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ORIGINAL RESEARCH

Analysis of the circRNA/lncRNA–miRNA–mRNA Networks of Action of Fire Needle Acupuncture in Relieving Chemotherapy-Induced Neuropathic Pain

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Purpose: The current treatment strategies of chemotherapy-induced peripheral neuropathy (CIPN) are not ideal. Fire needle acupuncture (FA) has been shown to have significant therapeutic effects on pain, including cancer-related pain. However, the potential mechanisms by which FA alleviates CIPN remain unclear.

Methods: A CIPN model was established using oxaliplatin, followed by FA treatment. Mechanical sensitivity in rats was assessed using the paw withdrawal threshold (PWT), and inflammatory cell infiltration in the L4–L6 spinal cord segments was analyzed via hematoxylin and eosin staining. Subsequently, L4–L6 spinal cord segments were collected and whole-transcriptome sequencing and small RNA sequencing were performed to identify differentially expressed genes. PPI networks, circRNAs/lncRNAs–miRNAs–mRNAs, and functional analyses of hub genes were performed to elucidate the potential mechanisms through which FA therapy improves CIPN.

Results: FA therapy rapidly increased the PWT and reduced inflammatory infiltration of the spinal cord in CIPN rats. Additionally, 656, 192, 15, and 657 differentially expressed circRNAs, lncRNAs, miRNAs, and mRNAs, respectively, were identified as being associated with FA treatment of CIPN. FA treatment is associated with extracellular matrix receptor interactions, the PI3K–Akt and IL-17 pathways. Furthermore, T cells, macrophages, and T follicular helper cells were closely associated with hub genes, suggesting that these immune cells play a pivotal role in the FA-mediated regulation of CIPN.

Conclusion: This study elucidates the potential mechanisms by which FA therapy alleviates CIPN. This study provides a direction for future research on FA therapy and lays a theoretical foundation for the clinical application of FA.

Keywords: chemotherapy, peripheral neuropathy, acupuncture, immune, inflammation

Introduction

Adjuvant chemotherapy is a key strategy for cancer treatment, in which tumor cells are effectively eradicated or their proliferation is suppressed, thereby reducing recurrence and improving long-term patient survival. However,

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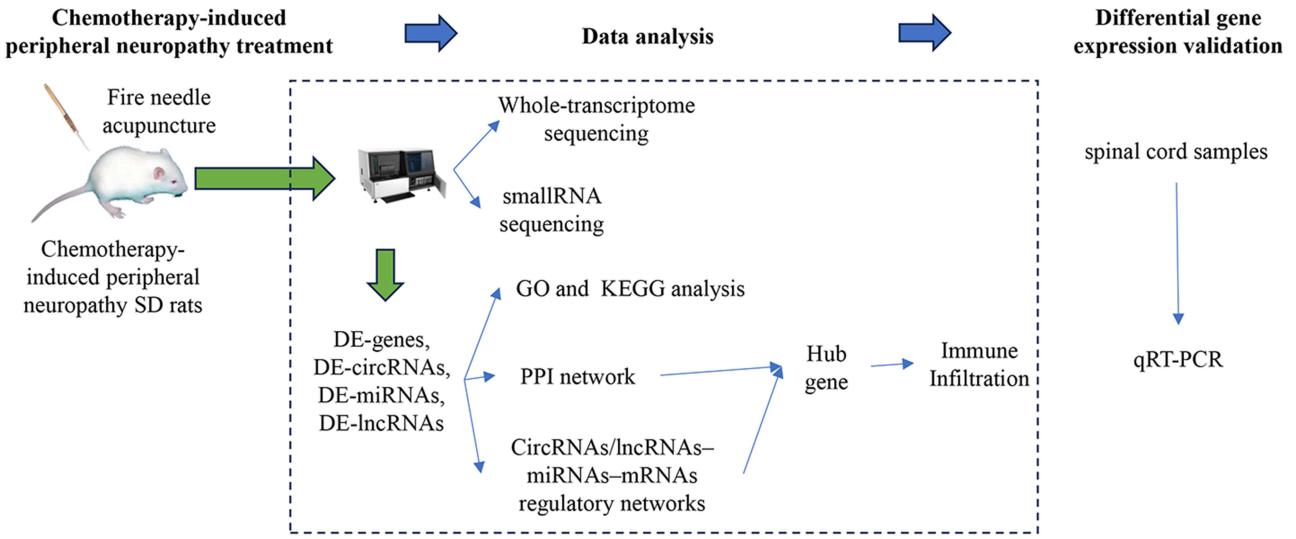
Graphical Abstract



chemotherapy has potential neurotoxic effects that can significantly affect patient health and quality of life.[1] Chemotherapy-induced peripheral neuropathy (CIPN), including that caused by chemotherapeutic drugs such as oxaliplatin (OXA) and paclitaxel, is the most prevalent and severe side effect.[2] Up to 68% of patients experience CIPN within a month of completing their chemotherapy regimen, and nearly 30% of patients with cancer continue to experience CIPN for 6 months or more following treatment.[3,4] The emergence of CIPN during a chemotherapy regimen may lead to a reduction in the chemotherapy dosage and may even lead to the discontinuation of the regimen, further affecting the efficacy of cancer treatment and survival rates.[5,6] Although CIPN has a significant impact on patients with cancer, there is currently no effective treatment protocol to alleviate or eliminate CIPN.[7,8] Therefore, the development of new treatment methods is an urgent requirement to improve the prognosis of patients with CIPN.

At present, there is greater focus on non-pharmacological interventions in clinical practice, such as rehabilitation, acupuncture, and moxibustion, which are designed to enhance the support available to patients with CIPN.[9,10] Importantly, acupuncture or electroacupuncture can effectively alleviate CIPN and improve the quality of life.[11,12] Fire needle acupuncture (FA) is a type of acupuncture and moxibustion that combines acupuncture and hyperthermia and has the advantages of mechanical and thermal stimulation compared with acupuncture and moxibustion. FA therapy can effectively improve pain caused by knee osteoarthritis and acute herpes zoster.[13–15] Additionally, FA therapy significantly alleviates cancer-related pain.[16] Further, it has been reported that FA increased mechanical pain threshold, reduced peripheral inflammatory cytokine levels, and improved CIPN in a rat model of OXA-induced neuropathic pain.[17] However, the mechanism through which FA ameliorates CIPN remains unclear.

circRNAs, lncRNAs, miRNAs, and mRNAs are key potential therapeutic targets for CIPN.[18–20] Nerve injury–specific lncRNAs in the dorsal root ganglion affect CIPN progression[21] miRNAs, including miR-3184-5p and miR-30d, are closely associated with CIPN and can serve as diagnostic biomarkers and therapeutic targets.[22–24] In spinal cord neurons, miR-124 improves CIPN by preventing microglial activation.[25] However, the changes that occur in the complete regulatory network during the improvement of CIPN with the application of FA remain unclear.

Hence, this study aimed to use whole-transcriptome and smallRNA sequencing to analyze changes in the circRNA/lncRNA–miRNA–mRNA regulatory network in the spinal cord of CIPN rats after FA intervention. Functional enrichment was also used to identify potential therapeutic targets and potential mechanisms underlying the analgesic effects of FA therapy.

