Propofol Alleviates Neuropathic Pain in CCI Rat Model via the miR-140-3p/JAG1/Notch Signaling Pathway

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Research Article

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Abstract

As a renowned anesthetic, propofol exerts excellent analgesic function in nerve injury. However, the underlying mechanism of propofol on neuropathic pain (NP) remains unknown. The research aims to analyze propofol's analgesia mechanism to alleviate NP in CCI rats. The chronic constriction injury (CCI) of sciatic nerve was used to established NP rat models. CCI rats were treated with propofol and its paw withdrawal mechanical threshold (PMWT) and paw withdraw thermal latency (PWTL) were measured. The expressions of TNF-α, IL-1β and IL-10 were detected. CCI rats with propofol treatment were injected with antagomiR-140-3p. After the targeting relationship between miR-140-3p and JAG1 was checked, JAG1 expression was detected. Propofol-treated CCI rats were further injected with Ad-JAG1. Finally, the levels of JAG1 and Notch pathway-related proteins were detected. As a result, propofol could alleviate NP, including thermal hyperalgesia and mechanical pain threshold, and ameliorate neuroinflammation. Mechanically, propofol enhanced the level of miR-140-3p in CCI rats. JAG1 was a direct target of miR-140-3p. The downregulation of miR-140-3p or upregulation of JAG1 could reduce the protective effect of propofol against NP. Propofol inhibited activation of Notch signaling via miR-140-3p/JAG1. Overall, Propofol could inhibit the neuroinflammation and Notch signaling pathway via miR-140-3p/JAG1 to alleviate NP.

Introduction

Neuropathic pain (NP) refers to a chronic pain originating from pathology of somatosensory system (Gierthmuhlen et al. 2016). NP is categorized into central type leading to symptoms including spinal cord injury and central lesion, and peripheral type leading to nerve injury (Basu et al. 2020). The crux of novel therapies to NP needs to be solved by the analysis of analgesia success (St John Smith 2018). As a fast-acting intravenous anesthetic and sedative agent, propofol is utilized by hospital for its effectively anesthetic and sedative function in surgical procedures and intensive care for its effects to reduce cerebral blood, cerebral metabolism and intracranial pressure (Folino et al. 2021; Guo et al. 2020). It has been widely applied to the treatment of craniocerebral and other neuronal injuries and demonstrated to relieve NP by prolonged paw withdrawal mechanical threshold (PMWT) and paw withdraw thermal latency (PWTL) of chronic constriction injury (CCI) rats (Jiang et al. 2021). However, the discussions on underlying mechanism of propofol to relieve NP are insufficient and unsatisfied.

MicroRNAs (miRNAs) are deemed as a class of small noncoding RNAs and could negatively regulate the level of mRNAs (Saliminejad et al. 2019). With short ribonucleic acids in chemical structure, miRNAs are utilized to develop medicines for NP treatment (Tang et al. 2021). According to the literature, miR-140 was reported to ameliorate NP and neuroinflammation in CCI rats (Li et al. 2020b). Especially, the expression of miR-140-3p was found to be reduced in the tissue of patients and mice with rheumatoid arthritis (RA) (Zu et al. 2021). Propofol regulates the expression of miRNAs for the treatment of many diseases, including cancers and myocardial damage (Li et al. 2019; Xu et al. 2020). However, whether propofol could regulate miR-140-3p in NP condition and the correlation between miR-140-3p and NP needs to be further analyzed and validated.

Furthermore, miRNAs controlled by propofol continues to regulate the expression of Jagged-1 peptide (JAG1) in NP condition (Li et al. 2020a). Moreover, JAG1, a ligand of notch signaling pathway is associated with the many pain models. For instance, the expression of JAG1 was boosted in the condition of degenerated discs which causes low back pain (Li 2020a). Especially, JAG1 could aggravate NP by activating the Notch signaling (Xie et al. 2015b). Notch signaling is reported to possess importance in many biological processes, including cell proliferation, cell death and differentiation (Arumugam et al. 2018). Previous research has confirmed that the expression of Notch signaling is augmented and activated after NP (Duan et al. 2021). From all above information, we hypothesized that propofol could regulate the expression of miR-140-3p and JAG1 to play a role in alleviating NP, and the paper aims to figure out the underlying mechanism of propofol to relieve NP and seek a novel therapeutic target of NP.

