Identification of IncRNA expression profile in the spinal cord of mice following spinal nerve ligation-induced neuropathic pain

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Abstract

**Background**: Neuropathic pain that caused by lesion or dysfunction of the nervous system is associated with gene expression changes in the sensory pathway. Long noncoding RNAs (lncRNAs) have been reported to be able to regulate gene expression. Identifying lncRNA expression patterns in the spinal cord under normal and neuropathic pain conditions is essential for understanding the genetic mechanisms behind the pathogenesis of neuropathic pain.

**Results**: Spinal nerve ligation (SNL) induced rapid and persistent pain hypersensitivity, characterized by mechanical allodynia and heat hyperalgesia. Meanwhile, astrocytes and microglia were dramatically activated in the ipsilateral spinal cord dorsal horn at 10 days after SNL. Further lncRNA microarray and mRNA microarray analysis showed that the expression profiles of lncRNA and mRNA between SNL and sham-operated mice were greatly changed at 10 days. The 511 differentially expressed (>2 fold) lncRNAs (366 up-regulated, 145 down-regulated) and 493 mRNAs (363 up-regulated, 122 down-regulated) were finally identified. The expression patterns of several lncRNAs and mRNAs were further confirmed by qPCR. Functional analysis of differentially expressed (DE) mRNAs showed that the most significant enriched biological processes of up-regulated genes in SNL include immune response, defense response, and inflammation response, which are important pathogenic mechanisms underlying neuropathic pain. 35 DE lncRNAs have neighboring or overlapping DE mRNAs in genome, which is related to Toll-like receptor signaling, cytokine–cytokine receptor interaction, and peroxisome proliferator-activated receptor signaling pathway.

**Conclusion**: Our findings uncovered the expression pattern of lncRNAs and mRNAs in the mice spinal cord under neuropathic pain condition. These lncRNAs and mRNAs may represent new therapeutic targets for the treatment of neuropathic pain.

**Keywords**: LncRNA, Spinal cord, Spinal nerve ligation, Neuropathic pain

**Background**

Neuropathic pain is one of the most common chronic pain in humans and characterized by an increase in the responsiveness of nociceptive neurons in the peripheral and central nervous system (CNS) [1]. Peripheral and central sensitization represents the altered functional status of nociceptive neurons and results from changes of a vast amount of functional protein and signaling pathways in the neuron and glial cell [2, 3]. Recent pharmaceutical research and discovery activities focus on well-characterized molecular targets, such as ion channels, G-protein-coupled receptors, and kinases in neurons and glial cells localized along the nociceptive pathways, which are regarded as direct contributors to the sensitization of pain signaling systems [4, 5]. However, the transcriptional
or translational regulatory mechanisms underlying the expression and functional changes of these molecules are poorly defined.

RNAs that do not code for a protein (noncoding RNAs, ncRNAs) consist of two major classes: the small ncRNAs, which include microRNAs (miRNAs) and other noncoding transcripts of less than 200 nucleotides, and long noncoding RNAs (lncRNAs), which are a novel class of non-protein coding transcripts longer than 200 nucleotides [6]. LncRNAs were initially considered as transcriptional by-products, but recent data suggest that lncRNAs can regulate gene expression via interfering with transcription, post-transcriptional processing, chromatin remodeling, miRNA sequestration, and generating small ncRNAs [7, 8]. Also, lncRNAs are involved in various aspects of cell biology and disease etiology, such as development [9], immune [10], cardiovascular disease [11], oncogenesis [12], and neurological disease [13]. LncRNAs are highly expressed in the CNS, and their expression profiles are associated with specific neuroanatomical regions, cell types, or subcellular compartments suggesting their potential functional roles in the nervous system [14–16]. It was reported that sciatic nerve resection induced differential expression of lncRNAs in dorsal root ganglia (DRG) [17]. Moreover, Zhao et al. have recently identified a functional lncRNA Kcna2, which contributed to neuropathic pain by silencing Kcna2 in DRG neurons [18]. These findings indicate the involvement of lncRNAs in neuropathic pain.

The spinal cord is responsible for receiving input from nociceptors and projecting to the brain, and plays an important role in the integration and modulation of pain-related signals. To clarify the molecular mechanisms underlying neuropathic pain and explore novel approaches for analgesic strategies, herein, we investigated the genome-wide expression of lncRNAs in the spinal cord after SNL. We then detected the expression profiles of lncRNAs and mRNAs in the L5 spinal cord at 10 days after SNL by microarray. First, we obtained a graphically overview of the expression signatures of lncRNAs and mRNAs by using scatter plot and hierarchical clustering analyses. The scatter plots showed that a large number of lncRNAs and mRNAs were differentially expressed between SNL and sham-operated mice (Figure 2a, b). Hierarchical cluster analysis of all lncRNAs or mRNA showed that the 3 sham or 3 SNL samples were clustered together respectively, and signal intensity was consistent in sham or SNL group (Figure 2c, d). The heatmap of DE lncRNAs or mRNAs whose expression were up-regulated or down-regulated by twofold were magnified (Figure 2e, f), indicating the high level of concordance in either SNL or sham samples. These data suggest that neuropathic pain is associated with the changes of lncRNAs and mRNAs in the spinal cord.

Overview of lncRNAs and mRNA expression profiles after SNL

We then detected the expression profiles of lncRNAs and mRNAs in the L5 spinal cord at 10 days after SNL by microarray. First, we obtained a graphically overview of the expression signatures of lncRNAs and mRNAs by using scatter plot and hierarchical clustering analyses. The scatter plots showed that a large number of lncRNAs and mRNAs were differentially expressed between SNL and sham-operated mice (Figure 2a, b). Hierarchical cluster analysis of all lncRNAs or mRNA showed that the 3 sham or 3 SNL samples were clustered together respectively, and signal intensity was consistent in sham or SNL group (Figure 2c, d). The heatmap of DE lncRNAs or mRNAs whose expression were up-regulated or down-regulated by twofold were magnified (Figure 2e, f), indicating the high level of concordance in either SNL or sham samples. These data suggest that neuropathic pain is associated with the changes of lncRNAs and mRNAs in the spinal cord.

Differentially expressed lncRNAs and mRNAs

We further analyzed differentially expressed (DE) lncRNAs using significance analysis of microarrays method, following the criteria q-value <0.05, and fold change >2. The results showed that 511 lncRNAs, containing 366 up-regulated and 145 down-regulated, were significantly changed in SNL group, comparing with the sham group. The most up-regulated lncRNAs were: uc009egw.1, Speer7-ps1, MM9LINCRAEXON12113+, ENSMUST00000118074, and uc009nzx.1, of which uc009egw.1 showed the largest up-regulation (Log2 fold change = 7.332.4243). The most down-regulated lncRNAs were: AK045739, AK020832, AK047380, ENSMUST00000171761 and uc008dwx.1, of which AK045739 showed the largest down-regulation (Log2 fold change = −45.320816). Detailed information including the top 20 up-regulated and 20 down-regulated lncRNAs was listed in Table 1.