Materials and Methods

Experimental Animals

The study was approved by the ethics committee of The First Affiliated Hospital, Guangzhou University of Chinese Medicine, in accordance with the ARRIVE guidelines and Laboratory Animal Guidelines for ethical review of animal welfare (GB/T 35892–2018) in China (TCMF1-2021059). A cohort of SPF Sprague–Dawley (SD) rats (8-weeks old; Experimental Animal Center of Guangzhou University of Chinese Medicine, SYXK (Yue) 2018–0092, Guangzhou, China) with a body weight of (180 ± 20) g were used for this study. Rats were housed at 20–25 °C, with a 12-h light/dark cycle in the SPF-grade animal laboratory of the First Affiliated Hospital of Guangzhou University of Chinese Medicine.

Grouping and FA Intervention

A total of 18 SD rats were randomly assigned to three groups: blank (n = 6), OXA (n = 6), and FA (n = 6). The CIPN modeling (OXA group) method was based on a study by Mihara et al, with an intraperitoneal injection of 4 mg/kg OXA (Hengrui, Jiangsu, China).[26] Rats in the OXA and FA groups received one intraperitoneal injection each on days 1, 2, 8, 9, 15, 16, 22, and 23. The blank group received intraperitoneal injections of 5% glucose solution on days 1, 2, 8, 9, 15, 16, 22, and 23 at the same dose used in the OXA modeling method (1 mL/kg). A 50% decrease in the paw withdrawal mechanical threshold (PWT) was considered to indicate successful establishment of the model. The blank group received no further treatment, whereas the OXA group received no treatment after successful creation of the model. The FA group received FA intervention (0.5 mm×4.0 cm) after successful creation of the model. Rats were anesthetized using 1% pentobarbital (40 mg/kg) via intraperitoneal injection. FA intervention was performed once daily on days 1, 3, and 5 after successful creation of the model (ie, on days 24, 26, and 28). The rats were shaved locally and the FA tip and body were heated to a red–white color. The horizontal line connecting the hip nodules on both sides of the rat spine, which corresponds to the L6 spinous process of the lumbar vertebra, and the L4–6 Jiaji, bilateral Zusanli, and Hegu acupoints were selected for FA intervention.[11] Each acupoint was punctured three to four times per second, at a depth of 0.1–1 mm, for approximately 3 s. On the first day after the third intervention, the rats were euthanized using 1% pentobarbital (150 mg/kg) via intraperitoneal injection and placed in a prone position on ice. The lumbar region was then exposed to separate the L4–L6 spinal cord segments.

PWT Assay

PWT measurements began 1 day before model establishment was initiated (recorded as day 0). During the creation of the model, PWT was analyzed 1 h after each session. During the intervention period, PWT was measured 1 h after the FA intervention. von Frey filaments were used to vertically stimulate the skin on the lateral edge of the right hind paw (avoiding the footpad). The pressure value of the von Frey filaments was gradually increased until a withdrawal response was observed in the hind foot (such as retraction, licking, or shaking of the leg). The minimum stimulus intensity that elicited a withdrawal response was recorded as the PWT. Each rat underwent three measurements, and the mean value was recorded. The interval between two PWT measurements was 5 min. Measurements were conducted in a single-blind design and performed by independent evaluators who were blinded to the experimental group assignments.

Hematoxylin and Eosin (H&E) Staining

Rat L4–L6 spinal cord tissues fixed in 4% paraformaldehyde solution were embedded in paraffin and sectioned for H&E staining. After dewaxing with xylene and rehydration through a graded ethanol series, the sections were stained with hematoxylin (Beyotime, Shanghai, China), followed by differentiation with hydrochloric acid–ethanol for a few seconds. Subsequently, they were blued in hot water at 50 °C, stained with eosin (Beyotime) for 1 min, dehydrated, cleared, and mounted. The sections were then scanned using a digital pathology slide scanner (Beijing Hamamatsu Photon Techniques Inc., Beijing, China) to observe structural organization.

Whole-Transcriptome and Small RNA Sequencing and Analysis

From each group, three rats were randomly chosen, and total RNA was isolated from the L4–L6 spinal cord tissues using an RNA extraction kit according to manufacturer's instructions. Subsequently, library construction, whole-transcriptome analysis, and small RNA sequencing were performed (Oebiotech, Shanghai, China). Differentially expressed (DE)-genes, DE-circRNAs, DE-miRNAs, and DE-lncRNAs in the blank vs OXA and OXA vs FA groups were screened using the $|\log_2 \text{FoldChange}| > 1$ and $P < 0.05$ criteria. Clustering among groups was visualized using heat maps and volcano plots generated using the Oebiotech Cloud Platform (https://cloud.oebiotech.com/). The DE-genes in the normal vs OXA and OXA vs FA groups were imported into the Oebiotech Cloud Platform to identify common DE-genes. GO and KEGG enrichment analyses were conducted on the DE-genes to determine the primary biological functions or pathways affected by these genes. The PPI network was constructed and Hub genes were identified using STRING and Cytoscape 3.7.1 (with the Hub app). CircRNA/lnRNA–miRNA interactions were predicted using the miRanda database, whereas miRNA–mRNA interactions were analyzed using ENCORI (https://rnasysu.com/encori/). CircRNAs/lncRNA–miRNA–mRNAs were constructed using Cytoscape 3.7.1. The effect of FA on the immune infiltration microenvironment of rats with CIPN was analyzed using The CIBERSORT algorithm and single-sample gene-set enrichment analysis (ssGSEA). Spearman correlation analysis was used to investigate the correlation between immune cells and hub genes.

qRT-PCR

Total RNA was extracted from–L4–L6 spinal cord samples of rats using TransZol (TransGen, Beijing, China). RNA yield was determined in terms of OD260/OD280 using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Reverse transcription was performed using TransScript All in One First Strand cDNA Synthesis SuperMIX for qPCR (Transgen) in a GeneAmp PCR System 9700 (Applied Biosystems, USA). qPCR was performed using PerfectStart Green qPCR SuperMix (Transgen) in a LightCycler 480 II Real-time PCR Instrument (Roche, Switzerland). The primer sequences (Tsingke, Beijing, China) are listed in Table S1. The expression levels of mRNAs were normalized to ACTB and calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

All data were analyzed using SPSS 23.0. mRNA expression levels determined using qRT-PCR were presented as mean ± SD. One-way analysis of variance (ANOVA) was used to compare differences among the Blank, OXA, and FA groups, followed by post-hoc comparisons using Tukey's test. Statistical significance was set at $P < 0.05$.

Results

FA Intervention Improves CIPN

A flowchart illustrating the administration of OXA and FA is shown in Figure 1A. Compared with the blank group, the PWT in the OXA and FA groups exhibited a notable decline on days 9, 15, 16, 22, and 23 during the modeling period. On days 24, 26, and 28, PWT in the OXA group remained significantly lower than that in the blank group. However, the PWT in the FA group was significantly higher than that in the OXA group on days 24, 26, and 28 (Figure 1B). Additionally, the quantification of cell density in the spinal dorsal horn was performed based on H E staining. The OXA group showed a significant increase in cell density compared to the Blank group, indicating severe inflammatory cell infiltration. Treatment with FA significantly reduced cell density compared to the OXA group (Figure 1C). These results indicated that FA therapy can rapidly increase PWT and reduce inflammatory cell infiltration.

