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The gene regulatory network in different brain regions of neuropathic pain mouse models

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Abstract. – OBJECTIVE: Neuropathic pain is directly developed from lesions or somatosensory nervous system diseases that are associated with emotion regulation. In general population, the incidence of neuropathic pain ranges from 7% to 10%, but the underlying mechanism remains largely unknown. Neuropathic pain is often associated with structural and functional abnormalities in multiple brain regions, and its regulation has been shown to correspond with the forebrain, including nucleus accumbens (NAc), medial prefrontal cortex (mPFC) and periaqueductal gray (PAG).

MATERIALS AND METHODS: To investigate the molecular mechanism of neuropathic pain across different brain regions, we identified the differentially expressed genes (DEGs) between the spared nerve injury model (SNI) mice suffering neuropathic pain and the control Sham mice in NAc, mPFC and PAG three brain regions, and mapped these genes onto a comprehensively functional association network. Thereafter, novel neuropathic pain genes in these three regions were identified using With Random Walk with Restart (RWR) analysis, such as Asic3, Cd200r1 and MT2, besides well-known Capn11 and CYP2E1.

RESULTS: Interactions or cross talks among DEGs in NAc, mPFC and PAG three brain regions were discovered.

CONCLUSIONS: Our results provide novel insights into neuropathic pain and help to explore therapeutic targets in the treatment.

Key Words:
Neuropathic pain, Spared nerve injury model, Functional association network, Random walk with restart, Cross talk.

Introduction

As defined by the International Association for the Study of Pain (IASP), neuropathic pain is the direct result caused by lesions or somatosensory nervous system diseases that are associated with emotion regulation[1,2]. In general population, the incidence of neuropathic pain is 7-10% and the underlying mechanisms are heterogeneous[3,4]. Many patients with neuropathic pain often experience depression and anxiety disorders, leading to the low quality of life[5,6].

Currently, recommended treatment approaches for neuropathic pain are pharmacological methods, such as the use of antidepressants, anticonvulsants and topical anesthetics[7,8]. In some cases, however, medical therapy alone cannot fully work on chronic pain. Some non-pharmacological approaches, including psychological approaches, physical therapy, interventional therapy and surgical procedures, have been shown to be effective for neuropathic pain[8]. In addition, it's also important to clarify the distinction between nociceptive and neuropathic pain, because different treatment methods are usually required for different types of pain.

Chronic pain is often related to structural and functional abnormalities in the brain[9,10]. Increased activity of the forebrain neurons results in enhanced inflammatory and neuropathic pain[11,12]. Besides, the forebrain, including nucleus accumbens (NAc), medial prefrontal cortex (mPFC) and paraventricular nucleus (PVN), has been shown to be associated with the regulation of neuropathic pain[13,14]. Nucleus accumbens (NAc), known to be related to emotional dysfunctions following neuropathic pain

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regulation, is a key component of the brain reward system[15-17]. Goffer et al[18] demonstrated that chronic pain induced depressive behaviors in rats selectively increased the level of AMPA-type glutamate receptors in the NAc, suggesting a crucial role of NAc in the regulation of neuropathic pain-induced depression[18].

Neuropathic pain leads to morphological and functional changes in the mPFC and its important role in the regulation of emotional processes and chronic pain has also been identified[19,20]. Animal and human imaging studies have proved that synaptic changes in the PFC occur in both chronic pain and acute models[21,22]. In addition, the nucleus accumbens (NAc) is the key output target of the PFC. Functional connections between NAc and mPFC were also reported to predict the prognosis of chronic pain after medical treatment[23,24]. Lee et al[22] suggested that the activation of mPFC-NAc projections could regulate the affective symptoms of neuropathic pain.

Patients with chronic pain also exhibit brain abnormalities in descending modulation of pain[25,26], especially in the periaqueductal gray (PAG), which may be associated with dysfunctions of pain regulation[27-29]. Neuropathic pain activates neurons in the periaqueductal gray (PAG), which project to the rostral ventromedial medulla (RVM) and then to the spinal cord, resulting in the inhibition or remission of the pain[30].

