Bioinformatics analysis of the regulatory lncRNA-miRNA-mRNA network in patients with neuropathic pain and prediction of potential therapeutics

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Abstract

Background: Neuropathic pain (NP) is the main form of chronic pain, caused by damage to the nervous system and dysfunction. Here, we aimed at exploring the key molecules involved in NP pathogenesis via the identification of its regulatory lncRNA-miRNA-mRNA network.

Methods: We downloaded NP-related data from public databases and identified differentially expressed long noncoding RNAs (lncRNAs), microRNAs (miRNAs) and mRNAs through differential gene expression analysis. The lncRNA and miRNA target predictions were performed and an integration of the three datasets was used for construction of the lncRNA-miRNA-mRNA network. Subsequently, functional enrichment analysis and protein-protein interaction (PPI) analysis were performed to explore the role and the interactions of the mRNAs. The drug prediction was performed based on the mRNAs in the lncRNA-miRNA-mRNA network.

Results: A total of 8,251 differentially expressed mRNAs (4,193 upregulated and 4,058 downregulated), 959 differentially expressed miRNAs (455 upregulated and 504 downregulated), 2,848 differentially expressed lncRNAs (1,324 upregulated and 1,524 downregulated) were identified by integrating the results of the three microarray datasets. We found that differentially expressed mRNAs were mainly enriched in blood circulation, metal ion transmembrane transporter activity, and synaptic membrane. The most significant pathway of mRNAs in lncRNA-miRNA-mRNA network were GTPase, cell cycle, and platelet activation. A total of 1,200 drugs were predicted as potential therapeutics for NP based on the regulatory genes.

Conclusion: Our study predicted drugs that may be effective for NP based on the NP regulatory network. This information will help further reveal the pathological mechanism of NP and provide more treatment options for NP patients.

Background

Neuropathic pain, the main form of chronic pain, is caused by damage to the nervous system and dysfunction (1, 2). Currently, the treatment of NP is limited to symptomatic treatment and its prognosis is poor. To better understand the pathogenesis of NP, it is essential to develop effective prevention strategies and improve the efficacy of NP treatment. This necessitates the deepening of the molecular genetics of NP.

lncRNA is a transcript that is more than 200 nucleotide (nt) long; it regulates a variety of processes, including DNA methylation, transcription, and post-transcriptional RNA processing. lncRNA participates in various gene transcriptional processes via interacting with transcription factors, coactivators, and/or inhibitors. For example, lncRNAs can bind miRNAs and affect the expression of downstream targeted mRNAs. Accumulating evidences show that lncRNAs play an important role in the pathogenesis of nervous system disease (3, 4). For instance, Zhou and colleagues (5) found that a total of 134 lncRNAs, 12 miRNAs, 188 circRNAs, and 1066 mRNAs were significantly regulated after spared nerve injury (SNI)

surgery. Wei and colleagues (3) found that lncRNA X inactive-specific transcript (XIST) promotes NP progression in rats via down-regulation of miR-154-5p and up-regulation of TLR5S; this pathway represents a novel therapeutic target for NP treatment.

It is known that miRNAs and lncRNAs regulate gene expression and can be used as potential biomarkers. Dayer and colleagues (6) reported that chronic pain may induce the abnormal and specific dysregulation of miRNA expression and that hsa-miR-320a and hsa-miR-98-5p (two circulating miRNAs signatures) can serve as biomarkers for pain-type classification. The epigenetic intervention of miRNAs may also be a new therapeutic approach for complications such as injurious hypersensitivity caused by peripheral nerve injury. Pan and colleagues (7) reported that miR-23a may be involved in NP through the TXNIP/NLRP3 inflammasome axis in spinal glial cells by directly targeting CXCR4.