Screening of DEGs After FA Intervention in CIPN

To identify DEGs involved in the effects of FA intervention on CIPN, we collected spinal cord tissues for whole-transcriptome and small-RNA sequencing. The volcanic and heat maps of DEGs (including DE-circRNAs, DE-lncRNAs, DE-miRNAs, and DE-mRNAs) in the OXA vs Blank and FA vs OXA groups are shown in Figures 2A and S1–S4. In this study, we focused on identifying specific therapeutic targets of FA. If a DEG was significantly downregulated or upregulated in the OXA group and FA treatment reversed its expression, we considered it to be the most relevant

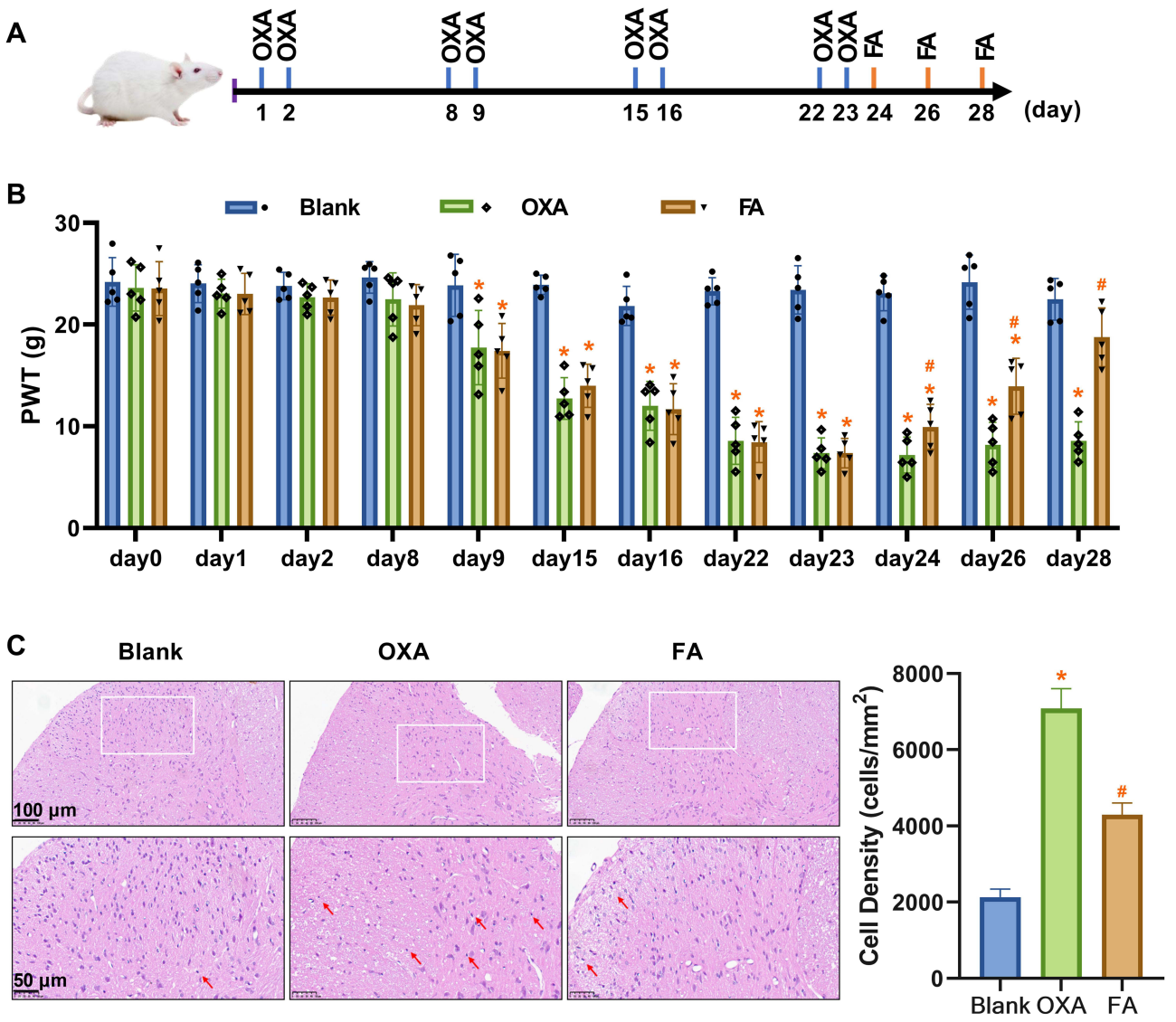


Figure 1 Fire needle acupuncture (FA) treatment ameliorated the chemotherapy-induced peripheral neuropathy (CIPN) of rats. (**A**) Experimental flowchart. (**B**) The paw withdrawal mechanical thresholds (PWTs) were measured during the creation of the CIPN model and during treatment. Data are presented as mean ± SD. (**C**) The infiltration of inflammatory cells in in L4–L6 spinal cord segments of rats in each group was detected by hematoxylin and eosin staining (50 μm). *P<0.05, vs Blank, #P<0.05, vs OXA.

gene associated with FA therapy. Hence, these DEGs were divided into four groups: OXA–Blank up (DEGs significantly upregulated in the OXA group compared with the blank group), OXA–Blank down (DEGs significantly downregulated in the OXA group compared with the blank group), FA-OXA up (DEGs significantly upregulated in the FA group compared with the OXA group), FA–OXA down (DEGs significantly downregulated in the FA group compared with the OXA group). Next, the intersection between "FA–OXA down" and "OXA–Blank up" and that between "FA–OXA up" and "OXA–Blank down" was analyzed (Figure 2B). In the intersection between "FA–OXA down" and "OXA–Blank up", DEGs were significantly upregulated in the OXA group and FA treatment reduced its expression, leading us to hypothesize that FA may mitigate CIPN by suppressing these DEGs. In the intersection between "FA–OXA up" and "OXA–Blank down", DEGs were significantly downregulated in the OXA group and FA treatment increased their expression, suggesting that FA may exert therapeutic effects by restoring their expression. In this study, 656 (414+242) DE-circRNAs, 192 (77+115) DE-lncRNAs, 15 (3+12) DE-miRNAs, and 657 (143+514) DE-mRNAs were identified (Table S2).

