PDCD4 Inhibition Alleviates Neuropathic Pain by regulating Spinal Autophagy and neuroinflammation

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Abstract

Neuropathic pain is still a clinical challenge. Inflammatory responses and autophagy in the spinal cord are important mechanisms for the occurrence and maintain of neuropathic pain. PDCD4 is an important molecule that regulates inflammatory responses and autophagy. However, the regulatory role of PDCD4 is unknown in pain modulation. In this study we found that the expression of PDCD4 in the spinal cord of CCI mice was increased. Inhibition of PDCD4 by intrathecal injection of adeno-associated virus alleviated neuropathic pain and enhanced autophagy in CCI mice, and inhibited the activation of MAPK pathway and glia, as well as the expression of inflammatory factors. Intrathecal injection of autophagy inhibitor 3-MA reversed PDCD4 inhibition induced pain relief and change of autophagy. Our results indicate that spinal cord inhibition of PDCD4 alleviates pain sensitization in neuropathic pain mice, and PDCD4 may be developed into a therapeutic target.

Introduction

Neuropathic pain (NP), a chronic pain condition, arises from damage or injurious stimulation to the somatosensory nervous system, and can also be caused directly by diseases\(^1,2\). NP can affect various parts of the nervous system, encompassing a clinical syndrome that includes types of pain such as allodynia, hyperalgesia, and spontaneous pain\(^3\). Patients with neuropathic pain often experience a range of distinct symptoms, such as burning and electrical sensations, as well as pain triggered by non-painful stimuli like light touch\(^4\). When these symptoms persist, neuropathic pain transform into chronic pain. People with neuropathic pain commonly suffer from sleep disturbances, anxiety, depression, and other conditions, severely impacting their quality of life\(^5\). Due to the complex etiology and mechanisms involved, the specific mechanism of neuropathic pain remains incompletely understood. Currently, traditional clinical treatment options primarily consist of antidepressants, antiepileptic drugs, opioid receptor agonists, and nonsteroidal anti-inflammatory drugs. However, the efficacy of pharmacological treatments is finite, and the toxic side effects associated with these medications impose certain restrictions on their clinical application\(^6,7\). Therefore, it is particularly important to investigate the mechanisms underlying the occurrence and development of neuropathic pain.

PDCD4, a tumor suppressor and protein translation inhibitor, exerts its effects by binding to translation initiation factor eIF4A, transcription factors, and other binding factors. PDCD4 inhibits cell growth, promotes apoptosis, and suppresses tumor invasion and metastasis\(^8,9\). As a cancer-inhibiting protein, PDCD4 is involved in the occurrence and development of tumors\(^10\). Recent years, numerous studies have reported the role of PDCD4 in other diseases such as polycystic ovary syndrome, obesity, diabetes, and coronary artery atherosclerosis\(^11-14\). PDCD4 also plays a crucial regulatory role in inflammation\(^15\). For instance, in pdc4d gene knockout mice, the JNK and NF-κB signaling pathways were activated, leading to elevated expression of TNF-α and IL-6\(^16\). Additionally, PDCD4 expression is increased in LPS-induced inflammation of central nervous system\(^17\). Based on the aforementioned evidence, PDCD4 may be an important molecule involved in regulating inflammatory responses of pain modulation.
However, there are few reports on the role of PDCD4 in pain regulation. A previous study reported elevated PDCD4 expression in the spinal cord of CCI mice, which is regulated by miR-330-3p, but the behind mechanisms remain unclear[18].

Autophagy refers to the process in which abnormal synthesized or misfolded proteins, as well as dysfunctional organelles and other substances within the cell, are enveloped by double-membrane structures and ultimately transported to lysosomes for degradation[19]. Autophagy is crucial for maintaining cellular homeostasis. Increasing evidence suggests that dysregulation of autophagy may be associated with the development of various diseases, in addition to its role in maintaining physiological states[20–22]. Studies have found that autophagy in spinal cord is involved in pain regulation, with weakened autophagy in chronic pain and relief of pain with autophagy inducer rapamycin[23, 24]. Previous research has identified PDCD4 as an important molecule in regulating autophagy. PDCD4 regulates the autophagy process by modulating the translation of autophagy-related proteins and is involved in the regulation of processes such as tumor development and epilepsy[25, 26]. However, whether PDCD4 participates in the regulation autophagy in chronic pain modulation has not been reported yet. This study aims to delve into the spinal mechanisms by which PDCD4 regulates neuropathic pain, providing a theoretical basis for the treatment of neuropathic pain.