With the rapid development of high-throughput sequencing, data mining, and the wide application of precision medicine, it becomes more feasible to extract lncRNA and mRNA data of NP from the microarray datasets. Although previous investigations have reported the role of some lncRNAs in the regulation of NP pathological pathways, the pathogenesis of NP has yet to be fully understood. In this study, three datasets (GSE107180, GSE145199, and GSE24982) were downloaded to identify differentially expressed mRNAs, miRNAs, and lncRNAs. Then, we performed GO and KEGG pathway analysis of differentially expressed mRNAs and constructed a PPI network to investigate gene interactions. Moreover, the lncRNA-miRNA-mRNA network was constructed to explore the interaction of lncRNAs, miRNAs, and mRNAs in NP. Finally, drugs were predicted based on the differentially expressed mRNAs in the regulatory network of NP. Our study will contribute to the understanding of NP pathogenesis and provide new strategies for targeted drugs and therapies.

Methods

Raw data collection

Raw data were obtained from Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) with the following search strategy: "Neuropathic Pain" AND ("lncRNA" OR "miRNA" OR "mRNA"). From the search results, we chose several lncRNA, miRNA, and mRNA expression profiles in *Mus musculus* with NP. Eligible datasets met the following inclusion criteria: 1) The dataset contains NP samples and control samples; 2) Each sample has a group label; 3) The microarray data type has been specified; 4) Gene symbol or GeneBank ID in the dataset file for each probe has been provided; 5) The original data is available. Finally, the included data accessions were GSE107180, GSE145199, and GSE24982.

Raw data preprocessing and screening for differentially expressed lncRNAs, miRNAs, and mRNAs.

The average expression of a gene was kept when its gene symbol mapped multiple probes, with genes containing the missing value (or zero value) being removed. Data preprocessing and differentially expressed analyses were performed with the R limma package(8). Data normalization was implemented by the quantile method with the R limma package and data scaling was performed by logarithmic

conversion in R. Differentially expressed lncRNAs, miRNAs, and mRNAs were identified with the significance thresholds of $|\log_2FC| > 0.5$ and $P\text{-value} < 0.05$. The R ggplot2 package(9) was used for data visualization. The ten most significant genes (sorted by P-values) of the up-regulated cluster and down-regulated cluster were extracted to make a heatmap that was done using the R pheatmap package (<https://cran.r-project.org/web/packages/pheatmap/index.html>). GO and KEGG pathway analysis was performed by using the R clusterProfiler package(10). The pathways were sorted according to gene count and the top ten pathways were used to draw the bubble plot. The pathways with $P\text{-value} < 0.05$ were considered significant.

Protein-protein interaction (PPI) network of differentially expressed mRNAs

Search Tool for the Retrieval of Interacting Genes (STRING, version 11.0, <https://string-db.org/>) is a database of known and predicted protein-protein interactions(11). Currently, the STRING database contains 24,584,628 proteins from 5,090 organisms, and interactions in the database are derived from genomic context predictions, high-throughput lab experiments, automated text mining, co-expression, and previous knowledge in databases. The identified top 50 differentially expressed mRNAs were input into the STRING database to construct the PPI network, in which individual non-connected proteins were eliminated.

Construction of lncRNA-miRNA-mRNA network

The targeted miRNAs regulated by the differentially expressed lncRNAs were predicted through the NPInter (<http://bigdata.ibp.ac.cn/npinter4/>) databases. Then lncRNA-miRNA interactions were constructed through the intersection of targeted miRNA and differentially expressed miRNAs, eliminating miRNAs positively correlated with lncRNAs. R multiMiR package(12) was used to predict target mRNAs regulated by differentially expressed miRNAs. Here, target mRNAs with negative correlation with miRNA were used for network construction. The miRNA-mRNA interactions were established based on the intersection of target mRNAs and differentially expressed mRNAs. The interaction of lncRNA-miRNA-mRNA was constructed based on the results of the two interactions above. Then, GO and KEGG pathway analysis was performed to investigate the biological function of mRNAs in lncRNA-miRNA-mRNA network. We extracted the subnets of the lncRNA-miRNA-mRNA network, and retained the mRNAs associated with miRNAs with the correlation greater than 0.8. The lncRNA-miRNA-mRNA sub-network was visualized by Cytoscape software(13) and the connectivity of nodes was used to evaluate the importance of nodes in the sub-network. Additionally, we performed GO and KEGG analysis to explore the biological function of mRNA in the lncRNA-miRNA-mRNA sub-network as described above.