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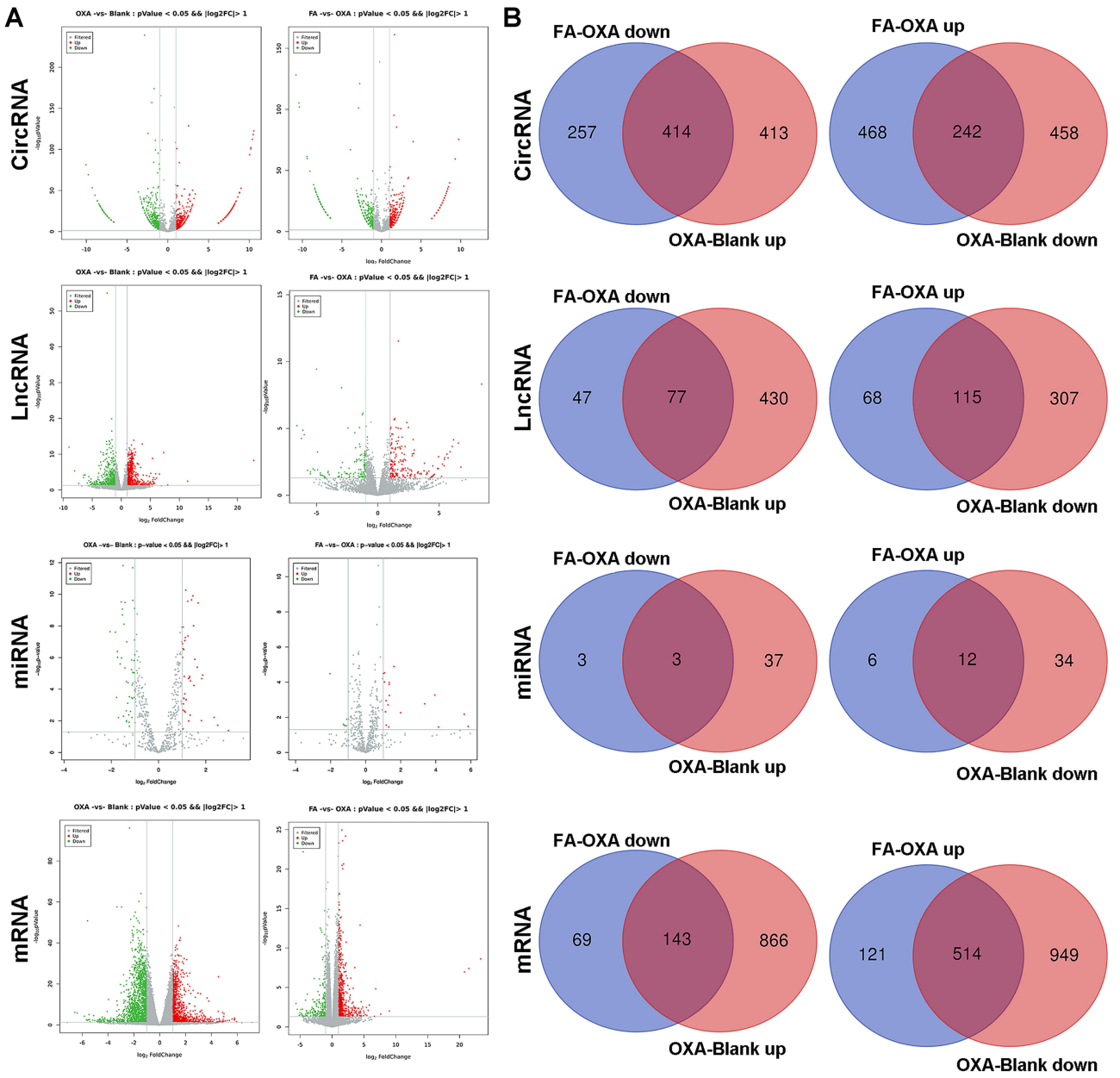

Figure 2 Differential expression of circRNA, lncRNAs, miRNAs, and mRNAs were identified. The L4–L6 spinal cord segments were collected from each group, followed by transcriptome sequencing and miRNA sequencing to analyze differentially expressed genes. (**A**) The differentially expressed circRNAs, lncRNAs, miRNAs, and mRNAs between OXA and Blank groups and between FA and OXA groups are shown in a volcano plot image. (**B**) Venn diagram displays the same genes between the two datasets. "FA–OXA up" indicates that the circRNA, lncRNAs, miRNAs, and mRNAs were significantly upregulated in the FA group compared with the OXA group. Conversely, "FA–OXA down" indicates that those were significantly downregulated. "OXA–Blank up" indicates that the circRNA, lncRNAs, miRNAs, and mRNAs were significantly upregulated in the OXA group compared with the Blank group. Conversely, "OXA–Blank down" indicates that those were significantly downregulated.

Functional Enrichment Pathway Analysis

We performed GO and KEGG analyses of the 657 DE mRNAs to elucidate the potential mechanisms of FA therapy for CIPN (Figure 3). The DE-mRNAs were mainly involved in extracellular matrix, gliogenesis, inflammatory cell infiltration (leukocyte migration and cell chemotaxis), external side of the plasma membrane, cytokine binding, fibronectin binding, integrin binding, cell adhesion molecule binding, and receptor ligand activity. The signaling pathways involved in DE-mRNAs mainly included extracellular matrix (ECM)–receptor interaction, the PI3K–Akt signaling pathway, and inflammation-related diseases (IL-17 signaling pathway, inflammatory bowel disease, and rheumatoid arthritis).

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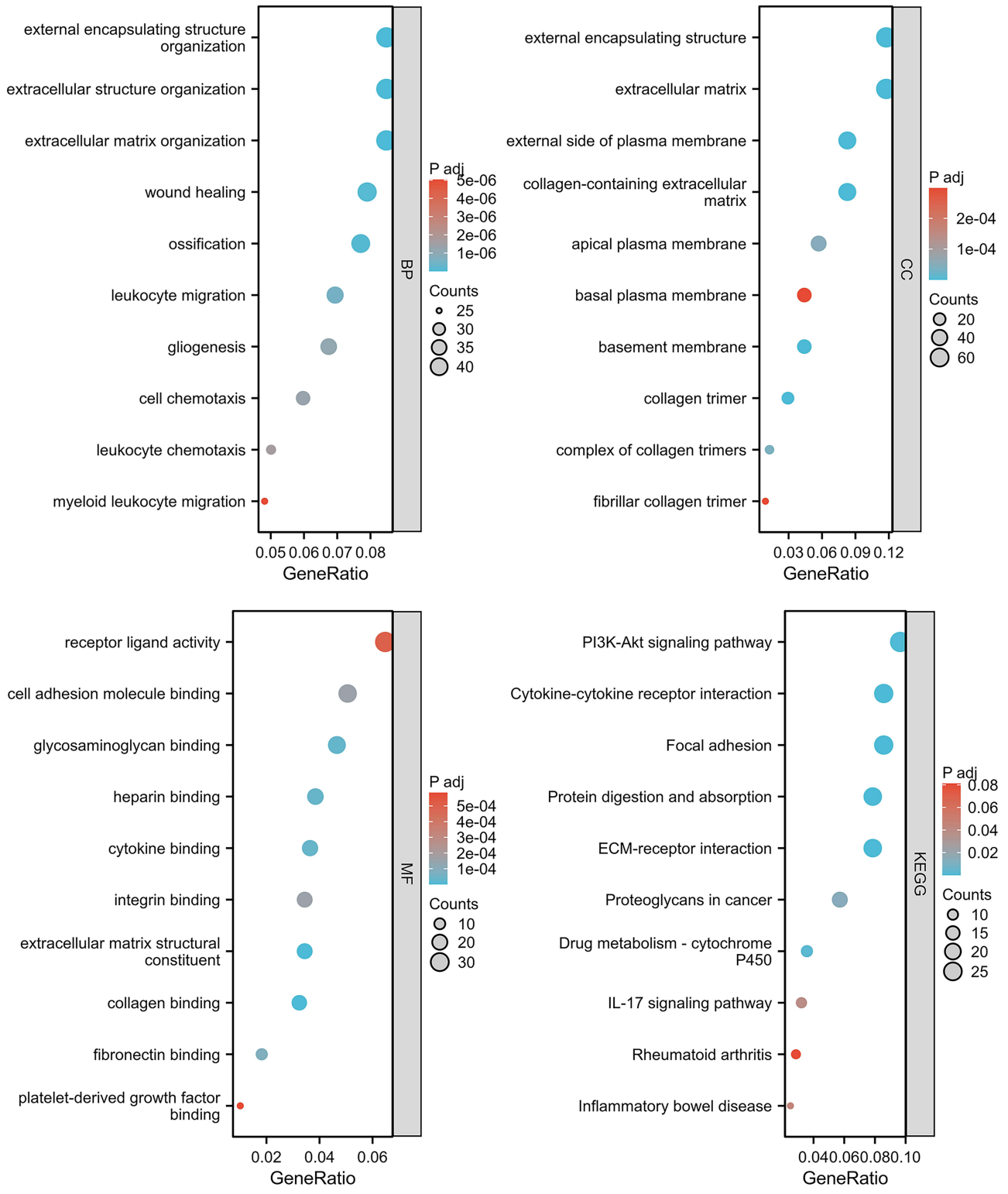