In the DE mRNAs, there are 493 genes whose mRNA change was more than twofold, and the number of up-regulated (363) mRNAs was larger than down-regulated (122) mRNAs in SNL. These DE mRNAs contain many known genes involving in pain processing, including Cacna1g (calcium channel, voltage-dependent, T type, alpha
1G subunit, 16.0978 fold increase) [22], 

Trpv1 (transient receptor potential cation channel, subfamily V, member 1, 9.31-fold increase) [23], Ccl5 (chemokine (C-C motif) ligand 5, 3.93-fold increase) [24], Cx3cr1 (chemokine (C-X3-C) receptor 1, 2.51-fold increase) [25], and Irf5 (interferon regulatory factor 5) [26]. Besides, a lot of other genes, whose roles in pain have not been identified, were dramatically changed. Further analysis showed that 39 genes whose expression were changed >tenfold, including 38 up-regulated genes and 1 down-regulated gene, such as Sprr1a (small proline-rich protein 1A, 148.7-fold), Anxa10 (annexin A10, 76.3-fold), and Kng1 (kininogen 1, 38.4-fold); 66 genes whose expression was changed between 5- and 10-fold, including 64 up-regulated and 2 down-regulated genes. Detailed information about the top 20 up-regulated and 20 down-regulated mRNAs was listed in Table 2.

**Real-time quantitative PCR (qPCR) validation of lncRNA and mRNA expression**

To validate the reliability of the microarray results and also analyze the temporal changes of lncRNA and mRNA
**Figure 2** SNL results in the expression profiling changes of lncRNA and mRNA. Scatter plot comparing global lncRNA (a) or mRNA (b) gene expression profiles in the spinal cord between the SNL and sham mice. *Green lines* indicate twofold differences in either direction in lncRNA and mRNA expression. Heat map showing hierarchical clustering of overall lncRNAs (c) or mRNA (d) expression pattern of reliably measured probe sets. Heat map showing hierarchical clustering of LncRNAs (e) or mRNA (f), whose expression changes were more than twofold. In clustering analysis, up- and down-regulated genes are colored in red and green, respectively.
expression after SNL, the up-regulated lncRNAs including *Speer7-ps1* and *uc007pbc.1*, the down-regulated lncRNAs, including *ENSMUST00000171761* and *ENSMUST00000097503*, the up-regulated mRNA *Cyp2d9*, and the down-regulated mRNA *Mnx1* were randomly selected and analyzed by qPCR. The spinal cord tissues were collected from naive animals, and SNL animals at 1, 3, 10, and 21 days. *Speer7-ps1* and *uc007pbc.1*, which are intergenic lncRNAs, were both significantly increased at 10 days and peaked at 21 days (Figure 3a, b). ENSMUST00000171761 and ENSMUST00000097503 are antisense overlap and bidirectional lncRNA with matching gene *Tagap* (T-cell activation Rho GTPase-activating protein) and Zfp236 (zinc finger protein 236). They were significantly decreased at 10 days and persisted to 21 days (Figure 3c, d). *Cyp2d9*, a member of cytochrome P450, family 2, subfamily d, was increased more than 12-fold at 10 days (Figure 3e). *Mnx1* is a sequence-specific DNA binding transcription factor. It decreased from 1 to 21 days (Figure 3f). In addition, the fold changes of these lncRNAs and mRNAs detected by qPCR at SNL 10 days were consistent with the results from microarray (Figure 3g), further supporting the reliability of the array data.

**Class distribution of changed lncRNAs**

lncRNAs were shown to regulate the expression of adjacent or overlapping mRNAs in genome [18, 27, 28]. Thus, the associations of DE lncRNAs with coding genes were analyzed and classified according to the method described by Li et al. [29]. lncRNAs are classified into four groups: intergenic lncRNAs (lncRNAs are located and transcribed from intergenic regions, and do not overlap with known protein coding genes or other types of genes in genome. It is also called lincRNAs), antisense lncRNAs (LncRNA exon is transcribed from the antisense strand and overlaps with a coding transcript exon), sense lncRNAs (LncRNA exon overlaps with a coding transcript exon on the same genomic strand), and bidirectional lncRNAs (LncRNA is oriented head to head with a coding transcript within 1,000 bp). As shown in Figure 4, among the DE lncRNAs, intergenic lncRNAs were the largest category, with 236 up-regulated and 90 down-regulated lncRNAs. The other DE lncRNAs included 100 antisense lncRNAs (78 up-regulated and 22 down-regulated), 59 sense lncRNAs (37 up-regulated and 22 down-regulated), and 26 bidirectional lncRNAs (15 up-regulated and 11 down-regulated).

**Functional prediction of DE mRNAs in SNL**

To explore the molecular mechanism in neuropathic pain, we further did GO and pathway analysis of deregulated genes in SNL versus sham. The GO results showed that the most significant enriched molecular function of up-regulated genes in SNL was chemokine activity, CCR
chemokine receptor binding, chemokine receptor binding, and cysteine-type endopeptidase inhibitor activity (Figure 5a). The most significant enriched biological processes of up-regulated genes in SNL were immune response, immune system process, defense response, and regulation of immune system process (Figure 5b).

The most noteworthy enriched cellular components of up-regulated genes in SNL were extracellular region, extracellular space, extracellular region part, and external side of plasma membrane (Figure 5c). The most significant enriched molecular function of down-regulated genes in SNL were binding, receptor binding, calcium

Table 2 The detail information of the top 20 up-regulated and 20 down-regulated mRNAs