Drug prediction of differentially expressed mRNAs

The Drug Gene Interaction Database (DGIdb, www.dgiddb.org) has been used to integrate, organize and display drugs, gene interactions, and gene-drug information from published articles and web resources (14). It is easily accessed through an intuitive Web user interface, an application programming interface (API), and publicly cloud-based server images. Here, we uploaded the differentially expressed mRNAs list

identified in the lncRNA-miRNA-mRNA networks onto the DGIdb to predict their potential targeting drugs effective for NP.

Results

Detection of differentially expressed lncRNAs, miRNAs and mRNAs

After data preprocessing, the raw data of each dataset was normalized. The boxplots in **Figure 1A-1C** show the difference of samples before and after data normalization. The volcano plots of lncRNA, miRNA and mRNA expression profiles in GSE107180, GSE145199, and GSE24982 were as depicted in **Figure 2A-2C**. A total of 2,848 differentially expressed lncRNAs (1,324 upregulated and 1,524 downregulated) were identified in GSE107180. A total of 959 differentially expressed miRNAs were identified in GSE145199, of which 455 miRNAs were upregulated and 504 were downregulated. Finally, 8,251 differentially expressed mRNAs (4193 upregulated and 4058 downregulated) were identified in GSE24982. The heatmaps in **Figure 2D-2F** displayed the top ten differentially expressed mRNAs sorted by P-values in the upregulated and downregulated clusters in GSE24982.

GO and KEGG pathway analysis of differentially expressed mRNAs

The R clusterProfiler package was used for GO and KEGG pathway analysis of 8,251 differentially expressed mRNAs (**Figure 3**). GO and KEGG pathway analysis has a wide range of applications in bioinformatics and can explain biological mechanisms and functional pathways in genomics and transcriptomics. We found that the differentially expressed mRNAs were enriched in blood circulation, regulation of membrane potential, regulation of ion transmembrane transport, regulation of metal ion transport in the category of biological process; metal ion transmembrane transporter activity, amide binding, gated channel activity, ion gated channel activity in the category of molecular function; synaptic membrane, postsynaptic specialization, neuron to neuron synapse and asymmetric synapse in the category of cellular component. Additionally, we found that differentially expressed mRNAs mainly participated in neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, MAPK signaling pathway, Rap1 signaling pathway, cAMP signaling pathway, and Calcium signaling pathway in the KEGG pathway analysis.

Construction of PPI network and lncRNA-miRNA-mRNA network

To explore the molecular mechanism of NP from a systematic perspective and discover novel drug targets, PPI analysis was performed. The PPI network obtained in the STRING database revealed the interactions between proteins encoded by the top 50 differentially expressed mRNAs. We eliminated the unconnected nodes in the network and finally got a network with 28 nodes and 45 edges (**Figure 4A**).

Additionally, Cytoscape was used to visualize the biomolecular interactions of lncRNA-miRNA-mRNA sub-network. When the correlation of miRNA and mRNA was set to be greater than 0.8, a total of 104 miRNAs were found, which jointly regulated 324 mRNA targets. The top five miRNAs were miR-940, miR-200C-3p,

miR-5192, miR-2277-3p, and miR-6882-3p, respectively. The top five target mRNAs were PRRC2B, IWS1, RAB15, RAB32 and GABPB1, respectively. A total of 140 lncRNA were found in the lncRNA-miRNA-mRNA sub-network which jointly regulated 104 miRNAs. The top five lncRNAs were CDCA3, SCAMP1, RPL18, NDUFA4, and CCDC132, respectively. The interaction network in **Figure 4B** showed that lncRNAs and miRNAs were highly clustered, among which miR-940, miR-2277-3p, and miR-5192 had the most lncRNA binding sites.

GO and KEGG pathway analysis of mRNAs in the lncRNA-miRNA-mRNA network and sub-network

To explore the biological function of mRNAs in the lncRNA-miRNA-mRNA network, we performed the GO and KEGG pathway analysis (**Figure 5**). We found that the mRNAs were enriched in the mRNA processing, response to peptide, positive regulation of cell cycle, adherens junction, chromatin, nuclear chromosome part, cell-substrate junction, neuron spine, neuron projection cytoplasm, GTPase binding, Ras GTPase binding, ubiquitin-like protein ligase binding. Through the KEGG pathway analysis, we found that the mRNAs were enriched in the pathway of the MAPK signaling pathway, proteoglycans in cancer, cell cycle, neurotrophin signaling pathway, and platelet activation.