Figure 3 Potential mechanism of PA treatment for CIPN. To elucidate the mechanisms of FA therapy for CIPN, GO analysis and KEGG analysis was performed on DE-genes in L4–L6 spinal cord segments.

Hub Gene Identification

To further identify the hub genes involved in the therapeutic effect of FA on CIPN, we screened the 657 DE-mRNAs and constructed a PPI network, and performed hub gene analysis. PPI networks are shown in Figure 4A. Based on the Hub

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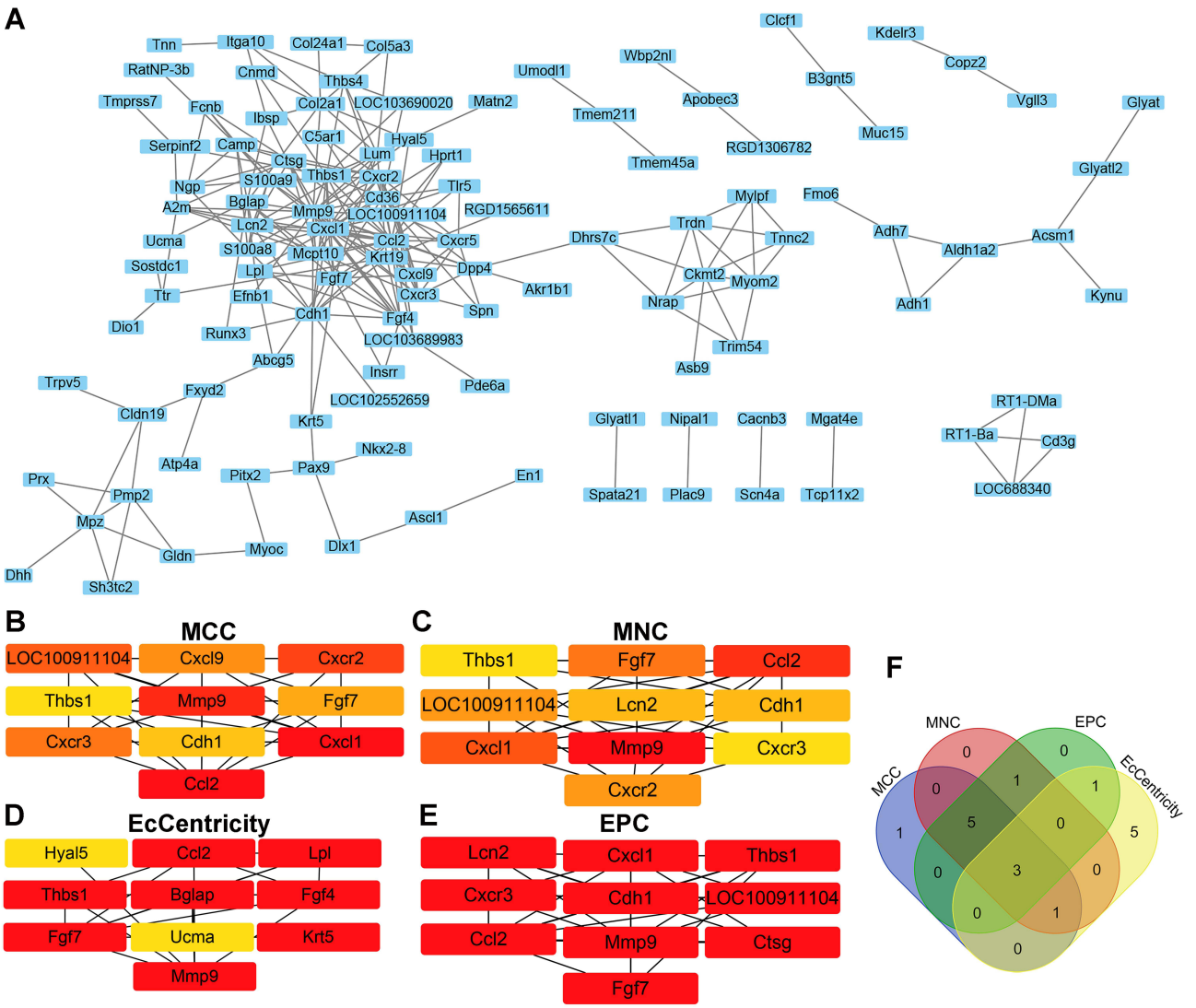

Figure 4 PPI network of the DE-genes in L4–L6 spinal cord segments construction and identified the Hub genes identification. (**A**) PPI networks of the DE-genes were constructed using STRING and Cytoscape. (**B**–**E**) Hub DE-genes were analyzed using MCC, MNC, EPC, and EcCentricity. (**F**) The intersection of the four sets of hub genes was taken.

gene analysis methods of MCC, MNC, EPC, and EcCentricity, three hub genes, *Mmp9, Fgf7*, and *Ccl2*, were identified (Figure 4B–F).

