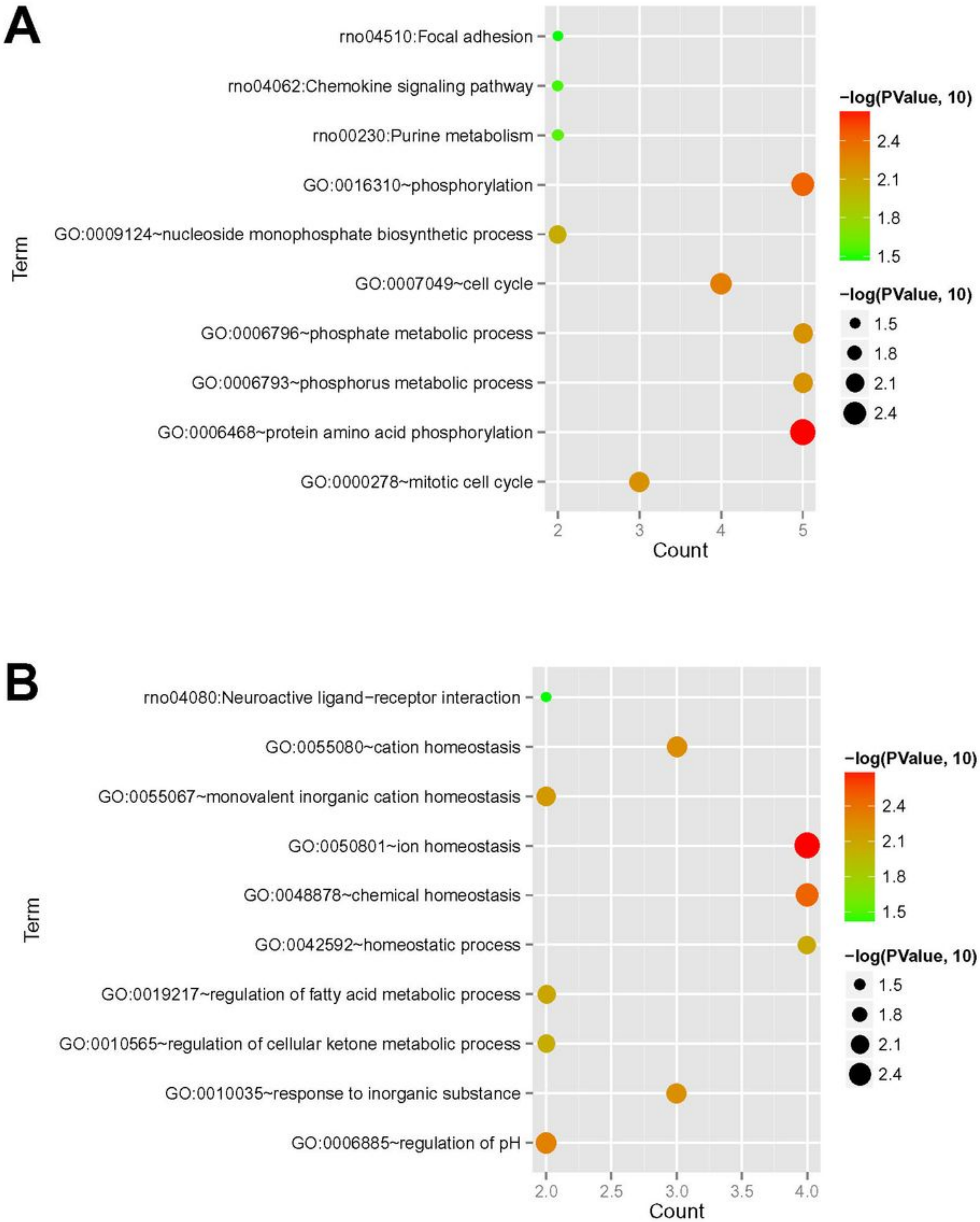

Figure 5

The KEGG pathways and GO terms enrichment for the DEGs in cluster 1 and (A) cluster 2 (B); The horizontal axis represents the number of genes, and the vertical axis represents the name of the item. The size and color of the point represent the significance, and the larger the point and the closer the color is to red, the higher the significance.