**Methods and materials**

**Animals**

C57BL/6 male mice (8 weeks old, weighing 21–25 g) were obtained from Xuzhou Medical University (SYXK 2016-0028). All mice were housed in groups of 4–6 individuals per cage in a standard condition with access to food and water ad libitum in a colony room kept at 19–22°C and 40–60% humidity, under a 12-h light/dark cycle (light from 08:00 to 20:00). The animals were acclimatized for 7 days before the experiments and were group-housed with the same cage mates throughout the acclimation and experiment. The study protocol was approved by the Ethics Committee of Xuzhou Medical University and all procedures were performed in accordance with the approved guidelines. All experiments were conducted in compliance with the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines.

**Intrathecal injection**

The drugs injected in this study were prepared as follows. After anesthesia, mice were bent at the waist. The 3-Methyladenine (3-MA) was dissolved in 2% dimethyl sulfoxide in saline (autophagy inhibitor; MCE, HY-19312, 5 µg/d/mouse) and intrathecally administered 10 µl on postoperative day 7, 8 and 9 during the stage of neuropathic pain. pAAV-U6-shRNA (Pdcd4)-CMV-EGFP-WPRE was designed and manufactured by Obio. Ltd. (Shanghai, China). pAAV-U6-shRNA (Pdcd4)-CMV-EGFP-WPRE and Adeno-associated virus (AVV) vector was injected into the cerebrospinal fluid through the L5-L6 lumbar intervertebral space using a 10 µL microsyringe. The accuracy of each injection was assessed by observing a reflexive flick of the tail.
Enzyme-linked immunosorbent assay (ELISA)

The concentrations of IL-1β, TNF-α, IL-6 levels in spinal cord tissues measured by ELISA Kits (KE10007 for IL-6 and KE10002 for TNF-α, Proteintech; HA722166 for IL-1β, Huabio). Spinal cord tissues were homogenized with lysis buffer including the protease inhibitor. ELISAs were performed and the standard curve was depicted according to the manufacturer's protocol.

Pain behavioral quantification

Thermal hyperalgesia was assessed by measuring the paw withdrawal latency (PWL) in response to a radiant heat stimulation. A Plantar Analgesia Meter (IITC Life Science Inc., CA, USA) was used as a radiant source. Briefly, mice were placed individually in perspex observation chambers on an elevated glass platform, and a radiant heat source was applied to the glabrous surface of the paw through the glass plate. The nociceptive endpoints included the characteristic lifting or licking of the hind paw. The heat was maintained constant intensity, which produced a stable PWL of 12–14 s in normal animals. A 20-s cutoff was used to prevent tissue damage. After acclimation to the test chambers, both hind paws were tested independently with 3-min intervals between trials.

Paw withdrawal threshold was measured in response to von Frey filament stimulation (Aesthesio, Danmic Global, San Jose, CA, USA) to determine the presence of mechanical allodynia. Briefly, following 3 consecutive days of acclimatization, the mouse was kept on a wire net floor in a plexiglass chamber and allowed to familiarize for 10–15 min before the experiment is initiated. The mice's mid-plantar surface of the hind paw was applied with a series of filaments (0.4, 0.6, 1.4, 2, 4, 6, 8, 10, and 15 g) with sustaining pressure to elicit a paw withdrawal reflex or to bend the filament for 5 s within 5 s. Application of each of the filaments was done five times, followed by the calculation of the 50% threshold (g) employing the formula: maximum bending force value [(maximum bending force value minimum bending force value) (positive rate of the maximum bending force - positive rate of the minimum bending force)] (positive rate of the maximum bending force 50%).