CircRNAs/lncRNAs–miRNAs–mRNAs Regulatory Networks Analysis

CircRNAs and lncRNAs predominantly function as competitive endogenous RNAs (ceRNAs) by competitively binding to miRNAs, thereby relieving miRNA-mediated repression of target mRNAs and modulating gene expression networks. Hence, this study constructed key circRNA/lncRNA—miRNA–mRNA regulatory networks based on 656 DE-circRNAs, 192 DE-lncRNAs, 15 DE-miRNAs, and 657 DE-mRNAs. Two distinct regulatory networks were established according to miRNA upregulation and downregulation patterns (Figure 5A and Figure 5B). These two key regulatory networks encompassed 79 DE-mRNAs, which were primarily associated with the regulation of vasoconstriction, collagen-containing ECM, growth factor binding, ECM–receptor interactions, and the PI3K–Akt signaling pathway (Figure S5). Furthermore, a PPI network was constructed using these 79 DE-mRNAs, leading to the identification of 10 novel hub genes (Figure 5C and D). These 10 new Hub DE-mRNAs were regulated by four DE-miRNAs: rno-miR-133a/b-3p-

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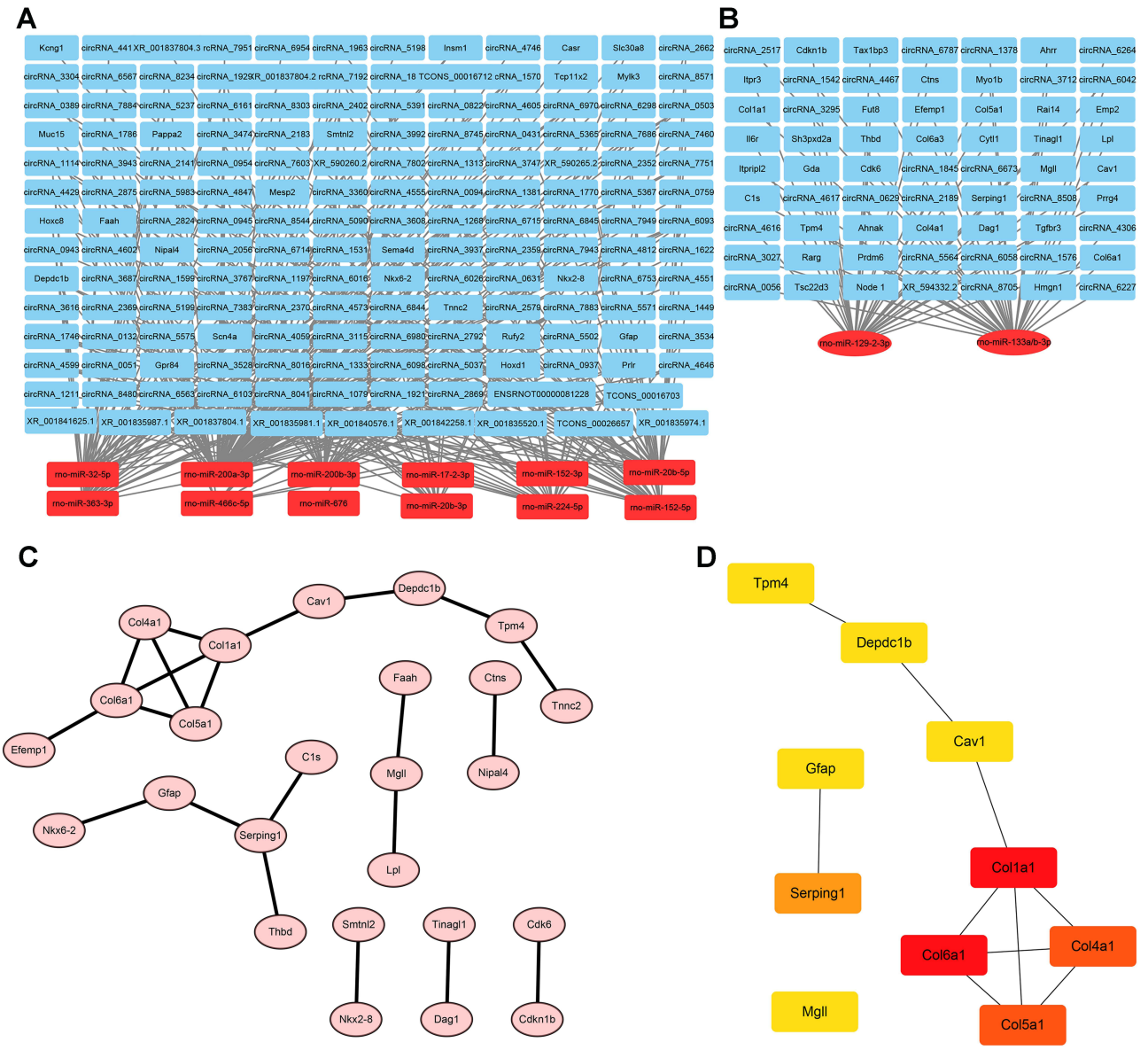

Figure 5 CircRNAs/lncRNAs–miRNAs–mRNAs regulatory networks in L4–L6 spinal cord segments was constructed. (**A**) A regulatory network centered on the upregulated DE-miRNAs in the FA vs OXA dataset was constructed. Red represents miRNA and green represents gene, circRNA, and lncRNA. (**B**) A regulatory network centered on the downregulated DE-miRNAs in the FA vs OXA dataset was constructed. Red represents miRNA and green represents gene, circRNA, and lncRNA. (**C**) PPI networks of the DE-mRNAs from two key regulatory networks were developed using STRING and Cytoscape. (**D**) Hub DE-mRNAs were analyzed using MCC.

Tpm4/Col1a1/Col4a1/Col6a1, rno-miR-129-2-3p-Col5a1/Col6a1/Cav1/Serping1/Mgll, rno-miR-152-5p-Gfap, and rno-miR-224-5p/rno-miR-200a-3p/rno-miR-17-2-3p-Depdc1b.

Immune Infiltration Analysis

To gain deeper insights into the effects of FA therapy on immune cell infiltration in rats with CIPN, we examined the relationship between the 13 hub DE-mRNAs (including three hub DE-mRNAs in Figure 4F and 10 hub DE-mRNAs in Figure 5D) and immune cell infiltration. Hub DE-mRNAs were mainly related to macrophages, T cells, and T follicular helper cells (TFH) in the OXA vs Blank (Figure 6A) and FA vs OXA datasets (Figure 6B). In both the OXA vs Blank and FA vs OXA datasets, the expression of *Cav1, Col6a1, Mgll, Serping1, Tpm4, Col5a1, Col4a1, Col1a1*, and *Fgf7* exhibited significant positive correlations with macrophage infiltration, whereas the expression of *Tpm4, Cav1*, and *Col5a1* was significantly and positively correlated with TFH infiltration (Figure 6C and D).