| Gene symbol | Description | Log$_2$ fold change (SNL/sham) | P-value |
|-------------|-------------|-------------------------------|---------|
| Up-regulated genes | | | |
| Sprr1a | Small proline-rich protein 1A | 148.7115 | 1.84E−10 |
| Anxa10 | Annexin A10 | 76.262054 | 1.61E−06 |
| 4933402N22Rik | RIKEN cDNA 4933402N22 gene | 46.512726 | 1.62E−10 |
| Vmn2r101 | Vomeronasal 2, receptor 101 | 44.090027 | 1.2E−08 |
| Kng1 | Kininogen 1 | 34.82939 | 2.14E−08 |
| Olfr803 | Olfactory receptor 803 | 31.403961 | 7.82E−08 |
| Gpr151 | G protein-coupled receptor 151 | 27.673513 | 5.95E−11 |
| LOC100048884 | Novel member of the major urinary protein (Mup) gene family | 24.719683 | 9.12E−09 |
| Mup11 | Major urinary protein 11 | 24.027332 | 8.26E−10 |
| Mup7 | Major urinary protein 7 | 23.902332 | 2.18E−08 |
| Mup12 | Major urinary protein 12 | 23.78707 | 2.79E−10 |
| Mup13 | Major urinary protein 13 | 23.254275 | 9.99E−08 |
| Mup19 | Major urinary proteins 11 and 8 | 23.019644 | 0.000000314 |
| Mup8 | Major urinary protein 8 | 22.686306 | 0.000000241 |
| Mup17 | Major urinary protein 17 | 21.82689 | 8.07E−10 |
| Atf3 | Activating transcription factor 3 | 19.8067 | 0.00000165 |
| Rreb1 | Ras responsive element binding protein 1 | 19.512457 | 0.0000258 |
| Olfr648 | Olfactory receptor 648 | 19.249556 | 0.00000434 |
| Clps | Colipase, pancreatic | 18.952599 | 0.000000801 |
| Vax2 | Ventral anterior homeobox containing gene 2 | 17.30259 | 0.00000187 |
| Down-regulated genes | | | |
| Lefty1 | Left right determination factor 1 | −10.109003 | 0.000000123 |
| Olfr866 | Olfactory receptor 866 | −7.406356 | 0.011693356 |
| Kcn5 | Potassium voltage-gated channel, shaker-related subfamily, member 5 | −5.9395947 | 0.0000537 |
| Tntt2 | Troponin T2, cardiac | −4.8715253 | 0.000213 |
| Csprs | Component of Sp100-rs | −4.639864 | 0.000183 |
| Gms5458 | Predicted gene 5458 | −3.9395294 | 0.000162 |
| Ypel4 | Yippee-like 4 (Drosophila) | −3.8847303 | 0.0000976 |
| Sell | Selectin, lymphocyte | −3.7629561 | 0.000967 |
| Mnx1 | Motor neuron and pancreas homeobox 1 | −3.702038 | 0.0003540842 |
| Fnip1 | Foliculin interacting protein 1 | −3.4727607 | 0.000226 |
| Epm2a | Epilepsy, progressive myoclonic epilepsy, type 2 gene alpha | −3.363634 | 0.00031 |
| H2-Ea-ps | Histocompatibility 2, class II antigen E alpha, pseudogene | −3.2939498 | 0.000021 |
| Chodl | Chordoreticin | −3.2821681 | 0.00000249 |
| Wtap | Wilms' tumour 1-associating protein | −3.1569881 | 0.0000001 |
| Pia4 | Paired-lg-like receptor A4 | −3.1222947 | 0.03241746 |
| Emi4 | Echinoderm microtubule associated protein like 4 | −3.117333 | 0.02077666 |
| Tntt2 | Troponin T2, cardiac | −3.0204759 | 0.0001 |
| Retnlg | Resistin like gamma | −2.9266624 | 0.00000051 |
| Mmp8 | Matrix metallopeptidase 8 | −2.9234846 | 0.000255 |
ion binding, and tropomyosin binding (Figure 5d). The most significant enriched biological processes of down-regulated genes in SNL were regulation of ATPase activity, monovalent inorganic cation transport, glucosamine-containing compound catabolic process, and amino sugar catabolic process (Figure 5e). The most significant enriched cellular components of down-regulated genes in SNL were extracellular region, striated muscle thin filament, extracellular space, and cell part (Figure 5f).

Similarly, different genes were analyzed in KEGG. The results showed that the up-regulated genes in SNL are involved in complement and coagulation cascades, Toll-like receptor signaling pathway, chemokine signaling pathway, cytosolic DNA-sensing pathway, and cytokine–cytokine receptor interaction, Changas disease, and...
NOD-like receptor signaling pathway (Figure 6a). Downregulated genes in SNL are involved in amyotrophic lateral sclerosis (ALS), prostate cancer, citrate cycle, glutamatergic synapse, osteoclast differentiation and NOD-like receptor signaling pathway (Figure 6b).

Comparison of our DE mRNAs with previously published microarrays

Previous studies have shown differential gene expression profile in the spinal cord in rats with neuropathic pain [30, 31]. In order to compare neuropathic pain-associated gene expression patterns in mice and rats, we did the overlap analysis between other’s microarray data from rat [30] and our current data from mice (Figure 7a). LaCroix-Fralish et al. reported that 88 genes were upregulated and 83 genes were downregulated in the spinal cord 7 days after L5 nerve root ligation in rats [30]. Surprisingly, compared to 361 up-regulated genes and 119 down-regulated genes in mouse, only 1 gene (Cd74) was upregulated and 2 genes (Nefm, Aco2) were downregulated in both rats and mice (Figure 7b). In addition, we compared our array data with 79 significantly regulated genes which were identified by meta-analysis from 20 independent microarray experiments from rats and mice after tissue inflammation or nerve injury [2]. We observed an overlap of 15 genes with the meta-analysis dataset (Figure 7c). These genes included 14 up-regulated genes (Ctss, C1qb, C1qc, Npy, Cd74, Gal, Aif1, Calca, Cxcl10, Atp3, Ccl2, Ctsk, Fcgr2b and Sprr1a) and 1 down-regulated gene (Nefm) (Figure 7d).

Relational analysis of IncRNAs and mRNAs

As some IncRNAs have been suggested to play key roles in regulating the expression of their neighboring or overlapping genes in genome wide, we further screened out DE mRNAs related to DE IncRNAs based on their location distributions on mouse chromosomes by UCSC Genome Browser. In the spinal cord, there are 39 DE IncRNA-mRNA pairs for 35 DE IncRNAs and 35 DE mRNAs. Among them, 32 pairs exhibited coordinated expression changes, and 7 pairs were non-coordinated, which may suggest a complex and various regulatory mechanisms across different IncRNAs and their target mRNAs. Intriguingly, all the seven non-coordinated IncRNA-mRNA pairs belong to intergenic IncRNA-mRNA pairs (Table 3). Further GO and pathway analysis showed that the high enriched molecular functions include pheromone binding, chemokine activity, high-density lipoprotein binding, and phosphatidylcholine-sterol O-acyltransferase activator activity (Figure 8a). Based on gene-pathway network graph analysis, we found that the DE mRNAs from IncRNA-mRNA pairs, such as Cxcl9 (chemokine (C-X-C motif) ligand 9), Cxcl10 (chemokine (C-X-C motif) ligand 10), Cxcl11 (chemokine (C-X-C motif) ligand 11), Trhr (thyrotropin releasing hormone receptor), and Apoa2 (apolipoprotein A-II), might involve in toll-like receptor signaling pathway, calcium signaling pathway, and PPAR signaling pathway (Figure 8b; Table 3), which have been proven to be involved in neuropathic pain pathogenesis [32–34].