Moreover, the GO and KEGG analysis of the mRNAs in the lncRNA-miRNA-mRNA sub-network (**Figure 6**) suggested that the biological function and pathway of the mRNAs were in utero embryonic development, cell cycle G1/S phase transition, DNA damage checkpoint, DNA integrity checkpoint, mitotic DNA damage checkpoint, endoplasmic reticulum-Golgi intermediate compartment, and the MAPK signaling pathway.

Drug prediction of Differentially expressed mRNAs

The DGIdb database provides the association between genes and their matching known or potential drugs. All differentially expressed mRNAs in lncRNA-miRNA-mRNA networks were used for drug prediction via the DGIdb database, and the results were presented in **Supplementary Table S1**. A total of 1,200 drugs were predicted. Among these drugs, fostamatinib had the highest number (66) of target genes. Other predicted drugs included cisplatin (37 target genes), copper (28 target genes), paclitaxel (26 target genes), dasatinib (24 target genes), erlotinib (23 target genes), arteminol (22 target genes), sorafenib (22 target genes), fluorouracil (21 target genes), gemcitabine (21 target genes), alcohol (18 target genes), carboplatin (18 target genes), gefitinib (18 target genes), docetaxel (17 target genes), imatinib (17 target genes), methotrexate (15 target genes), dexamethasone (14 target genes), everolimus (14 target genes), methyl dopa (14 target genes), palbociclib (14 target genes), progesterone (14 target genes), and tamoxifen (14 target genes). More predicted drugs can be obtained in **Supplementary Table S1**.

Discussion

Neuropathic pain is a debilitating condition caused by damage to the nervous system and chronic disease. As a kind of non-coding RNA, lncRNA-related research has become a hot spot in genetic research and is involved in many life activities such as epigenetic regulation, cell cycle regulation, and cell

differentiation. Several lncRNAs have been reported to be involved in various diseases, including Alzheimer's disease (AD) (15), Parkinson's disease (PD) (16), autoimmune thyroid disease (AITD) (17), and cancers (18, 19). An miRNA is a short non-coding single-stranded RNA molecule; it participates in the regulation of post-transcriptional gene expression in animals and plants (20, 21). At present, many miRNAs have been reported in neurodegenerative diseases (22), cancer (23, 24), bowel disease (25), and diabetic heart disease (DHD) (26). Although some lncRNAs (27, 28) and miRNAs (29, 30) have been reported in NP, there are still few reports on how lncRNAs, miRNAs and mRNAs jointly regulate the pathogenesis of NP. Zhou and colleagues (5) analyzed the expression profile of non-coding RNAs in the spinal cord following spared nerve injury-induced NP by sequencing, and constructed lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA networks in NP. However, they only analyzed 12 samples, which may have some limitations. In order to solve the bias that may be caused by the small sample size, different datasets were collected in this paper. Our research used three datasets from the GEO database, including 76 samples, which can explain the pathological mechanism of NP more comprehensively.

At present, the pathological mechanism of NP is still unknown. The differential expressed mRNAs can cause a variety of diseases. We found several differentially expressed mRNAs in GSE24982. The GO and KEGG enrichment analysis of these differentially expressed mRNAs can help us reveal their functional role. Here, the most significant biological processes were blood circulation, regulation of membrane potential, metal ion transmembrane transporter activity, synaptic membrane, and postsynaptic specialization. The enrichment of differentially expressed mRNAs in blood circulations was supported by a previous study, indicating that partial sciatic nerve ligation (PSL)-induced neuropathic pain can upregulate the synthesis of hypothalamic Oxytocin (OXT) and transport it to the OXT axon terminal through the systemic blood circulation (31). The detection and processing of pain by afferent sensory neurons depends on different types of voltage and ligand-gated ion channels, including sodium, calcium, and TRP pathways. This study found that differentially expressed mRNAs clustered in regulation of membrane potential and metal ion transmembrane transport pathway, suggesting that the expression and long-term changes of ion channels may be closely related to chronic pain states (32). Additionally, we found that the differentially expressed mRNAs were related to the synaptic membrane and postsynaptic specialization, indicating that the formation and regulation of synapses may be associated with neuralgia, which is consistent with reports in a previous study (33). We found that the main pathways for these differentially expressed mRNAs in NP were neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, MAPK signaling pathway, cAMP signaling pathway, and Calcium signaling pathway. A previous study reported that jct-801 relieves paclitaxel-induced neuropathic pain through the PI3K/Akt pathway (34). Our study suggested that differentially expressed mRNAs are involved in NP possibly through regulating the MAPK signaling pathway, which is consistent with a previous study (35). The Cyclic Adenosine Monophosphate (cAMP) signaling pathway is a key contributor to the development of chronic pain, and an existing study indicated that knockdown of the cAMP effector can relieve the pain-like responses in chronic pain models (36). Therefore, our study provided a reference for the molecular explanation of the NP pathology mechanism.