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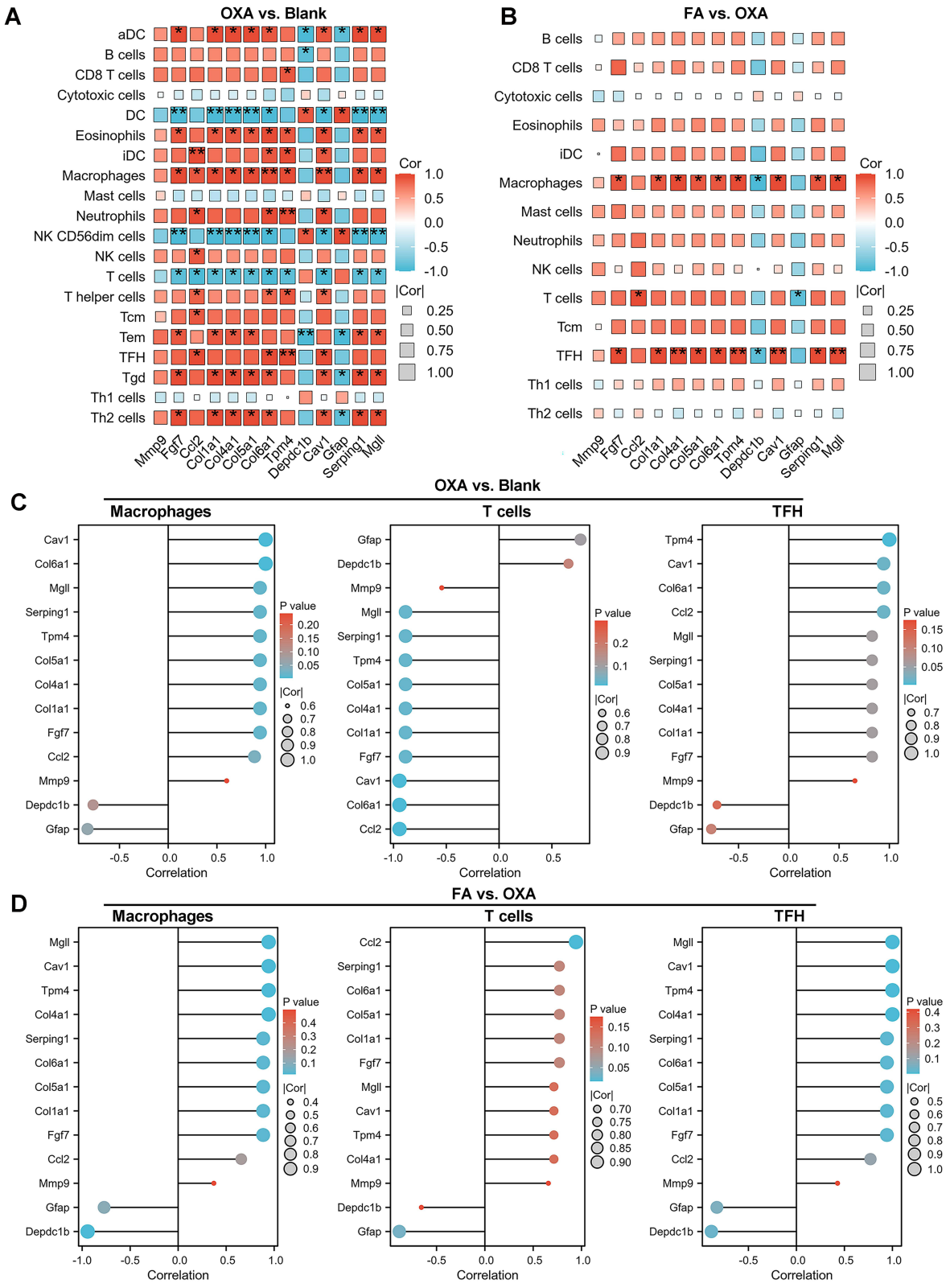

Figure 6 Investigating the relationship between Hub DE-genes and immune cell infiltration in L4–L6 spinal cord segments. (**A** and **B**) The relevance between Hub DE-genes and immune cell infiltration was analyzed based on the OXA vs Blank (**A**) and FA vs OXA (**B**) data. *P<0.05, **P<0.01. (**C** and **D**) The relationship between Hub DE-genes and macrophages, T cells, and TFH was analyzed based on the OXA vs Blank (**C**) and FA vs OXA (**D**) data.

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The Validation of the Expression of DE-Genes

We randomly selected nine genes for validation of expression and found that *Gfap* expression was significantly increased in the OXA group and decreased after FA treatment, whereas *Sfrp1, Wnt6, Rspo1, Wisp1, Gpc3, Ccl6, Ccr1*, and *Cxcl13* expression was significantly reduced in the OXA group and increased after FA treatment (Figure 7). The expression trends of all genes were consistent with the sequencing results.

Discussion

Presently, there has been a growing focus on CIPN due to its significant impact on both health and economic burdens. However, there is a lack of effective treatments specifically targeting CIPN.[27] This study has discovered that FA treatment can alleviate CIPN in rats and may represent a promising therapeutic strategy for for improving CIPN.

A total of 657 DE-mRNAs were identified as potentially involved in the ameliorative effect of FA on CIPN in rats. These are primarily associated with the ECM, inflammatory cell infiltration, and inflammation-related diseases, as well as ECM–receptor interactions and PI3K–Akt and IL-17 signaling pathways. ECM–receptor interactions, such as those with integrins, protect neurons from chemotherapeutic damage by modulating the interaction between neurons and the ECM.[28] Additionally, JTC-801 was found to mitigate paclitaxel-induced CIPN by modulating the PI3K/Akt pathway.[29] Inhibition of the PI3K/Akt/mTOR signaling pathway alleviates CIPN in rats and reduces glutaminase levels.[30] IL-17 secreted by dorsal root ganglion glial cells and astrocytes increases the excitability of primary sensory neurons via the IL-17 receptor, inducing mechanical allodynia, thereby driving CIPN.[31] This signaling pathway has been recognized as an important pathway in CIPN management. FA improves CIPN outcomes potentially by targeting ECM receptor interactions and the PI3K–Akt and IL-17 pathways.

Among 657 DE-mRNAs, three hub genes (*Mmp9, Fgf7*, and *Ccl2*) were identified. MMP9 exacerbates neuropathy by regulating ECM remodeling and inflammatory responses in CIPN.[32] Ccl2, commonly known as MCP-1, is a chemokine that facilitates the recruitment of monocytes and macrophages to areas experiencing inflammation. The upregulation of Ccl2 and its receptor Ccr2 in the dorsal root ganglion is involved in OXA-induced PWT reduction, thereby contributing to CIPN pathogenesis.[33] Meanwhile, Ccl2 can increase Mmp9 expression.[34] The function of Fgf7 in CIPN remains unclear. FGF7 is predominantly found in the neurons of the dorsal root ganglion and can be translocated to the dorsal spinal cord. FGF7 enhances the acute pain response triggered by formalin, indicating that it plays a role in the excitatory modulation of nociceptive afferent pathways.[35] In summary, Mmp9, Fgf7, and Ccl2 are integral to the pathophysiological mechanisms underlying CIPN. Sequencing results revealed that FA treatment reversed OXA-induced expression changes in Mmp9, Fgf7, and Ccl2, suggesting these genes may serve as key therapeutic targets for FA-mediated amelioration of CIPN. Future studies should employ genetic knockdown/overexpression approaches to determine whether FA's therapeutic effects on CIPN are mechanistically dependent on its regulation of Mmp9, Fgf7, and Ccl2 expression.