Materials And Methods

Experimental animals

Healthy male Sprague-Dawley rats were bought from Southern Medical University, Guangzhou, China (Approval No: SCXK(Guangdong) 2016-0041) and raised at 23 ± 2℃ and 55 ± 5% humility under a 12-h light/dark cycle. All rats can freely eat food and drink. The treatment of animals in experiments was in accordance to the standards of animal ethics.

CCI rat model establishment

AntagomiR-140-3p and antagomiR-NC (negative control) were purchased from GenScript Biotech Company (Nanjing, Jiangsu, China), and construction of adenovirus plasmids Ad-JAG1 and Ad-NC (empty plasmids without the target gene) were constructed and packaged by Vector Gene Technology Company Ltd. (Beijing, China). Rats were injected via caudal veins for consecutive three days before modeling (Antago: 40 μg/g; Ad-JAG1 and Ad-NC: $5{\cdot}10^9$ pfu).

The CCI method was applied to establish a rat model (Bennett et al. 1988). Briefly, after anesthesia by intraperitoneal injection of 1% sodium pentobarbital (50 mg/kg), sciatic nerves of rat right hind limbs were exposed. Next, the sciatic nerve was ligated with a 4 − 0 catgut into 4 consecutive channels with an interval of 1.0 mm. Sight depression of the nerve is appropriate for ligation intensity and the blood supply of sciatic nerve was not blocked when observed under the microscope. After surgery, the muscles and skin were seamed layer by layer and disinfected using iodophor, and each rat was raised in a single cage. A group of rats were subjected to sham surgery, in which sciatic nerves were exposed but not ligated. Another group of rats were intraperitoneally injected with propofol (50 mg/kg) 12 h after surgery for 7 consecutive days. Then, the rats were assigned into 7 groups with 6 rats per group: sham group (sciatic nerves were exposed but not ligated), CCI group (SD rats with CCI surgery), CCI + Pro group (CCI rats were injected with propofol), CCI + Pro + antago-NC group (CCI rats were injected with propofol and antagomiR-NC), CCI + Pro + antagomir group (CCI rats were injected with propofol and antagomiR-140-3p), CCI + Pro + Ad-NC group (CCI rats were injected with propofol and Ad-NC), and CCI + Pro + Ad-JAG1 group (CCI rats

were injected with propofol and Ad-JAG1). At 14 days post modelling, ipsilateral dorsal root ganglions were taken for protein RNA extraction.

PWMT

Each group of rats was placed in cages in a quiet environment 10 min before the experiment. Then, von Frey filaments (4, 6, 8, 10, 15, 26, 60, and 100 g) from the bottom of the cage were used to stimulate the left foot of the rat. The experiment was stopped when a positive reaction appeared. The von Frey stimulation test for each rat was repeated 5 times. If a rat lifted or licked its paw 3 or more times under the filament stimulation, the result was regarded as positive; otherwise, it was negative. The force that triggered a positive reaction was called PWMT (He et al. 2010; Wang et al. 2020). The test was finished when a positive reaction was observed. There was an interval of 5-min rest between each test and the test was performed 3 times to get the mean value as the final result. If a positive reaction arose from a von Frey filament with a force of less than 100 g, the PWMT of the rat was considered 100 g. Adaptability training was performed one week in advance, and the PWMT of each rat was assessed 1 d before modelling and 1, 3, 7, and 14 d after modelling.

PWTL

The hot pad apparatus was preheated to 55℃. The rat was locked in a cage under 25℃ and a timer was started simultaneously. The time when the rat attempted to lift or lick its foot was recorded was PWTL (Shi et al. 2016; Wang 2020). There was an interval of 5 min between each test and the test was performed 3 times. The average time was considered as PWTL, referring to the rat's reaction time to thermal stimulation. The experimental environment remained quiet to keep the rat free of scare. To avoid burning rats, a cutoff time was set to 20 s. Adaptability training was performed one week in advance, and PWMT of each rat was assessed 1 d before modelling and 1, 3, 7, and 14 d after modelling.