Discussion

Chronic neuropathic pain is a somatosensory disorder caused by nerve injury or disease that affects the nervous system [35]. Evidence suggested that the particular patterns of gene expression at different levels of the nociceptive system play important roles in the development and maintenance of neuropathic pain [2, 36]. Over the past decades, the molecular mechanisms underlying neuropathic pain have been extensively studied; however, the pathophysiological process of pain is still vague. LncRNAs were recently shown to regulate gene expression [37] and traffic cellular protein complexes, genes, and chromosomes to appropriate locations [8]. Their function in regulating gene expression switching in the maintenance phase of neuropathic pain is poorly understood. In this study, we for the first time identified the global expression changes in IncRNAs and analyzed their characteristics and possible relation with coding genes in the spinal cord under neuropathic pain condition. The 24,833 IncRNAs were detected in the spinal cord of mice. Among them, 366 IncRNAs were up-regulated and 145 IncRNAs were down-regulated at 10 days after SNL. These DE IncRNAs are consistently altered in a high

Figure 4 Distribution of various types of DE IncRNAs. Four classes (sense overlap IncRNAs, antisense overlap IncRNAs, bidirectional IncRNAs and intergenic IncRNAs) were analyzed.
percentage of analyzed spinal cords from SNL and sham mice, suggesting that lncRNAs may be involved in neuropathic pain processing. So far, most DE lncRNAs have not been functionally characterized. Although it was still too early to translate this knowledge into the development of novel analgesic agents for better pain relief, these findings may likely provide novel insight into the molecular basis of pain.

In this study, the expression profiles of mouse genome-wide mRNAs were also detected using IncRNA Microarray Chip at the same time. Among DE mRNAs, the up-regulated mRNAs are far more numerous than the down-regulated in SNL samples, which reflects the emergence of new biology processes and pathways in pathological conditions. A number of reported pain-related genes, including Cacna1g, Trpv1, Ccl5, Cx3cr1 and Irf5 were dramatically increased after SNL. Moreover, a lot of other mRNAs, such as Sprr1a, Anxa10, Kng1, and Gpr151 (G-protein-coupled receptor 151), whose functions are unclear in the spinal cord were also screened out. As the expression changes for some genes may be related to nerve damage and homeostatic responses to denervation, further studies are needed to identify whether they are involved in neuropathic pain processing.
Figure 6  Pathway analysis for 366 up-regulated and 127 down-regulated mRNAs with fold changes >2. a The significant pathways for up-regulated genes in SNL group. b The significant pathways for down-regulated genes in SNL group.

Figure 7  Gene overlap analysis between the present data and previously published microarrays in pain model. a Venn diagram showing the number of common up- and down-regulated genes in our present mice model (mice-up, mice-down) and previously published rat model (rat-up, rat-down) after SNL. Only three genes were shared with the same tendency between the two microarray experiments. b The detailed information of the overlap genes that were significantly regulated in both the mice and rat spinal cord. c Venn diagram showing the overlap between gene-sets of our present data and previously published microarrays (Up-P up-regulated genes of the previous studies, Down-P down-regulated genes of the previous studies, Up-A up-regulated genes of the author’s data, Down-A down-regulated genes of the author’s data). d The detailed information of 14 up-regulated and 1 down-regulated overlapped genes between our present data and previously published microarrays.
### Table 3  DE lncRNAs and their neighboring or overlapping DE mRNAs