CircRNAs, lncRNAs, and miRNAs play crucial roles in the development of CIPN by regulating various signaling pathways and neuroinflammatory processes.[18–20,36] The study identified 656 DE-circRNAs, 192 DE-lncRNAs, and 15 DE-miRNAs, contributing to our understanding of the potential mechanism by which FA improves CIPN. Further, four key regulatory networks were identified: rno-miR-133a/b-3p-Tpm4/Col1a1/Col4a1/Col6a1, rno-miR-129-2-3p-Col5a1/Col6a1/Cav1/Serping1/Mgll, rno-miR-152-5p-Gfap, and rno-miR-224-5p/rno-miR-200a-3p/rno-miR-17-2-3p-Depdc1b. The overexpression of miR-133a-3p contributes to diabetes-induced neuropathic pain, peripheral nerve injury, and chronic constriction injury.[37–39] MiR-200a-3p overexpression attenuates neuropathic pain.[40] This study identified the regulatory networks that could play a role in CIPN progression, providing research directions for exploring the mechanisms of action of FA. However, the functional roles of these pathways in CIPN pathogenesis and FA-mediated therapeutic interventions are yet to be experimentally validated.

The effects of immune cells, glial cells, and neuroinflammation on CIPN are well-established.[41,42] TFH is associated with paclitaxel-induced peripheral neuropathy.[18] Microglia (macrophages), which are present in circulation or reside within the neuronal microenvironment, significantly influence the neuronal milieu and contribute to CIPN progression by releasing cytokines, chemokines, and neuroactive molecules. These substances can modulate neuronal signaling and pain perception, thereby playing a crucial role in CIPN development and progression.[43,44] PWT is significantly reduced in

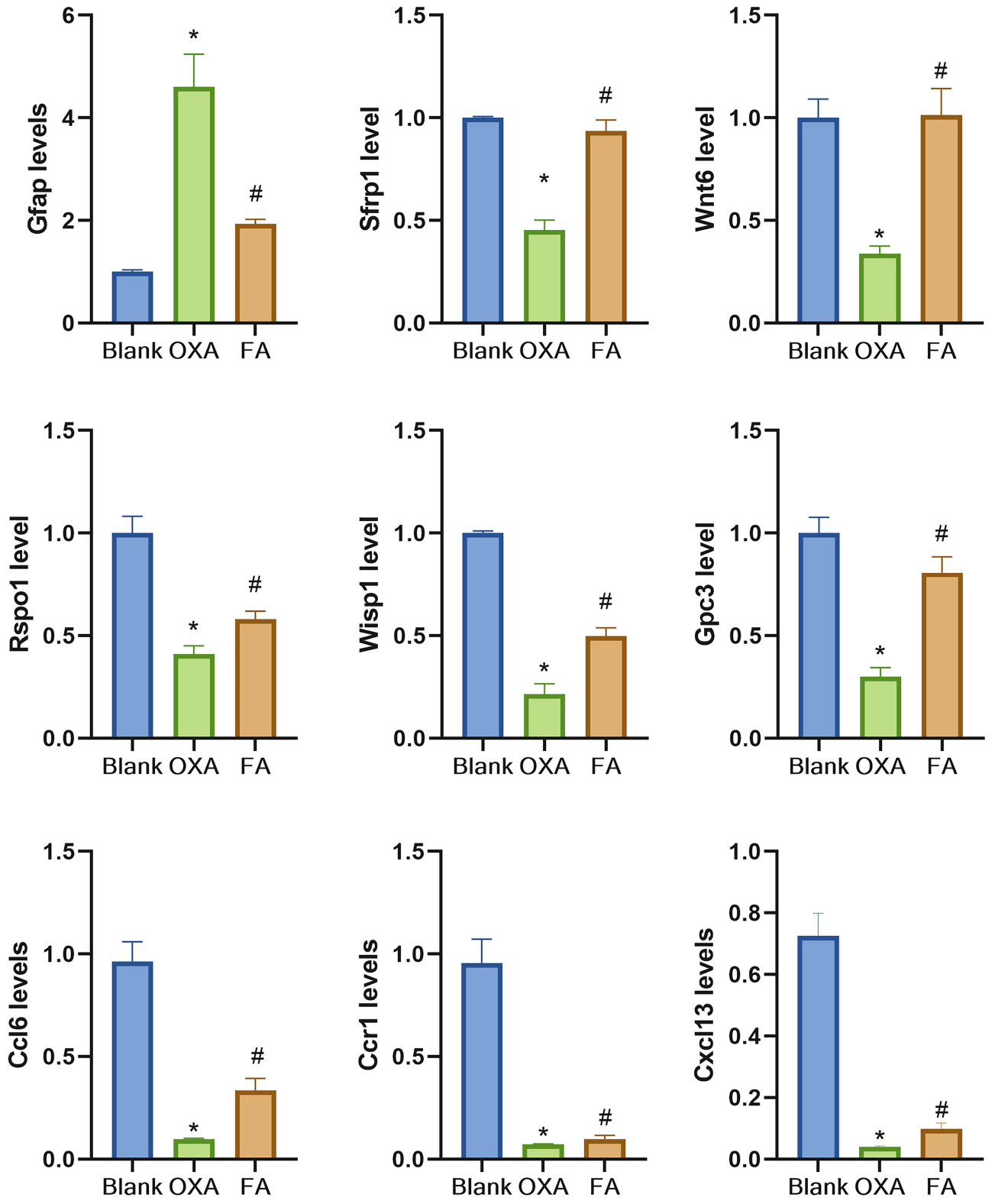

Figure 7 The expression level of DE-genes in L4–L6 spinal cord segments was measured using RT-qPCR after FA treatment. *P < 0.05, OXA vs Blank; #P < 0.05, FA vs OXA.

T cell–deficient mice.[45,46] Therefore, these cells were confirmed to be the drivers of CIPN. Consistently, this study found that in rats with CIPN, the Hub genes were related to macrophages, T cells, and TFH. Similarly, the amelioration of CIPN in rats by FA was related to macrophages, T cells, and TFH. Additionally, astrocyte activation contributes to the

progression of CIPN through the secretion of synaptogenesis-related proteins, such as SPARCL1 and Hevin.[47] This study found that GFAP, a marker of activated astrocytes, was upregulated in rats with CIPN and reduced following FA treatment indicating that these immune cells are closely associated with hub genes and may play a pivotal role in FA-mediated regulation of CIPN.

Nevertheless, this study has several limitations. First, the existence of the circRNAs/lncRNAs–miRNAs–mRNAs regulatory network requires further validation through luciferase reporter assays, RNA immunoprecipitation, and miRNA pull-down experiments. Additionally, the functional role of the circRNAs/lncRNAs–miRNAs–mRNAs regulatory network requires further validation via in vitro and in vivo experiments, and it needs to be investigated whether FA can ameliorate CIPN through this network. Furthermore, the absence of a sham FA control group limited our ability to distinguish between the specific therapeutic effects of acupuncture and non-specific factors. This design limitation suggests that the current findings may reflect both physiological regulatory effects and nonspecific responses. Future clinical studies should incorporate a sham FA control group to further validate the specific efficacy of acupuncture.

Conclusion

In this study, circRNAs/lncRNAs–miRNAs–mRNAs regulatory networks were found to be associated with the treatment of CIPN using FA. This study identified potential transcriptomic targets and pathways through which FA therapy alleviates CIPN, providing further directions for elucidating mechanisms through which FA improves CIPN.

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Disclosure

The authors report no conflicts of interest in this work.

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