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits were utilized to detect the expression of tumor necrosis factor-α (TNF-α) (ab11564, Abcam, Cambridge, MA, USA), interleukin-1β (IL-1β) (ab255730, Abcam) and interleukin-10 (IL-10) (ab214566, Abcam). All procedures were followed by the instructions. In short, samples to be tested (containing antibodies) was connected with the antigen, forming a compound. Then, the labeled enzyme was combined with the compound, forming an antigen-antibody-labeled enzyme compound. Finally, the substrate of the enzyme was added to produce a colored product, and the value of optical density was measured by spectrophotometer.

Quantitative real-time polymerase chain reaction (qRT-PCR) assay

According to manufacturer's instruction, TRIzol (Takara, Tokyo, Japan) was used to extract total RNA from dorsal root ganglion. Next, 500 ng total RNA was added to synthesize complementary DNA (cDNA) in the first strand cDNA synthetic reaction using the PrimeScript RT Teagent kit. CFX 96 QPCR was used

for reverse transcription PCR. SYBR RT-PCR kit was applied for qRT-PCR assay. The internal reference set for PCR was GAPDH or U6. *The* $2^{-\triangle\triangle ct}$ method was employed to calculate the relative expression of miR-140-3p and JAG1. The test of each sample was repeated 3 times independently. The primers of PCR were shown in Table 1.

Table 1
Primer sequence information

Name of primer	Sequences
miR-140-3p-F	GGGCTACCACAGGGTAGAA
miR-140-3p-R	GTGCAGGGTCCGAGGT
JAG1-F	ATGCGGTCCCCACGGACGCGC
JAG1-R	GCAGGGCGAGCAGAAGGCTCA
GAPDH-F	CAAGGTCATCCATGACAACTTTG
GAPDH-R	GTCCACCACCCTGTTGCTGTAG
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT

Dual-luciferase assay

miR-140-3p and JAG1 specific binding sites were analyzed through TargetScan website (http://www.targetscan.org/vert_72/). The bound and mutated sequences were cloned into luciferase reporter vector pGL3 (Promega, Madison, WI, USA) respectively to produce the wild-type luciferase reporter plasmids (JAG1-wt) and mutant reporter plasmids (JAG1-mut). The 293T transfected cells (ATCC, Manassas, VA, USA) were seeded into 6-well plates (2×10^5 cells/well). According to the instructions of Lipofectamine 2000 (11668-019, Invitrogen, Carlsbad, CA, USA), 293T cells were transfected with constructed luciferase reporter plasmids and inhibitor-NC or miR-140-3p inhibitor (Shanghai Genechem Co., Ltd., Shanghai, China) (miRNA-inhibitor 100 nM). After 24 h, Dual-Lucy Assay Kit was used to assess luciferase activity. Each cell experiment was repeated 3 times independently.

Western blot

Protein lysates of the dorsal root ganglion was produced using RIPA lysis buffer (P1003B, Beyotime, Shanghai, China) and 1% phenylmethylsulfonyl fluoride and then quantified by bicinchoninic acid (BCA) kit (Beyotime). Equal amounts of proteins (15–50 μg) were separated using 4–20% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride (PVDF) membranes with pore size of 0.45 μm or 0.22 μm. The PVDF membranes were blocked with 5% skim milk power for 1 h at room temperature and incubated with the primary antibodies at 4˚C overnight and ultimately incubated with the secondary antibody anti-rabbit IgG (1: 2000, ab6721, Abcam) at room temperature for 1 h. Then, the PVDF membranes were treated with enhanced chemiluminescence

(#34080, Thermo Fisher, Waltham, Massachusetts, USA) and imaged on ImageQuant LAS 4000 (General Electric Company, Schenectady, NY, USA). The primary antibodies were as follows: JAG1 (1:1000, PA5-72843, Thermo Fisher), Notch1 (1: 1000, ab167441, Abcam), HES1 (1:1000, ab108937, Abcam), and β-actin (1: 1000, ab8227, Abcam).