Immunofluorescence

Under deep anesthesia, the L4-5 spinal segment of mice was dissected and post-fixed in 4% PFA for 3 hours, and subsequently transferred into Tris-buffered saline (TBS) containing 30% sucrose and conserved at 4°C until use. The spinal sections were transversely cut 25 µm thick in a cryostat and stored in TBS. The sections were first blocked with 5% donkey serum in 0.3% Triton X-100 for one hour at room temperature and then incubated overnight at 4°C with the following primary antibodies: rabbit anti-PDCD4 polyclonal antibody (1:500, 9535S, CST, USA), rabbit anti-p-ERK polyclonal antibody (1:100, 28733-1-AP, Huabio), rabbit anti-p-JNK polyclonal antibody (1:400, 80024-1-RR, Huabio), rabbit anti-p-p38MAPK polyclonal antibody (1:200, 28796-1-AP, Huabio). The sections were rinsed 3 times for 10 min in TBS and then incubated for one hour at room temperature with corresponding secondary antibodies (conjugated to Alexa Fluor 488 or 594, Invitrogen, Carlsbad, CA, USA) overnight at 4°C. For double immunostaining, the stained sections were incubated secondly with the same immunostaining
procedure described above. The immunofluorescent images were captured with a confocal scanning laser microscope (FluoView FV1000, Olympus Co., Tokyo, Japan). For obtaining the semiquantitative determination of c-Fos and p-CREB fluorescence, 20 fields spreading the whole section of dorsal horn in each group were scanned and analyzed at the same exposure time to generate the data of green fluorescence intensity.

**Western blot**

The dorsal horn of the L4-5 spinal cords were quickly removed from mice under deep anesthesia, then the tissue samples were dissected and stored in liquid nitrogen. Then equivalent amounts of protein (80 µg) were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked with 5% non-fat milk or BSA at room temperature for 2 h followed by overnight incubation with the following primary antibodies at 4 °C: rabbit polyclonal anti-PDCD4 antibody (1:500, 9535S, CST, USA), Beclin1 (1:200, HA721216, Huabio, China), p62 (1:500, HA72117, Huabio, China) and glyceraldehyde-3-phosphate dehydrogenase (1:10000, GAPDH, G9545, Sigma, USA), respectively. After washing with TBST 3 times, the blots were incubated with HRP conjugated secondary antibody and detected in SuperLumia ECL solution (MedChemExpress, Monmouth Junction, NJ, USA). Band intensity was quantified using Quantity One Analysis Software (Version 4.6.5, Bio-Rad Laboratories, Hercules, CA, USA). The target protein was expressed as the fold change of the control group after normalization against GAPDH. The fold change of the control group was set as 1 for quantifications.

**Statistical analyses**

GraphPad Prism 9 (GraphPad Software Inc., CA, USA) was used to conduct all statistical analyses. Alterations of detected protein expression were tested using one-way ANOVA with repeated measures, followed by the Dunnett multiple comparison test, and changes of behavioral response to radiant heat stimuli over time among groups were tested using two-way ANOVA with repeated measures, followed by Bonferroni post hoc test. All data are expressed as means ± SEM. Statistical differences were considered significant if P<0.05.

**Results**

**1. Expression and distribution of PDCD4 in the spinal cord of CCI mice**

We first examined the expression of PDCD4 in the spinal cord of CCI mice at different time points after surgery. Western blot results revealed a significant increase in PDCD4 expression at 3, 7–14 and 21 days post-CCI (Fig.1 A). To determine the cellular localization of PDCD4 in the spinal cord of CCI mice, we performed immunofluorescence co-staining of PDCD4 with neuronal marker NeuN, astrocyte marker GFAP, and microglia marker IBA1. The immunofluorescence results demonstrated predominant expression of PDCD4 in spinal cord neurons and microglia, with minimal expression observed in astrocytes (Fig.1 B).
2. The impact of intrathecal adenovirus-mediated PDCD4 inhibition on behavioral outcomes in CCI mice.

Previous investigations have revealed an upregulation of PDCD4 expression in the spinal cord of CCI mice. In this part, we employed an innovative tool, AAV-shPDCD4, to suppress the expression of PDCD4. The construction of AAV-encoded shPDCD4-EGFP or control EGFP was shown in Fig. 2A. Immunofluorescence staining results showed the expression of GFP in the spinal cord dorsal horn (Fig. 2B). Then, the expression of PDCD4 following AAV-shPDCD4-EGFP and AAV-EGFP delivery has been examined in the spinal cord (Fig. 2C). Results showed that PDCD4 protein was significantly suppressed by AAV-encoded shPDCD4 EGFP in the spinal cord compared with the AAV-control EGFP group. There was no significant difference in pain behaviors among the groups before or after shPDCD4-EGFP or control injection. Timecourse of pain behaviors showed that PDCD4 inhibition had a significant inhibitory effect on the PWL (Fig. 3B) and PWT (Fig. 3C) evaluation revealed that mechanical allodynia and thermal allosthesia were attenuated in AAV-shPDCD4-EGFP treated mice at all time points after CCI compared with AAV-control-treated mice. However, there was no significant change in pain behaviors in the AAV-control group during the whole experiment. These results revealed that inhibition of PDCD4 in the spinal cord is responsible for pain relief induced by CCI.