| LncRNAs               | Fold change | Regulation | Relationship    | mRNAs | Fold change | Regulation | Function prediction of DE IncRNAs with related mRNAs | Pathway                        |
|-----------------------|-------------|------------|-----------------|-------|-------------|------------|----------------------------------------------------|--------------------------------|
| ENSMUST00000160110    | 3.9130898   | Down       | Antisense overlap | Phf1  | 2.1720073   | Down       | GO:0003677 DNA binding                              |                                 |
| AK136749              | 2.089502    | Up         | Antisense overlap | Asap2 | 8.8652115   | Up         | GO:0005215 transporter activity                     | GO:0005550 pheromone binding   |
| ENSMUST00000121460    | 11.624642   | Up         | Antisense overlap | Mup2  | 16.324926   | Up         |                                                     |                                |
| mouselincRNA1303+     | 2.959626    | Up         | Intergenic       | Vmn1S4| 2.5373068   | Up         | GO:0005319 lipid transporter activity               |                                |
| MM9LINCR-NAEXON12110+ | 9.611986    | Up         | Intergenic       | Apoa2 | 5.3063893   | Up         | GO:00008035 high-density lipoprotein binding        |                                |
|                       |             |            |                  |       |             |            | GO:0017127 cholesterol transporter activity        |                                |
|                       |             |            |                  |       |             |            | GO:0042603 protein homodimerization activity        |                                |
|                       |             |            |                  |       |             |            | GO:0046982 protein heterodimerization activity      |                                |
|                       |             |            |                  |       |             |            | GO:0055102 lipase inhibitor activity                |                                |
|                       |             |            |                  |       |             |            | GO:0066228 phosphatidylcholine-sterol O-acyltransferase activator activity |                                |
| MM9LINCR-NAEXON11813− | 2.2022471   | Up         | Intergenic       | Ngfr  | 2.3073637   | Up         | GO:0005303 neurotrophin receptor activity           |                                |
|                       |             |            |                  |       |             |            | GO:0048406 nerve growth factor binding              |                                |
|                       |             |            |                  |       |             |            | GO:0048406 nerve growth factor binding              |                                |
|                       |             |            |                  |       |             |            | Neurodegenerative disorders                        |                                |
|                       |             |            |                  |       |             |            | Cytokine–cytokine receptor interaction              |                                |
|                       |             |            |                  |       |             |            |                                                     |                                |
| C75950                | 2.3177905   | Up         | Intergenic       | Gm5136| 2.2296717   | Down       | GO:0005244 voltage-gated ion channel activity      |                                |
| mouselincRNA1231−     | 2.3548565   | Up         | Intergenic       | Hvcn1 | 2.0545347   | Up         | GO:0030171 voltage-gated proton channel activity   |                                |
|                       |             |            |                  |       |             |            |                                                     |                                |
| ENSMUST00000133243    | 2.2177694   | Up         | Intergenic       | Usp11 | 2.747246    | Up         | GO:0004221 ubiquitin thiolesterase activity         |                                |
|                       |             |            |                  |       |             |            |                                                     |                                |
| MM9LINCR-NAEXON11661+ | 20.514269   | Up         | Intergenic       | Asap2 | 8.8652115   | Up         | GO:0003700 transcription factor activity           |                                |
| humanlincRNA1070+     | 6.5686955   | Up         | Intergenic       | Vax2  | 17.30259    | Up         |                                                     |                                |
| humanlincRNA2255−     | 6.4199057   | Up         | Intergenic       | Trhr  | 2.1457152   | Down       | GO:0005515 protein binding                          |                                |
| mouselincRNA1631+     | 2.131738    | Up         | Intergenic       | Krh15 | 2.0060081   | Up         |                                                     |                                |
|                       |             |            |                  |       |             |            |                                                     |                                |
| LncRNAs                  | Fold change | Regulation | mRNAs         | Fold change | Regulation | Function prediction of DE lncRNAs with related mRNAs | Pathway                                      |
|-------------------------|-------------|------------|---------------|-------------|------------|-----------------------------------------------------|----------------------------------------------|
| humanlincRNA1443—       | 4.366208    | Up         | Intergenic    | 7.495438    | Up         | GO:0005021 vascular endothelial growth factor receptor activity |                                |
|                         |             |            |               |             |            | GO:0005515 protein binding                           |                                |
|                         |             |            |               |             |            | GO:0005524 ATP binding                               |                                |
| MM9LINCRAEXON12110+     | 9.611986    | Up         | Intergenic    | 2.2202826   | Down       | GO:0003677 DNA binding                               |                                |
|                         |             |            |               |             |            | GO:0005515 protein binding                           |                                |
| MM9LINCRAEXON10576—     | 5.209898    | Up         | Intergenic    | 5.6018896   | Up         | GO:0008009 chemokine receptor activity              |                                |
|                         |             |            |               |             |            | Cytokine–cytokine receptor interaction                |                                |
|                         |             |            |               |             |            | Toll-like receptor signaling pathway                 |                                |
| MM9LINCRAEXON11308+     | 3.7596319   | Up         | Intergenic    | 2.1100945   | Down       | GO:0003677 DNA binding                               |                                |
|                         |             |            |               |             |            | GO:0008270 zinc ion binding                           |                                |
| BM248967                | 6.0079184   | Up         | Intergenic    | 3.3316648   | Up         | GO:0004143 diacyl-glycerol kinase activity           |                                |
| MM9LINCRAEXON11616+     | 2.5639145   | Up         | Intergenic    | 2.0003252   | Up         | GO:0004553 hydrolase activity, hydrolyzing O-glycosyl compounds |                                |
|                         |             |            |               |             |            | GO:0004563 beta-N-acetylhexosaminidase activity       |                                |
|                         |             |            |               |             |            | GO:00042803 protein homodimerization activity         |                                |
|                         |             |            |               |             |            | GO:00043169 cation binding                           |                                |
|                         |             |            |               |             |            | GO:0046982 protein heterodimerization activity        |                                |
|                         |             |            |               |             |            | N-Glycan degradation                                |                                |
|                         |             |            |               |             |            | Aminosugars metabolism                              |                                |
|                         |             |            |               |             |            | Glycosaminoglycan degradation                        |                                |
|                         |             |            |               |             |            | Glycosphingolipid biosynthesis—globoseries           |                                |
|                         |             |            |               |             |            | Glycosphingolipid biosynthesis—ganglioseries         |                                |
|                         |             |            |               |             |            | Glycan structures—degradation                        |                                |
|                         |             |            |               |             |            |                                              |                                |
| uc008808.1               | 2.1543121   | Down       | Intergenic    | 2.5731633   | Up         | GO:0005200 structural constituent of cytoskeleton   |                                |
| MM9LINCRAEXON12066—     | 4.066157    | Down       | Intergenic    | 3.0204759   | Down       |                                              |                                |
| MM9LINCRAEXON10576—     | 5.209898    | Up         | Intergenic    | 2.7319772   | Up         | GO:0008009 chemokine activity                       |                                |
|                         |             |            |               |             |            | Cytokine–cytokine receptor interaction                |                                |
|                         |             |            |               |             |            | Toll-like receptor signaling pathway                 |                                |
| MM9LINCRAEXON10576—     | 5.209898    | Up         | Intergenic    | 6.9877048   | Up         | GO:0008009 chemokine activity                       |                                |
|                         |             |            |               |             |            | Cytokine–cytokine receptor interaction                |                                |
|                         |             |            |               |             |            | Toll-like receptor signaling pathway                 |                                |
| AK0354438               | 2.3012707   | Up         | Intergenic    | 9.431554    | Up         | GO:0005515 protein binding                           |                                |
Based on the GO term enrichment analyses of DE mRNA, we found that significantly enriched molecular functions and biological processes of up-regulated gene in SNL vs sham were mainly involved in chemokine activity, inflammation, and immunity. These findings are consistent with previous studies showing that neuroinflammation, manifested as infiltration of immune cells [38], activation of glial cells [39] and production of inflammatory mediators [40] in the peripheral and CNS, plays an important role in the induction and maintenance of chronic pain [41]. Additionally, our immunostaining of GFAP and IBA-1 showed dramatic glial activation in the spinal cord at 10 days after SNL. From significant pathway analyses of DE gene, the third most significant enriched pathway of the up-regulated genes in SNL vs sham is the toll-like receptor signaling pathway. Indeed, Tlr2 [42], Tlr4 [43], and Tlr7 [44] have been implicated as potential therapeutic targets in neuropathic and other pain models. The data collectively indicate that anti-neuroinflammation may be an effective strategy for the treatment of neuropathic pain.

Previous studies utilizing cDNA microarrays to analyze gene expression profiles primarily focus on pain models in rats, rarely in mice [2]. The overlap analysis showed little overlap between rat and mice spinal cord gene expression patterns under neuropathic pain states, suggesting the species difference in gene expression. However, we found that there were 15 overlap genes between our current data and meta-analysis results reported by LaCroix-Fralish et al. [2]. These overlap genes including Atf3, Sprr1al and Nefm can be induced by nerve damage, which contribute to chronic pain [45–47]. In addition, gene ontology-based functional annotation clustering analyses of the previous gene chip study revealed strong evidence for regulation of immune-related genes in pain states, which was consistent with our data.

Although lncRNAs play important roles in the regulation of gene expression [48], there is a large gap between the number of existing lncRNAs and their known association with a particular molecular or cellular function [49]. Regulatory mechanisms and major functional principles of lncRNAs are complex and quite obscure. Unlike