LncRNA, miRNA, and mRNA often coordinate the occurrence and prognosis of diseases in organisms. Up to date, only one study analyzed the lncRNA-mRNA-miRNA network in NP (5). The authors (5) found that the PI3K-Akt signaling pathway may be related to NP, which is consistent with our study. Though their study gave insights into the regulatory mechanisms of NP, their study may have some limitations due to the limited sample size. The accumulation of transcriptome data in public databases can help overcome these limitations and allow the discovery of more insightful information. Herein, we constructed the lncRNA-mRNA-miRNA network in NP. The lncRNAs can be used as candidate indicators for NP (30). Various studies have provided new mechanisms for the molecular roles of miRNAs in the pathogenesis of NP (37-39). Several vital lncRNAs were found in the present NP lncRNA-miRNA-mRNA network, and these lncRNAs have not been reported in NP. For example, lncRNA SCAMP1 played an important role in the regulatory network of this study, and it had been reported in a previous study that it may be associated with malignant biological behavior (40). In addition, lncRNA SCAMP1 discovered in the present study were related to the regulation of cancer (41). The identified differentially expressed miRNAs have not been reported in previous NP-related studies. For the first time, we reported that miR-940 and miR-2277-3p may play an important role in NP pathology based on the regulatory network of NP, while these miRNAs were previously reported mainly in cancer research (42-44). Additionally, miR-5689 was identified in the regulatory network of NP and it was reported as a biomarker for predicting the development of new distant metastasis (45). We have found some differentially expressed mRNAs that may be involved in the NP regulatory network. However, few studies have reported the functions of these genes in NP. Here, we believe that IQGAP1 may play an important role in NP pathogenesis, which may explain the significant correlation between chronic pain at 3 months after surgery (46). In addition, in previous studies, BTAF1 has been reported to be related to immunity (47) whereas LRPAP1 and MXRA7 are considered to be related to the tumorigenesis (48, 49). Compared with previous works, we identified a larger number of lncRNAs, miRNAs, and mRNAs, which has enriched our knowledge in this field and provided a new insight into the pathogenetic mechanism and the identification of targeted drugs. In short, we have discovered for the first time some lncRNAs, miRNAs and mRNAs that may be involved in NP, but their specific function in NP needs more validation studies.

To further explore the biological function of the lncRNA-miRNA-mRNA network, we performed the GO and KEGG pathway analysis. We found that the mRNAs in the network were involved in the mRNA processing, response to peptide, positive regulation of cell cycle, GTPase, chromatin, cell-substrate junction, neuron spine, and neuron projection cytoplasm. Through the KEGG pathway analysis, we found that the mRNAs in the network were enriched in the pathway of the MAPK signaling pathway, proteoglycans in cancer, cell cycle, neurotrophin signaling pathway, and platelet activation. Our research suggested that the lncRNA-miRNA-mRNA network may participate in the regulation of NP through GTPase. Several studies have reported that GTPase plays a key regulatory role in NP (50, 51). For example, a study reported that spinal Rheb-mTOR signaling plays an important role in the regulation of NP after spinal cord sensitization (52). The lncRNA-miRNA-mRNA network may regulate the occurrence of NP by the cell cycle. Studies have reported that the cell cycle is activated in spinal cord injury (SCI), which may regulate chronic pain after SCI (53, 54). We found that the mRNAs in the network were enriched in cell cycle and chromatin,