3. The influence of intrathecal AAV-shPDCD4 injection on spinal cord autophagy in CCI mice

Enhanced autophagy has been shown to alleviate neuropathic pain in mice\(^{[27]}\). PDCD4 is an important molecule in regulating autophagy, and its regulation is involved in various physiological and pathological processes, such as gastric cancer, endometriosis, and pancreatic cancer\(^{[28-30]}\). Our findings thus far suggested that PDCD4 regulated neuropathic pain in CCI mice. To uncover the underlying mechanisms, here we investigated the relationship between PDCD4 and autophagy in neuropathic pain regulation. As shown in Fig. 4, western blot results indicated that intrathecal AAV-shPDCD4 injection significantly enhanced the expression of Beclin1 and LC3B, while reducing the expression of p62. These results suggested that PDCD4 may participate in neuropathic pain regulation through the modulation of autophagy.

4. The impact of intrathecal AAV-shPDCD4 injection on MAPK pathway activation

Studies have demonstrated that PDCD4 is a crucial regulator of inflammatory responses, and the MAPK pathway plays a pivotal role in regulating neuroinflammation\(^{[31]}\). Previous researches have indicated that PDCD4 modulated the MAPK pathway in processes such as tumor development and fulminant hepatic failure\(^{[32, 33]}\). In this study, we examined the effects of PDCD4 inhibition on MAPK pathway activation. Immunofluorescence results indicated that intrathecal AAV-shPDCD4 injection suppressed the expression of p-ERK, p-JNK, and p-p38MAPK in the spinal cord after CCI (Fig. 5). These findings suggested that PDCD4 may regulate neuropathic pain through the modulation of the MAPK pathway activation.

5. The impact of intrathecal AAV-shPDCD4 injection on glial activation and neuroinflammation
PDCD4 plays an important role in regulating neuroinflammation, studies have shown that PDCD4 was upregulated in the cerebral cortex of LPS induced neuroinflammation, and inhibition of PDCD4 inhibits microglial activation through the MAPK pathway\cite{17}. Glial activation is one of the important manifestations of neuroinflammation\cite{34}. Given the important regulatory role of PDCD4 in neuroinflammation, we investigated the effect of PDCD4 inhibition on glial activation. As shown in Figure 6, the expression of activated markers GFAP and IBA1 in astrocytes and microglia is inhibited by adeno-associated virus inhibition of PDCD4 within the subarachnoid space. At the same time, we also tested the expression of inflammatory factors, ELISA results further demonstrated a reduction in the expression of inflammatory factors IL-6, IL-1β, and TNF-α following AAV-mediated PDCD4 inhibition (Fig.5 B-D). These findings suggested that PDCD4 may regulate neuropathic pain through the modulation of the activation of astrocytes and microglia and neuroinflammation.

6. Autophagy activation was required for PDCD4 inhibition induced analgesia

Above studies have confirmed that inhibiting spinal cord PDCD4 alleviated pain hypersensitivity in CCI mice, possibly through activation of autophagy. To further clarify the regulatory relationship between PDCD4 and autophagy, we confirmed it through the following experiments. Two weeks following direct injection of AAV-shPDCD4, the mice were established neuropathic pain model by CCI. To assess the contribution of autophagy in the spinal cord of CCI mice, an autophagy inhibitor 3-MA (i.t., 10μL, 5 μg/d/mouse) or vehicle (10 μL) was given within the sheath once daily from day 7 to day 9 after CCI (Fig. 7A). As shown in Fig. 7 B-C, treatment with 3-MA markedly reversed the analgesic effect of PDCD4 inhibition, the change of p62, LC3B and Becline1 induced by PDCD4 inhibition in CCI mice were also reversed by 3-MA (Fig.7 D-F). Moreover, the reduction of inflammatory cytokines was reversed by 3-MA as well (Fig.7 G-I). These results going further suggested PDCD4 may regulated neuropathic pain via autophagy.