**Table 3 continued**

| LncRNAs             | Relationship | mRNAs          | Function prediction of DE lncRNAs with related mRNAs |
|---------------------|--------------|----------------|----------------------------------------------------|
| **Sequence name**   | **Fold change** | **Regulation** | **GeneSymbol** | **Fold change** | **Regulation** | **Molecular Function** | **Pathway** |
| MM9LINC-NAEXON10268 | +6.8239675 Up Intergenic | Irf8 | 2.335659 Up Go:0003700 transcription factor activity |
| MM9LINC-NAEXON11735 | +2.4868224 Down Intergenic | Ppp2r5c | 2.1656942 Down Go:0008601 protein phosphatase type 2A regulator activity |
| DV650983            | +2.0293975 Down Intergenic | Olf1416 | 2.2257524 Down Go:0004984 olfactory receptor activity |
| MM9LINC-NAEXON11795 | +2.5727692 Down Intergenic | Cd68 | 2.6843183 Up |
| MM9LINC-NAEXON11795 | +2.671865 Up Intergenic | Cd68 | 2.6843183 Up |
| ENSMUST00000120184  | +2.5531633 Down Sense overlap | Amy2b | 2.4439986 Down |
| uc007xpp.1           | +2.1796808 Down Sense overlap | Tbr | 2.1457152 Down |
| uc009pmr.1           | +3.02046 Down Sense overlap | Elmod1 | 2.2530112 Down |
| uc007ceu.1           | +6.378551 Down Sense overlap | Trnt2 | 3.0204759 Down |
| ENSMUST00000049306  | +4.188862 Down Sense overlap | H2-Ea-2s | 3.2939498 Down |
| uc008bzw.1           | +2.2820547 Up Sense overlap | Laptm5 | 2.1142242 Up |
| ENSMUST00000117412  | +2.5116289 Up Sense overlap | Gm10147 | 2.2881203 Up |
| ENSMUST00000119882  | +3.1487308 Up Sense overlap | Gm10486 | 2.4736855 Up |
| ENSMUST00000119882  | +3.1487308 Up Sense overlap | Gm14819 | 3.018787 Up |
| uc008tbr.1           | +10.098583 Up Sense overlap | Mup17 | 21.82689 Up |
microRNA, there are no common languages that can be used to predict lncRNAs' target genes and function by their sequence information or secondary structure. Accumulating evidence suggests that a number of lncRNAs function locally to activate or repress their neighboring or overlapping genes' expression [18, 27, 50]. In this study, we found that intergenic lncRNAs (lincRNAs) were the largest category in all DE lncRNAs after SNL. In reality, lncRNAs are found to be conserved across multiple vertebrate species [51] and perform important functions
in many cellular processes, from cell proliferation to cancer progression [52]. Furthermore, lncRNAs can function through different types of mechanisms, including cis or trans transcriptional regulation, translational control, splicing regulation, and other post-transcriptional regulation [33]. We examined whether their neighboring or overlapping protein-coding genes in the genome are simultaneously DE in the spinal cord after SNL, and found that there are 39 DE lncRNA-mRNA pairs. Our further analysis showed that an up-regulated lncRNA, MM9LINCRNAEXON10576—in the spinal cord after SNL was found to be located near Cxcl10, Cxcl9 and Cxcl11 gene cluster in mice chromosome 5. All the four RNAs have the same expression trends and increased more than twofold after SNL. Recently, studies using animal models have shown that upregulation of chemokines in the spinal cord play a vital role in the development and maintenance of chronic pain [41, 53, 54]. Indeed, recent research found that Cxcl10 and its receptor Cxcr3 were involved in inflammatory pain and cancer pain [55–57]. Therefore, lncRNA MM9LINCRNAEXON10576—may contribute to neuropathic pain through regulation of chemokines Cxcl10, Cxcl9 and Cxcl11.

In our microarray results, 12 DE mRNA have their corresponding DE sense-overlap lncRNAs, and the change patterns of these lncRNAs were same as that of their accompanying protein-coding genes. Di et al. found that a sense-overlap lncRNA arising from the CCAAT/enhancer-binding protein alpha (Cebpα) gene locus can bind to DNA methyltransferase 1 (DNMT1) and prevent Cebpα gene locus methylation, then to increase the expression of Cebpα gene. Their deep sequencing of transcripts associated with DNMT1 combined with genome-scale methylation and expression profiling extend the generality of this finding to numerous gene loci. [27]. Given that the 12 DE mRNA and their DE sense-overlap lncRNAs were both increased after SNL, it’s possible that the DE sense-overlap lncRNAs regulate the expression of their sense-overlapping mRNAs via demethylation after SNL.

Conclusion
Our results demonstrated that lncRNA transcripts were highly enriched and hundreds of lncRNAs were differentially expressed in the spinal cord after SNL. Dozens of DE lncRNAs were observed to have neighboring or overlapping DE mRNAs in genome. These lncRNAs may locally regulate their related protein-genes expression and play key roles in the pathogenesis of neuropathic pain. Further studies are required to clarify the molecular and cellular functions of DE lncRNAs and determine whether they can serve as novel analgesic targets in neuropathic pain.

Methods
Animals and surgery
Adult male ICR mice (male, 8 weeks) were maintained on a 12:12 light–dark cycle at a room temperature of 22 ± 1°C with free access to food and water. The experimental procedures were approved by the Animal Care and Use Committee of Nantong University and performed in accordance with the guidelines of the International Association for the Study of Pain. To produce a SNL, animals were anesthetized with isoflurane and the L6 transverse process was removed to expose the L4 and L5 spinal nerves. The L5 spinal nerve was then isolated and tightly ligated with 6-0 silk threads [58]. For sham operations, the L5 spinal nerve was exposed but not ligated.

Behavioral test
Animals were habituated to the testing environment daily for at least 2 days before baseline testing. The room temperature remained stable for all experiments. For testing mechanical sensitivity, animals were put in boxes on an elevated metal mesh floor and allowed 30 min for habituation before examination. The plantar surface of each hindpaw was stimulated with a series of von Frey hairs with logarithmically incrementing stiffness (0.02–2.56 g, Stoelting, Wood Dale, IL, USA), presented perpendicular to the plantar surface (2–3 s for each hair). The 50% paw withdrawal threshold was determined using Dixon’s up-down method [59]. For testing heat sensitivity, animals were put in plastic boxes and allowed 30 min for habituation. Heat sensitivity was tested by radiant heat using Hargreaves apparatus (IITC Life Science Inc., Woodland Hills, CA, USA) and expressed as paw withdrawal latency (PWL). The radiant heat intensity was adjusted so that basal PWL is between 10 and 14 s, with a cutoff of 18 s to prevent tissue damage.

Immunohistochemistry
At 10 days after SNL or sham-operation, animals were deeply anesthetized with isoflurane and perfused through the ascending aorta with PBS followed by 4% paraformaldehyde with 1.5% picric acid in 0.16 M PB. After the perfusion, the L4–L5 spinal cord segments were removed and postfixed in the same fixative overnight. Spinal cord sections (30 μm, free-floating) were cut in a cryostat. The sections were first blocked with 5% goat serum for 2 h at room temperature. The sections were then incubated overnight at 4°C with the following primary antibodies: GFAP antibody (mouse, 1:6,000; Millipore, Billerica, MA, USA), IBA-1 antibody (Mouse, 1:3,000, Serotec, Kidlington, UK). The sections were then incubated for 2 h at room temperature with FITC-conjugated secondary antibodies (1:1,000, Jackson ImmunoResearch). The stained sections
Tissue collection and RNA isolation
We prepared nine mice for SNL and nine mice for sham-operation. At 10 days after operation, the animals were deeply anesthetized with isoflurane and perfused through the ascending aorta with saline. After the perfusion, the L4–L5 spinal cord segments were collected. Total RNA was extracted from the spinal cord dorsal horn tissue using Trizol reagent (Invitrogen, Carlsbad) according to the manufacturer’s protocol. The RNA concentration and purity were assayed by the absorbance values at 260 and 280 nm using the NanoDrop 1000 Spectrophotometer (Thermo). RNA integrity was checked by electrophoresis on 2% (m/v) agarose gels. After these testing, equal mRNA from three mice under the same treatment was mixed as one sample. Therefore, six samples (3 for SNL and 3 for sham) were sent for microarray analysis.