suggesting that the regulatory network may be involved in the pathway of NP through regulating the cell cycle. In addition, we found that platelet activation was enriched in the regulatory network, which was consistent with a previous study that blockade of the platelet-activating factor-pain loop can relieve neuropathic pain (55). The GO and KEGG analysis of lncRNA-miRNA-mRNA sub-network suggested that the mRNAs in the sub-network may participate in the pathway of NP by cell cycle G1/S phase transition, DNA damage checkpoint, and the MAPK signaling pathway. The cell cycle G1/S phase transition, DNA damage checkpoint were vital for cell cycle, indicating that the lncRNA-miRNA-mRNA sub-network may regulate the NP pathway through cell cycle.

Neuropathic pain is a chronic disease, and there is still no effective treatment drug. DGldb database can infer the targeted drugs of genes in diseases based on existing resources. The drugs included in this database contain the US Food and Drug Administration (FDA) certified drugs, which will provide more professional and reliable assurance for target drug prediction. For example, Nambou and Anakpa (56) recently discovered nicotinamide adenine dinucleotide (NAD) and ChEMBL1161866 with potential therapeutic value for corona virus disease 2019 (COVID-19) through the DGldb database, which provided novel insight for the treatment of COVID-19. In addition, Yang and colleagues predicted candidate drugs for lung adenocarcinoma (LUAD) via the DGldb database as well (57). Here, we predicted a series of drugs related to NP treatment based on mRNAs in the regulatory networks of NP. It is reported that inhibition of spleen tyrosine kinase (Syk) can alleviate mechanical allodynia (58). Fostamatinib is an oral Syk inhibitor that has been approved by the FDA for the adult treatment of Chronic immune thrombocytopenia (ITP)(59). Although there are differences between the pathological processes of mechanical pain and neuropathic pain, the number of genes targeted by this drug is high (66 target genes), so we recommend that doctors try fostamatinib in patients with NP to see its potential therapeutic effects. Dexamethasone is a synthetic glucocorticoid with anti-inflammatory activity and minimal glucocorticoid effect, which is widely used in the treatment of various inflammation disorders (60). It has been reported that low doses of ibuprofen and dexamethasone have a synergistic therapeutic effect on trigeminal neuropathic pain in rats, and can significantly inhibit mechanical allodynia (61). Dexamethasone is effective in the treatment of NP caused by tumor-related spinal cord compression, and pregabalin is effective for malignant painful radiculopathy (62). Nevertheless, the long-term use of dexamethasone also has certain side effects (63). In the past, opioids such as morphine were often ineffective in the treatment of neuropathic pain, but a study showed that the combination of imatinib and morphine can completely relieve pain (64), which provides more treatment options for patients. A recent study reported that in rats of infraorbital nerve ligation trigeminal neuralgia, c-Abl expression was significantly increased, and the downstream activation product p38 was also abnormally activated. Interestingly, imatinib mesylate (STI571), a specific c-Abl family kinase inhibitor, can reduce the expression of p38 and reduce the loss of dopaminergic neurons, suggesting that imatinib may alleviate the symptoms of NP through the c-abl-p38 signaling pathway (65). Additionally, our study predicted the treatment ability of progesterone (PROG) in NP and was supported by a previous study, demonstrating that PROG may provide new strategies for the treatment of NP(66). NP caused by chemotherapy can reduce the prognosis of patients with the quality of life. Tamoxifen can alleviate NP induced by paclitaxel,

vincristine, and bortezomib in chemotherapy through inhibiting protein kinase C/extracellular signal-regulated kinase pathway(67). Although we have only discussed the therapeutic effects of the drugs above, we cannot ignore the potential effects of other drugs that have not been mentioned. These predicted drugs can be verified in future studies, either individually or in combination. The number of genes corresponding to the targeting drugs was small, indicating that the therapeutic effect of these drugs may still be relatively weak. Therefore, we suggest doctors consider both the efficacy and side effects of drugs during the treatment.