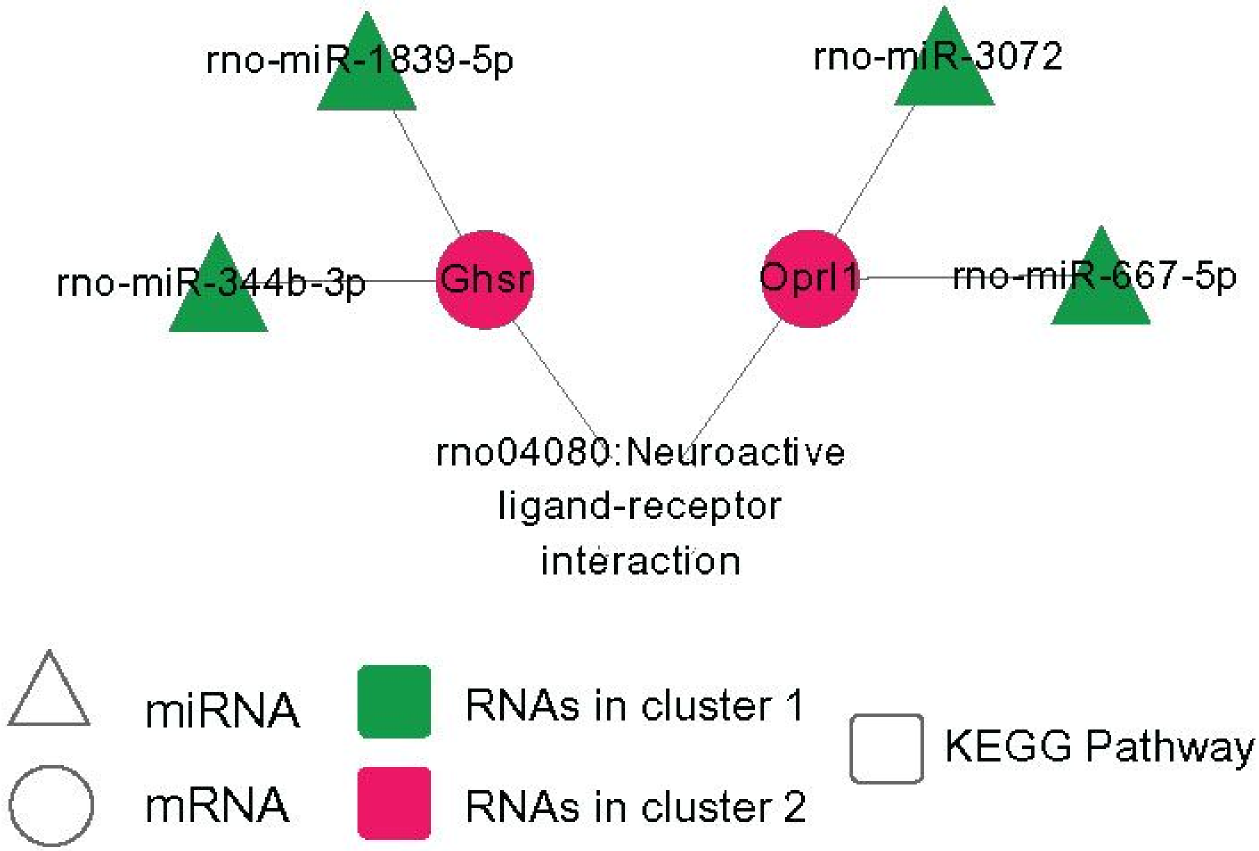

Figure 6

Pathway regulatory networks associated with central nervous injury.Triangle and circle represent miRNA and mRNA, respectively; Green and red represent RNAs elements from cluster 1 and 2, respectively; Square represents KEGG signaling pathway directly related to central nervous injury.