Microarray assay
The gene chip of the mouse lncRNA microarray V2.0 (8 k 60K, Arraystar), which includes 25,376 lncRNA probes and 31,423 coding gene probes, was used in the experiments. The total RNAs of sham and SNL groups were individually hybridized with gene chips. Briefly, RNA was purified from 1 μg total RNA after removing rRNA. The RNA sample was then transcribed into fluorescent cRNA along the entire length of the transcripts without 3′ bias utilizing random primers. The labeled cRNAs were hybridized to mouse lncRNA microarray. Finally, arrays were scanned by Agilent Scanner G2505B. The array images were analyzed by Agilent Feature Extraction software (version 10.7.3.1). The GeneSpring GX v11.5.1 software package (Agilent Technologies) was utilized to analyze quintile normalization and subsequent data processing. The microarray hybridization was carried out by Kangchen Bio-tech, Shanghai, China.

Bioinformatics analysis
Differentially expressed IncRNAs and mRNAs with statistical significance were identified through Volcano Plot filtering. The threshold used to screen up- or down-regulated RNAs was fold-change >2.0 (P < 0.05). Hierarchical clustering was carried out by Cluster 3.0, and the heat maps were generated in Java Treeview. The DE mRNAs which were adjacent to or overlap with the DE IncRNAs were recognized as DE IncRNAs related mRNAs using UCSC Genome Browser. The differentially expressed mRNAs or DE IncRNAs related mRNAs were analyzed by pathway annotation and gene ontology (GO) functional enrichment using CapitalBio® Molecule Annotation System V3.0 (MAS3.0). The −log10 (P-value) of the GO and pathway results were shown in the histogram.

Real-time reverse transcription-polymerase chain reaction (RT-PCR)
The microarray results were confirmed by RT-PCR. Total RNA was extracted from the spinal cord tissue as described above and total RNA was reverse transcribed using random hexamers primer (TaKaRa Bio Inc) according to the manufacturer’s description. The expression level of six genes was checked, including Speer7-ps1, uc007pbc.1, ENSMUST00000171761, ENSMUST0000097503, Cyp2d9, and Mnx1. The Gapdh was used as house-keeping gene. The sequences of all primers were shown in Table 4. RT-PCR was performed using the Fast Start Universal SYBR Green Master (TaKaRa Bio

| Table 4 Primer sequences used in Real-Time PCR |
|-------------------------------|-----------------|---------------|
| **Sequence name** | **Primer sequence** | **Amplicon size (bp)** |
| Speer7-ps1 | F: 5’-CATGCTTCATAGCTCACC-GA-3’ | 70 |
| | R: 5’-TACGCTGGAACAAACAAC-3’ | |
| uc007pbc.1 | F: 5’-CATCTAGACCCTAAAGCACC-3’ | 340 |
| | R: 5’-TGTAGGCAAGCATCCAAG-3’ | |
| ENSMUST00000171761 | F: 5’-TCGGAATCTTCTTCCCGT-3’ | 108 |
| | R: 5’-AAGACATGCGATGGGGGCA-3’ | |
| ENSMUST0000097503 | F: 5’-AGGTCATCCCACATTGTGAC-3’ | 77 |
| | R: 5’-GAGTTGGTTTGGCGCCGTC-3’ | |
| Cyp2d9 | F: 5’-TGTTACTCCTCCTGCACT-3’ | 71 |
| | R: 5’-GTGATGGCCCTCCTGGTCA-3’ | |
| Mnx1 | F: 5’-GAAACACCAGTCAAGCTCAACA-3’ | 129 |
| | R: 5’-GCTGCCGTCCATTCATTGCA-3’ | |
| Gapdh | F: 5’-TGTTCCCCCATTGTAAGTC-3’ | 129 |
| | R: 5’-GTGAGCCAAGATGCCCT-3’ | |
Inc) with 20-μl reaction system, according to the manufacturer’s protocol, in a Rotor-Gene 6000 instrument (Hamburg, Germany). The melting-curve analysis was performed in order to monitor the specificity of production. All experiments were replicated three times. The gene expression levels in the sham and SNL groups were analyzed with the \(2^{-\Delta\Delta CT}\) method.

**Statistical analysis**

The behavioral data were analyzed by two-way analysis of variance. The RT-PCR results were reported as mean ± SEM and analyzed by the one-way analysis of variance followed by Tukey’s multiple comparison test. The criterion for statistical significance was P < 0.05.

**Abbreviations**

| Abbreviation | Full Form |
|--------------|-----------|
| AOA10 | annexin A10 |
| APOA2 | apolipoprotein A-II |
| CCL5 | chemokine C-C motif ligand 5 |
| TAGAP | GTPase-activating protein |
| ANXA10 | annexin A10 |
| ATF3 | activating transcription factor 3 |
| CACNA1G | calcium channel, voltage-dependent, T type, alpha 1G |
| CD36 | CD36 |
| CEBPA | CCAAT/enhancer-binding protein alpha |
| CX3CR1 | chemokine (C-X3-C) receptor 1 |
| DACT | downstream activator of T-cell death |
| DPYSL3 | Drosophila plakoglobin-like protein |
| FMN | flavin mononucleotide |
| GPR151 | G-protein-coupled receptor 151 |
| HLA | human leukocyte antigen |
| HTRA1 | high temperature requirement A1 |
| IRE1 | unfolded protein response |
| INSR | insulin receptor |
| JUN | Jun | transcription factor |
| MERTK | mer receptor tyrosine kinase |
| MTHFD1 | methylenetetrahydrofolate dehydrogenase 1 |
| NFKB | nuclear factor kappa B |
| NFATC1 | nuclear factor of activated T-cells 1 |
| NGF | nerve growth factor |
| PAG | proteinase-activated receptor |
| PDE4D | phosphodiesterase 4D |
| PRKCA | protein kinase C, alpha |
| PTEN | phosphatase and tensin homolog |
| PTI | proteinase-activated receptor |
| PTPRC | protein tyrosine phosphatase, receptor type, C |
| PSA | prostate specific antigen |
| RORC | retinoic acid receptor-related orphan receptor C1 |
| SDHA | succinate dehydrogenase A |
| TLR | toll-like receptor |
| TRPA1 | transient receptor potential domain |
| VR1 | vanilloid receptor |
| ZFP236 | zinc finger protein 236 |

**Authors’ contributions**

BCI designed the microarray experiment, analyzed the data, and drafted the manuscript. WXX participated in the data analysis and prepared the figures. LNH did the real-time PCR analysis. DLC did the immunostaining. ZJZ prepared SNL model and did the behavioral test. YJG designed and supervised the overall experiment, revised the manuscript. All authors read and approved the final manuscript.

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**Compliance with ethical guidelines**

**Competing interests**

The authors declare that they have no competing interests.