Since the brain region corresponding to chronic pain is between the ventromedial PFC and PAG in humans, and mPFC-PAG in rodents, we would like to identify the genes that respond to neuropathic pain and investigate the molecular mechanisms[31,32]. The differentially expressed genes (DEGs) in the nucleus accumbens (NAc), the medial prefrontal cortex (mPFC), and the periaqueductal grey (PAG) of the spared nerve injury model (SNI) were mapped onto gene regulatory network. Novel neuropathic pain genes in different brain regions were identified using Random Walk with Restart (RWR) algorithm and the interactions or cross talks across different regions were analyzed.

Materials and Methods

The DEGs Between SNI and Sham Mice In NAc, mPFC and PAG

Descalzi et al[33] identified gene expression profiles from NAc, mPFC and PAG in SNI mice and Sham mice using RNA-sequencing[33]. SNI mice were the mouse models with neuropathic pain and Sham mice were used as control. Sham NAc, Sham PAG, Sham PFC, SNI NAc, SNI PAG and SNI PFC samples of equal sample size of 6 were collected, and matched Reads Per Kilobase per Million mapped reads (RPKM) gene expression profiles were accessed from GSE91396 in Gene Expression Omnibus (GEO) database. DEGs between SNI mice and Sham mice in NAc, mPFC and PAG were screened with the threshold of fold change greater than 1.5 and p-value smaller than 0.05, sequentially mapped onto STRING network for further analysis[34]. The p-value was calculated using function voom from limma in R package.

The Network Expansion of Neuropathic Pain Genes In NAc, mPFC and PAG Based on RWR Analysis

To investigate the interactions or cross-talks among different brain regions that were responsive to neuropathic pain, DEGs between SNI mice and Sham mice in NAc, mPFC and PAG were mapped onto STRING networks (a widely used network for bioinformatics studies[34-38]). Only the STRING networks with high confidence interactions were included, in other words, the confidence score of the interaction must be greater than 0.900.

RWR algorithm was applied to explore the cross talks among these three brain regions[35,39-42]. To illustrate how RWR can reveal the cross talk, STRING network was denoted as a graph comprised of a set of genes and a set of interactions .

The whole interaction network can be represented in an adjacency matrix. n referred to the genes total number. The value in row and column was 1 if gene and gene had interactions, whereas the value was 0 if there was no interaction.

(1) Normalization. The adjacency matrix was column-wise normalized.

$$A_{[i,j]}{}' = \frac{A_{[i,j]}}{\sum_{k=1}^{n} A_{[k,j]}} \quad (1)$$

(2) Iteration. Then, a random walk step was iterated. In each round of iteration, the state probabilities at time were based on previous state and the initial state.

$$P_{t+1} = (1-r)A'P_t + rP_0 \quad (2)$$

is previous state probability at time . is the restart probability and is the initial state probability, which was a column vector with for the seed genes (NAc, mPFC, PAG neuropathic pain genes, respectively), and to 0 for other genes on the network.

(3) Converge. The iteration process was ended when the difference between two states was smaller than 1×10^{-6}.

Genes on the network undergoing RWR analysis were given a probability of being visited by the seed genes.

The neuropathic pain genes in NAc, mPFC and PAG were considered as seed genes, respectively.

To evaluate how significant the probability was, we randomly chose the same number of seed genes 1000 times and calculated the RWR probabilities. If there were more than 50 times that the permutation proverbialities were greater than the actual proverbiality, the permutation *p*-value for that gene was greater than 50/1000=0.05 and that gene would be excluded.

According to the permutation *p*-value, novel neuropathic pain genes in NAc, mPFC and PAG based on RWR analysis were identified. Such expanded neuropathic pain genes in NAc, mPFC and PAG could be overlapped to show the cross talks among NAc, mPFC and PAG under neuropathic pain.