Statistical analysis

SPSS 21.0 software (IBM Corp, Armonk, NY, USA) was utilized for data analysis and GraphPad Prism 8.0 software was utilized for. The data were normally distributed and expressed by mean ± standard deviation (SD) as examined by Shapiro-Wilk. The comparison of differences among multiple groups was analyzed by one-way analysis of variance (ANOVA) or two-way ANOVA and checked by Tukey's multiple comparisons test. *P* value was from two-tailed test, and *P* value < 0.05 was considered significant in statistics.

Results

Protective effect of propofol on rats with NP

To analyze the effects of propofol on NP, a model of rats with NP (CCI rat model) was firstly established. Then, rats in the sham group, CCI group and CCI + Pro group were subjected to PMWT and PWTL tests (Fig. 1A, B). CCI treatment shortened the time of PMWL and PWTL in rats. After treatment of propofol, the situation was partially improved, but the time still did not recover to sham-operated rats ($P < 0.001$). There is a research evidenced that neuropathic pain is closely associated with neuroinflammation (Guo et al. 2021). Therefore, ELISA kits were used to detect the expression of cytokines related to neuro-inflammation of ipsilateral dorsal root ganglitis (Fig. 1C). CCI upregulated pro-inflammatory factors TNF-α and IL-1β and downregulated anti-inflammatory factor IL-10. After injection of propofol, the changes of above inflammation-related cytokines were reversed (all $P < 0.001$), suggesting that propofol exerts protective effects on rats with neuropathic pain.

Propofol exerts protective effect on CCI rats via miR-140-3p

miRNAs play a significant role in neuropathic pain (Tang 2021). Some papers pointed out that miR-140-3p can regulate rheumatoid arthritis (Zhong et al. 2020; Zu 2021). Accordingly, the propofol may protect CCI rats by regulating miR-140-3p. Firstly, the expression of miR-140-3p was detected by qRT-PCR. It was observed that miR-140-3p expression was significantly reduced in CCI rats and propofol improved its level ($P < 0.001$). CCI rats with the treatment of propofol was injected with antagomiR-140-3p to downregulate the level of miR-140-3p (Fig. 2A), and the changes of PWMT and PWTL were measured (Fig. 2B, C). The reduction of miR-140-3p level reversed the effects of propofol on PWMT and PWTL in CCL rats. Then, ELISA kits were utilized to detect the levels of inflammation-related cytokines (Fig. 2D). It was observed that antagomiR-140-3p reversed the protective effects of propofol on CCI rats. Altogether, propofol protects CCI rat by upregulating the expression of miR-140-3p.

MiR-140-3p targeted JAG1

To further research the role of miR-140-3p in CCI rats, the downstream target gens of miR-140-3p were predicted via TargetScan (http://www.targetscan.org/vert_72/), miRDB (http://mirdb.org/) and RNAInter (http://www.rna-society.org/raid/search.html) and intersected, and JAG1 was focused. A research confirmed that JAG1 plays a role in neuropathic pain to some extent (Li 2020a). Accordingly, miR-140-3p may affect CCI rats by targeting JAG1. miR-140-3p and JAG1 binding sites were predicted by TargetScan (Fig. 3B) and verified by dual-luciferase assay in 293T cells (Fig. 3B). Then, qRT-PCR assay was done to detect the level of JAG1 among different groups. It was observed that the mRNA level of JAG1 in CCI rats was significantly upregulated. The overexpression of miR-140-3p caused by propofol reversed the expression of JAG1and the level of JAG1's mRNA was reduced. In conclusion, miR-140-3p targets and inhibits the expression of JAG1.

JAG1 was upregulated to reverse the effects of propofol on CCI rats

Rats in the CCI + Pro group were injected with Ad-JAG1 to overexpress JAG1 and the efficiency of transfection was detected by qRT-PCR assay (Fig. 4A). The overexpression of JAG1 reversed the change of PWMT and PWTL caused by propofol (Fig. 4B, C). ELISA kit detected the change of inflammation-related cytokines (Fig. 4D). It was observed that upregulation of JAG1 reversed the change of inflammation-related cytokines caused by propofol, suggesting that upregulation of JAG1 could reverse the protective effect of propofol on CCI rats.