Discussion

Neuropathic pain remains a challenge in clinical practice, and its underlying mechanisms are not yet fully understood. In this study, we discovered the role of spinal dorsal horn PDCD4 in regulating neuropathic pain. (1) CCI induces upregulation of PDCD4 expression in spinal dorsal horn neurons and microglia; (2) Inhibiting PDCD alleviates pain hyperalgesia in CCI mice; (3) PDCD4 regulates neuropathic pain through autophagy; (4) PDCD4 regulates neuropathic pain by regulating the MAPK and glial activation.

In our study, we find an elevation of PDCD4 expression in the spinal cord of CCI mice, which started on postoperative day 3 and persisted until day 21, consistent with previous research\cite{18}. Further investigation reveals the expression of PDCD4 is in neurons and microglia in the spinal dorsal horn. Notably, inhibition of PDCD4 using AAV-shPDCD4 significantly attenuates pain hypersensitivity in CCI mice, highlighting the significance of PDCD4 as a key molecule in regulating neuropathic pain.
Autophagy serves as an immune defense mechanism by which the body combats external stimuli. It is a lysosome-mediated process that involves the degradation of cellular organelles, proteins, and RNA. This cellular response mechanism acts as a resistance mechanism against external survival pressures and plays a crucial role in maintaining cellular substance and energy balance. Morphologically, it is characterized by the appearance of autophagosomes in the cytoplasm, which subsequently fuse with lysosomes for degradation. Dysregulation of cellular autophagy has been implicated in the occurrence and progression of neuropathic pain. Activation of cellular autophagy has been shown to effectively alleviate neuropathic pain. These studies indicate autophagy plays an important role in pain regulation.

PDCD4, an important molecule involved in autophagy regulation, plays an important role in various physiological and pathological processes. In this study, it was observed that the expression of LC3B, Beclin-1 and p62 were increased in the spinal cord of CCI mice. However, inhibiting PDCD4 expression through AAV delivery increases the expression of Beclin-1 and LC3B, suppressed the expression of p62, indicating that PDCD4 regulates neuropathic pain in mice through autophagy.

Emerging lines of evidence indicated central sensitization through neuronal and non-neuronal immune cells centrally plays a central role in the initiation and maintenance of chronic pain processing. Spinal neuroinflammation is a crucial mechanism underlying chronic pain. In chronic pain models, activation of spinal glial cells leads to the release of various inflammatory mediators, which bind to receptors on neurons/glial cells, initiating intracellular signaling and amplifying pain signals, ultimately resulting in central sensitization. Previous studies have suggested a possible link between nociceptive behavior and glial activation after CCI. Moreover, glial activation could secrete glial mediators, including pro-inflammatory cytokines, which could enhance neuronal hyperactivity to further facilitate pain in the spinal cord of CCI mice. Recent studies have found that PDCD4 plays a regulatory role in LPS-induced neuroinflammation. In this study, it was found that PDCD4 is expressed in spinal neurons and microglia of CCI mice. Inhibiting PDCD4 through AAV delivery reduces the expression of inflammatory cytokines, and suppresses the activation of astrocytes and microglia, suggesting that PDCD4 regulates neuroinflammation in CCI mice.

The MAPK pathway is a crucial signaling pathway involved in the regulation of chronic pain and neuroinflammation. Spinal MAPK pathway was activated in chronic pain procession. Previous studies have shown that PDCD4 regulates MAPK pathway, which are implicated in the pathogenesis of tumors and other diseases. In this study, p-ERK, p-JNK, and p-p38MAPK are upregulated in spinal cord of CCI mice, inhibiting PDCD4 suppressed the expression of the phosphorylation and activation of all three substreams of the MAPK signaling including ERK, JNK, and p38MAPK, indicating that PDCD4 is involved in the regulation of neuropathic pain in mice through the modulation of the MAPKs.

In conclusion, our study reveals the significant role of PDCD4 in the regulation of neuropathic pain in mice. In the future, PDCD4 may emerge as an important therapeutic target for the treatment of...
neuropathic pain.

**Declarations**

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**Author contributions**

Ting Zhang designed and performed the experiments, Le Qi and Kai Sun analyzed the data, and wrote manuscript. Xiang Huan and Hao Zhang provided animals. Meiyan Zhou and Liwei Wang coordinated the study, interpreted the data, and wrote manuscript. All authors read and approved the final manuscript.