Results

The Neuropathic Pain Genes In NAc, mPFC and PAG Identified by Differential Analysis

The gene expression profiles of NAc, mPFC and PAG in SNI and Sham mice were analyzed and the DEGs were identified. Totally, 123, 89 and 795 DEGs were screened in NAc, mPFC and PAG, respectively.

Comparison was performed among these three DEG lists as shown in **Supplementary Table I**. Venn diagram was plotted (Figure 1), and two genes were found to be overlapped, containing Capn11 and Cyp2e1. These two genes might play important roles in neuropathic pain.

Capn11 (Calpain 11) encodes the intracellular calcium-dependent cysteine protease that has protease activity and calcium-binding capacity[43]. Calpains have been reported to participate in some neuronal processes, including synaptic plasticity, neurodegeneration, signal transduction and enhancement[44-46]. As well, calpains can be observed in diverse cell types in the central nervous system (CNS), such as spinal cord neurons, cortical neurons and glial cells[47,48]. The activity of calpains was markedly increased in neurodegenerative diseases, traumatic brain injury and neuropathic pain[49-51]. Blocking calpain signaling by its inhibitor MDL28170 or silencing calpain-1 level in the spinal cord attenuates the neuropathic pain and the inflammation following peripheral nerve injury[51,52]. Mahajan et al[53] suggested that calpain also mediated the editing of AMPA receptor subtypes[53]. Notably, depression is a well-known emotional feature of chronic neuropathic pain. Goffer et al[18] discovered that chronic pain could increase the AMPA-type glutamate receptor expression level at the synapses of NAc in a rat model of chronic neuropathic pain with depression-like behaviors[18]. In addition, the in-

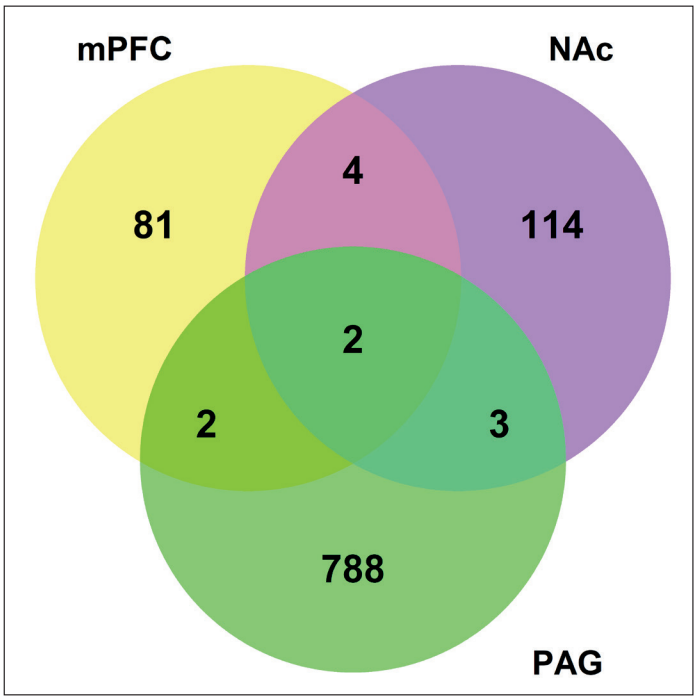

Figure 1. The Venn Diagram of DEGs in NAc, mPFC and PAG. Among the 123, 89 and 795 DEGs in NAc, mPFC and PAG, only two genes, Capn11 and Cyp2e1, were overlapped. These two genes played important roles in neuropathic pain, but there were many undiscovered neuropathic pain genes in the differential analysis.

creased level of GluA1 led to the formation of calcium-permeable AMPA receptors (CPARs), and the inhibition of these CPARs in the NAc could increase depressive symptoms associated with neuropathic pain[18]. Therefore, CPARs may present as a novel therapeutic target for the treatment of depressive symptoms in neuropathic pain.