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24. Ramaglia V, Daha MR, Baas F. The complement system in the peripheral nerve: friend or foe? Mol Immunol. 2008;45:3865–77.
25. Morgan BP, Gasque P, Singhrao SK, Piddlesden SJ. Role of complement in inflammation and injury in the nervous system. Exp Clin Immunogenet. 1997;14:19–23.
26. Ferrari S, Morbin M, Nobile-Orazio E, Musso A, Tomellini G, Bertolasi L, et al. Antisulfatide polynuropathy: antibody-mediated complement attack on peripheral myelin. Acta Neuropathol. 1998;96:659–74.
27. Di Ruscio A, Ebralidze AK, Benoukraf T, Amabile G, Goff LA, Terragni J, et al. DNMT1-interacting RNAs block gene-specific DNA methylation. Nature. 2013;503:371.
28. Guttman M, Amir M, Garber M, French C, Lin ME, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009;458:223–7.
29. Li D, Chen G, Yang J, Xu G, Gong Y, Xu G, et al. Transcriptome analysis reveals distinct patterns of long noncoding RNAs in heart and plasma of mice with heart failure. PLoS One. 2013;8:e77938.
30. Lacroix-Fralish ML, Tawfik VL, Tanga FY, Spratt KF, DeLeo JA. Differential spinal cord gene expression in rodent models of radicular and neuropathic pain. Anesthesiology. 2006;104:1283–92.
31. Levin ME, Jin JG, Ji RR, Tong J, Pomonis JD, Lavery DJ, et al. Complement activation in the peripheral nervous system following the spinal nerve ligation model of neuropathic pain. Pain. 2008;137:182–201.
32. Sauvageau M, Goff LA, Lodato S, Bonev B, Groff AF, Gerhardinger C, et al. Multiple knockout mouse models reveal lncRNAs are required for life and brain development. eLife. 2013;2:e01749.
33. Ulltisky I, Bartel DP. lncRNAs: genomics, evolution, and mechanisms. Cell. 2013;154:26–46.
34. Awad C, Cooper M, Odeleye A, Jensen BK, White MG, Vassoler FU, et al. Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system. J Neurovirol. 2014;20:39–53.
35. Imai S, Ikegami D, Yamashita A, Shimizu T, Narita M, Niikura K, et al. Epigenetic transcriptional activation of monocyte chemoattractant protein 3 contributes to long-lasting neuropathic pain. Brain. 2013;136:828–43.
36. Ulltisky I, Shkumatava A, Jan CH, Sive H, Bartel DP. Conserved function of lncRNAs in vertebrate embryonic development despite rapid sequence evolution. Cell. 2011;147:537–50.
37. Beckedorff FC, Ayaune AC, CroCCI-Souza R, Amaral MS, Nakaya H, Holtys DT, et al. The intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation. PLoS Genet. 2013;9:e1003705.
38. Diamond B, Volpe BT. A model for lupus brain disease. Immunol Rev. 2012;248:56–67.
39. Zhao ZJ, Cao DL, Zhang XJ, Ji RR, Gao YJ. Chemokine contribution to neuropathic pain: respective induction of CXCL1 and CXCR2 in spinal cord astrocytes and neurons. Pain. 2013;154:2185–97.
40. Wang Y, Gehringer R, Mousa SA, Hackel D, Brack A, Rittner HL. CXCL10 controls inflammatory pain via opioid peptide-containing macrophages in electroacupuncture. PLoS One. 2014;9:e94696.
41. Ye D, Bu H, Gao G, Shu B, Wang W, Guan X, et al. Activation of CXCL10/CXCR3 signaling attenuates morphine analgesia: implications of G protein. J Mol Neurosci. 2014;53:571–9.
42. Bu H, Shu B, Gao F, Liu C, Guan X, Ke C, et al. Small proline-rich repeat protein 1A and transcription. Nat Rev Genet. 2011;12:136–49.
43. Christofo D, Dumlaos DS, Stokes JA, Dennis EA, Svensson CI, Corr M, et al. Spinal TLR4 mediates the transition to a persistent mechanical hypersensitivity after the resolution of inflammation in serum-transferred arthritis. Pain. 2011;152:2881–91.
44. Park CK, Xu ZZ, Benta T, Han Q, Chen G, Liu XJ, et al. Extracellular micro-RNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron. 2014;82:47–54.
45. Seijffers R, Mills CD, Woolf CJ. ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration. J Neurosci. 2007;27:7911–20.
46. Bonilla LE, Tanabe K, Strittmatter SM. Small proline-rich repeat protein 1A is expressed by axotomized neurons and promotes axonal outgrowth. J Neurosci. 2002;22:1303–15.
47. Rodriguez Parkitna J, Korostynski M, Kaminska-Chovanec D, Obara I, Mika J, Przewlocka B, Przewlocki R. Comparison of gene expression profiles in neuropathic and inflammatory pain. J Physiol Pharmacol. 2006;57:401–14.
48. Fritah S, Nicola P, Azuaje F. Databases for IncRNAs: a comparative evaluation of emerging tools. RNA. 2014;20:1655–65.
49. Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA, et al. Activating RNAs associate with mediator to enhance chromatin architecture and transcription. Nature. 2013;494:497–501.
50. Fritah S, Nicola P, Azuaje F. Databases for IncRNAs: a comparative evaluation of emerging tools. RNA. 2014;20:1655–65.
51. Ulitsky I, Shkumatava A, Jan CH, Sive H, Bartel DP. Conserved function of lncRNAs in vertebrate embryonic development despite rapid sequence evolution. Cell. 2011;147:537–50.
52. Beckedorff FC, Ayaune AC, CroCCI-Souza R, Amaral MS, Nakaya H, Holtys DT, et al. The intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation. PLoS Genet. 2013;9:e1003705.
53. Diamond B, Volpe BT. A model for lupus brain disease. Immunol Rev. 2012;248:56–67.
54. Zhang ZJ, Cao DL, Zhang XJ, Ji RR, Gao YJ. Chemokine contribution to neuropathic pain: respective induction of CXCL1 and CXCR2 in spinal cord astrocytes and neurons. Pain. 2013;154:2185–97.
55. Wang Y, Gehringer R, Mousa SA, Hackel D, Brack A, Rittner HL. CXCL10 controls inflammatory pain via opioid peptide-containing macrophages in electroacupuncture. PLoS One. 2014;9:e94696.
56. Ye D, Bu H, Gao G, Shu B, Wang W, Guan X, et al. Activation of CXCL10/CXCR3 signaling attenuates morphine analgesia: implications of G protein. J Mol Neurosci. 2014;53:571–9.
57. Bu H, Shu B, Gao F, Liu C, Guan X, Ke C, et al. Small proline-rich repeat protein 1A and transcription. Nat Rev Genet. 2011;12:136–49.
58. Pauli A, Rinn JL, Schier AF. Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet. 2011;12:136–49.
59. Ornstein L, Azuaje F. Databases for IncRNAs: a comparative evaluation of emerging tools. RNA. 2014;20:1655–65.