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**Data availability**

The data used in the study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate.** All procedures regarding the care and use of animals were approved by the ethics committee of Xuzhou Medical University of China. All methods were performed in accordance with approved guidelines.

**Consent for publication**

Not applicable.

**Competing interests**

The authors have declared that no competing interests exist.

**References**


Figure 1

Expression and distribution of PDCD4 in spinal cord of CCI mice.

A: WB showed time-course of PDCD4 in the spinal cord of CCI mice. (**P < 0.01 compared with the sham group, n = 6 per group).

Expression of PDCD4 (PDCD4/GAPDH)
B: Distribution of PDCD4 in the spinal cord dorsal horn following CCI. PDCD4 was colocalized mostly with neurons (NeuN), and a minority with microglia (IBA1), but not astrocytes (GFAP) in the spinal cord dorsal horn. (n = 6 per group, scale bar = 100 μm).

Figure 2

Effects of intra-spinal injection of AAV encoded shPDCD4 (AAV-shPDCD4) on the expression of PDCD4 protein.

(A) Schematics of AAV constructs shPDCD4. CMV, cytomegalovirus promoter; EGFP, enhanced GFP; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element.

(B) EGFP immunoreactivity was detected in the superficial laminae of the dorsal horn at 4 weeks after AAV-shPDCD4 (5.0 \times 10^{12} \text{v.g/mL}) injection.

(C) Representative bands for the expression of PDCD4 in the spinal cord of mice at 4 weeks after intrathecal injection of AAV-shPDCD4 or AAV-control. (**P < 0.01 compared with the indicated group, n = 6 per group).
Figure 3

Effects of PDCD4 inhibition on existing hyperalgesia after CCI.

A Schematic timeline for drug administration, behavioral detection, and tissue extraction.

(B-C) Analgesic effects of i.t. AAV-shPDCD4 on thermal hyperalgesia (PWL) and mechanical hyperalgesia (PWT) in ipsilateral to CCI. (**P < 0.01 versus sham+AAV-control group. *P < 0.05, ###P < 0.001 versus CCI+AAV-control, n=8 per group).
Figure 4

The effects of PDCD4 inhibition on autophagy.

A-C: Beclin1 and LC3B were upregulated and p62 was downregulated after AAV-shPDCD4 intrathecal injection in CCI mice. (*P < 0.05, **P < 0.01, ***P < 0.001 compared with CCI+AAV-control; #P < 0.05, ##P < 0.01, ###P < 0.001 compared with Sham+AAV-control, n = 4 per group).
Figure 5

The effects of PDCD4 inhibition on MAPK activation

Immunofluorescence showed the expression of p-ERK, p-JNK and p-p38MAPK were inhibited by intrathecal injection of AAV-shPDCD4 in the L4-6 spinal cord (n = 3 per group, scale bar = 100 μm).
Figure 6

The effects of PDCD4 inhibition on glial activation and inflammatory factors

A. The expression of GFAP and IBA1 in the L4-6 spinal cord were detected inhibited by intrathecal injection of AAV-shPDCD4 in the L4-6 spinal cord (n = 3 per group, scale bar = 100 μm).

B-D. ELISA data showed the expression of pro-inflammatory cytokines in the L4-6 spinal cord were downregulated by intrathecal injection of AAV-shPDCD4 after CCI (**P < 0.01, ***P < 0.001 compared with the sham group, ##P<0.01 compared with CCI-AAV-control group, n = 3 per group).
Figure 7

Effect of the autophagy inhibitor 3-MA on pain-related behaviors, autophagy and inflammatory cytokines following AAV-shPDCD4 injection.

A: A schematic timeline for drug administration, behavioral detection, and tissue extraction.
B-C: Intrathecal injection of autophagy inhibitor 3-MA markedly reversed the analgesic effect of PDCD4 inhibition.

D-F: Intrathecal injection of autophagy inhibitor 3-MA suppressed the autophagy activation induced by PDCD4 inhibition.

G-I: Intrathecal injection of autophagy inhibitor 3-MA markedly reversed the suppression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) by PDCD4 inhibition. (**P < 0.01, ***P < 0.001 compared with the CCI+AAV-shPDCD4 group, n = 4 per group).