CYP2E1 (Cytochrome P450 2E1), a member of the cytochrome P450 superfamily, can be considered as the second enzymatic system involved in the ethanol metabolism in brain[54-59]. This enzyme can be widely expressed in various cell types and human brain regions, including the hippocampus, substantia nigra and medulla[60-62]. Further studies are needed to identify the role of Cyp2e1 in chronic pain and sensory symptoms of pain. Toselli et al[63] found that CYP2E1 was expressed in human AMG and PFC and might influence the drug effects on those regions.

As shown above, these two genes function via complex pathways and regulatory mechanisms. There are many genes that can facilitate neuropathic pain responses in different brain regions have not been identified. To find these hidden genes, we mapped these DEGs onto functional association networks of STRING.

The Novel Neuropathic Pain Genes In NAc, mPFC and PAG Identified by RWR Analysis on the Functional Association Network

To identify more novel neuropathic pain genes in NAc, mPFC and PAG and find their hidden links or cross talks, DEGs were mapped onto networks and RWR analysis was performed. After being considered as seed genes and permuted 1000 times, DEGs with permutating *p*-value smaller than 0.05 were identified significant., including 623, 888 and 507 novel neuropathic pain genes in NAc, mPFC and PAG, respectively (**Supplementary Table II**).

Venn Diagram of these novel neuropathic pain genes in NAc, mPFC and PAG on the network was plotted (Figure 2) and found 25 overlapped genes (Table I). These 25 overlapped genes showed great promise in linking the three brain regions and revealing the potential cross talk mechanisms among NAc, mPFC and PAG. Their functions were discussed in the next section.

Discussion

Among the 25 genes in Table I, many of them were involved in pathways or functions that were associated with neuropathic pain genes. Asic3, Cd200r1 and MT2 three genes were shown to be most promising.

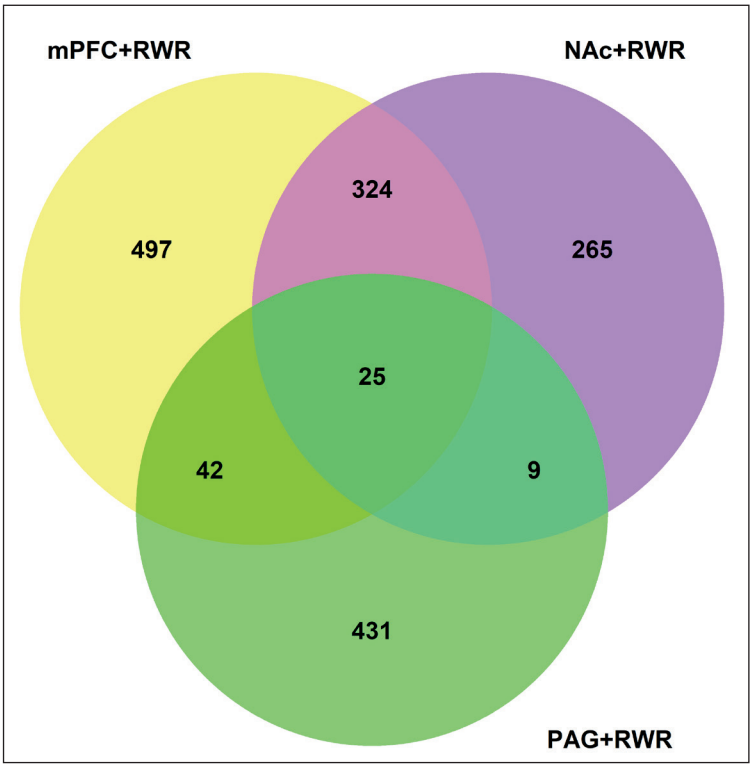

Figure 2. The Venn Diagram of novel neuropathic pain genes in NAc, mPFC and PAG on the network. Among the 623, 888 and 507 novel neuropathic pain genes in NAc, mPFC and PAG, 25 genes were overlapped. These genes linked the three brain regions and revealed the potential cross talk mechanisms among NAc, mPFC and PAG.

Table I. The 25 overlapped novel neuropathic pain genes in NAc, mPFC and PAG on the network.

Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol
Adat2	Ap5b1	Cd200r1	Igdcc3	Prlhr
Adat3	Ap5s1	Cic	Junb	Stoml3
Adgra3	Asic3	Ctu1	Mt1	Ush2a
Adgrv1	Atxn1	Ctu2	Mt2	Vezt
Agt	Cd200	Cyp2e1	Prlh	Whrn

Asic3 (Acid-sensing ion channels, ASICs) is a cationic channel expressed principally in central (CNS) and peripheral (PNS) nervous systems[64,65]. Ion channel modulation is the main approach to achieve novel neuropathic pain management[66]. Evidence from many experiments has suggested the involvement of ASICs in pain sensation[66,67]. Among the ASICs, ASIC3 is known to regulate inflammatory pain, ischemic pain and mechanical pain[64,68]. Inflammation is one of the pain symptoms that induces a significant increase of ASIC3 channel expression in sensory neurons, which demonstrates the crucial role of ASIC3 in the generation of pain-associated inflammation[69]. Therefore, inhibition of ASIC3 channel at the sensory system could significantly help to alleviate pain. In addition, Jeong et al[70] suggested that ASIC3 may be associated with the antinociceptive effects of amiloride and benzamil, inhibitors for ASIC channels, in neuropathic pain, and blocking ASIC3 channel may be a novel therapeutic strategy in neuropathic pain treatment[70-72].

Cd200r1, encoding the membrane glycoprotein of the immunoglobulin superfamily, is highly expressed in neurons in the central nervous system, while its receptor CD200R is restricted to the surfaces of myeloid lineage cells like macrophages and microglia[73,74]. The CD200-CD200R interaction has been reported to be closely associated with the macrophage-mediated damage in autoimmune disease and various neuroinflammatory diseases[75-78]. Animal models have also shown that loss of immunosuppression through CD200 has significant impact on neuroinflammation and neurodegeneration[79,80]. Hernangomez et al[80] reported that the CD200/CD200R regulatory system could suppress the neuroinflammatory reactions associated with peripheral neuropathic pain, and it might be used as a target for treating neuropathic pain.

MT2 (Metallothioneins II) is a major neuroprotective protein with a high affinity for metals[81,82]. MT2 has been found in many CNS (central nervous system) regions, such as cortex, hippocampus, brainstem and spinal cord[83-85]. A series of evidence has suggested that metallothioneins (MTs) are essential for the recovery from CNS damage[83,86]. Hidalgo et al[87] reported that MT-I/-II was capable of decreasing inflammatory responses associated with CNS injury, and provided credible evidence for supporting that MT-I/-II was able to protect neurons from death[87-89]. Kwon et al[90] evaluated the expression of MT-I/II in the spinal cord in rat models with inflammatory and neuropathic pain and found that increased MT-I/II participated in the initiation of inflammatory and neuropathic pain.

Conclusions

Neuropathic pain is a common nervous system disease with a low incidence (7-10%) in the general population, and its underlying mechanisms remain largely unknown. Neuropathic pain involves the structural and functional abnormalities in multiple brain regions. The regions in forebrain, including nucleus accumbens (NAc), medial prefrontal cortex (mPFC) and periaqueductal gray (PAG), have all been proved to correspond to neuropathic pain response. To investigate the molecular mechanism of neuropathic pain across different brain regions, we identified the DEGs between SNI mice (a widely used model for neuropathic pain) and the Sham mice (control) in NAc, mPFC and PAG three brain regions. Then, these DEGs were mapped onto STRING networks. RWR analysis was conducted and more novel neuropathic pain genes in NAc, mPFC and PAG were revealed in concomitant with more overlapped genes. These overlapped novel neuropathic pain genes can help us understand how different brain regions communicate with each other and coordinate the regulation of neuropathic pain. These genes were worth to be further validated and investigated as therapeutic targets.

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Authors' contributions

XL contributed to the study design. LX conducted the literature search. YX acquired the data. QY wrote the article. ZF performed data analysis and drafted it. MY revised the article. XL and LX gave the final approval of the version to be submitted.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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