Although this study found several lncRNAs, miRNAs, and mRNAs that may be related to NP through the public datasets, and analyzed the pathways that may participate in the pathological mechanism of NP through GO and KEGG pathway analysis, our research still has some limitations. Circular RNAs (circRNAs) are non-coding RNA molecules that have recently been shown to regulate the function of miRNAs and affect a variety of complex human diseases(68-70). Although there have been some studies on the circRNA regulation in NP (71-73), the present public databases still do not include the circRNA expression data of NP. Therefore, we used microarray sequencing data, including the sequencing results of lncRNA, miRNA, and mRNA in NP patients, which may not fully reflect the pathological mechanism of NP. This study found some lncRNAs, miRNAs and mRNAs that may be related to NP, but their relationship with NP is still unclear. We predicted several drugs that may have therapeutic effects on NP, but the effects of these drugs in clinical treatment have not yet been reported. In the future, we will collect samples from human NP patients, and obtain lncRNA, miRNA, mRNA, and circRNA data of NP patients through high-throughput sequencing technology. We plan to verify the functions of the genes and drugs identified here through more *in vitro* and *in vivo* experiments and strive to further reveal the pathological mechanism of NP.

Conclusions

Our study predicted drugs to treat NP based on the NP regulatory lncRNA-miRNA-mRNA network. Some of the predicted drugs have been reported to alleviate NP in previous studies, but the role of other drugs in NP therapy remains unknown. Future research will require more studies to validate these drugs. *In vitro* experiments on NP in cells, animals, and clinical trials are necessary to validate these drugs.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. Data is available at NCBI GEO: GSE107180, GSE145199 and GSE24982.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HZ analyzed the data, prepared figures and wrote the manuscript. LL and ZS analyzed the data and performed the experiments. DZ conceived and designed the study. All authors have read and approved the final version of the manuscript.

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Abbreviations

NP, Neuropathic pain; lncRNA, Long non-protein-coding RNA; XIST, X inactive-specific transcript; miRNA, MicroRNA; PPI, protein-protein interaction; DAVID, Database for Annotation, Visualization and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene ontology

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Figures

Figure 1

Box plot of each sample distribution in GEO data before and after normalization. The blue boxplots represent the distribution of the original data, while the red boxplots represent the distribution of the normalized data. (A) GSE107180, (B) GSE145199 and (C) GSE24982.

Figure 2

Differential analysis of lncRNAs, miRNAs, and mRNAs, respectively. Volcano plots of lncRNAs (A), miRNAs (B) and mRNAs (C). Differentially expressed genes were screened by the criteria of $|\log_2FC| > 1$ and $P\text{-value} < 0.05$, the green spots represented the downregulated molecules, while the red spots represented the upregulated molecules. The top ten lncRNAs (D), miRNAs (E) and mRNAs (F) with the

smallest P-values in the upregulated and downregulated clusters were selected for the drawing of a heatmap.

Figure 3

Bubble plot of GO and KEGG pathway analysis of the differentially expressed mRNAs. The bubble size represented the count of differential expressed mRNAs, and the depth of color of the bubble represented the significance of the differentially expressed mRNAs based on the P-values.

Figure 4

Molecular regulatory interaction network. (A) Protein-protein interaction network. Different colors represent different clusters, and the clustering result was obtained by the k-mean clustering method. (B) lncRNA-miRNA-mRNA sub-network. The blue nodes represent lncRNAs, the yellow represent miRNAs, and the red nodes represent mRNA; the red lines represent the positive regulatory relationships, the blue lines

represent the negative regulatory relationships, the node size represented the degree of association of a given molecule with other molecules.

Figure 5

Bubble plot of GO and KEGG pathway analysis of the mRNAs in lncRNA-miRNA-mRNA network. The bubble size represented the count of mRNAs, and the depth of color of the bubble represented the significance of the mRNAs based on the P-values.

Figure 6

Bubble plot of GO and KEGG pathway analysis of the mRNAs in the lncRNA-miRNA-mRNA sub-network. The bubble size represents the count of mRNAs, and the depth of color of the bubble represented the significance of the mRNAs based on the P-values.

Supplementary Files

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- [SupplementaryTableS1.xlsx](#)