Propofol exerts protective effect on CCI rats via the Notch signaling pathway

Some researches evidenced that JAG1 affected neuropathic pain via the Notch signaling pathway (Xie et al. 2015a; Xie 2015b). Accordingly, propofol may protect CCI rats via the Notch signaling pathway. Western blot was applied to detect the levels of proteins related to JAG1 and Notch signaling pathway among sham group, CCI group and CCI + Pro group (Fig. 5A). Compared with sham group, the levels of JAG1, Notch1 and HES1 proteins were significantly upregulated after CCI modelling. The treatment of propofol reversed the levels of these proteins to some extent. In conclusion, propofol could inhibit the Notch signaling pathway to relieve NP in CCI rats.

Discussion

NP as a common condition occurs in the general population with a prevalence of 6.9–10% (St John Smith 2018). Unfortunately, the present therapies and medicines utilized by hospitals are unsatisfying and not effective to reduce patients' pain because of the heterogeneity of NP mechanisms (Baron et al. 2010). In this research, we investigated the protective mechanisms of propofol on NP in CCI rat model, and got a conclusion that propofol could inhibit neuroinflammation and alleviate NP via miR-140-3p/JAG1 and the Notch signaling pathway.

The development of CCI-induced NP in rats could be demonstrated by the time of PMWT and PWTL (Wang 2020). Our results indicated that the CCI treatment significantly shortened PMWT and PWTL and the injection of propofol improved them to some extent. The result was supported by previous research, which demonstrated that propofol mitigated NP with the prolongation of PMWT and PWTL in CCI rats (Jiang 2021). NP was evoked by neuroinflammation and pro-inflammatory cytokines TNF-α and IL-1β (Jiang 2021; Sommer et al. 2018). In this research, the CCI treatment upregulated TNF-α, IL-1β and downregulated anti-inflammatory cytokine IL-10, and propofol reversed the expression of these cytokines. Similarly, propofol suppressed the inflammation in MIRI (myocardial ischemia-reperfusion injury) rats by promoting the level of IL-10 and inhibiting TNF-α and IL-6 (Liu et al. 2021). Based on the results, the research evidenced that propofol could ameliorate neuroinflammation to some extent in CCI rats.

According to the literature, miRNAs in the spinal microglia could regulate various microglial markers to participate in NP development and propofol with different dosage could up/down-regulate the expression of miRNAs in on adipocyte stem cells (Kim et al. 2014; Tang 2021). More importantly, miR-140-3p was believed to relieve RA which is associated with NP (Dubois-Mendes et al. 2021; Zhong 2020). Therefore, we hypothesized that propofol is likely to regulate the expression of miR-140-3p to realize its protective effect on CCI rats. In this research, the CCI treatment reduced the expression of miR-140-3p and propofol upregulated its expression, indicating the possibility that propofol could upregulates the expression of miR-140-3p in NP condition. Then, propofol-treated rats were transfected with antagomiR-140-3p, and we found that downregulation of miR-140-3p counteracted propofol's effects to PWMT, PWTL, and inflammation-related cytokines in CCI rats. The result is supported by the previous study proposing that miR-140 was overexpressed to ameliorate NP (Li 2020b). Taken together, propofol exerts protective effect on CCI rats via regulating miR-140-3p.

Then, we explored the miR-140-3p downstream target. Through database prediction and intersection, we focused on JAG1. A recent study has discovered that the inhibition of JAG1 could relieve mechanical allodynia and thermal hyperalgesia in CCI rat model (Li 2020a). Hence, JAG1 is considered to play a negative role in NP. In this research, the dual-luciferase assay and qRT-PCR showed that the inhibition of and miR-140-3p reduced the expression of JAG1. Taken together, miR-140-3p could target JAG1. To further explore the mechanism of JAG1, propofol-treated rats were injected with Ad-JAG1. Our results revealed that overexpression of JAG1 reversed the change caused by propofol of PWMT, PWTL, and inflammation-related cytokines. Collectively, upregulation of JAG1 could reverse the protective effect of propofol on NP. Additionally, JAG1 as a non-specific ligand of Notch receptors could activate the notch signaling pathway in neuro and glia (Xie 2015b). The inhibition of Notch signaling can relieve the mechanical allodynia and thermal hyperalgesia induced by JAG1 (Sun et al. 2012). In this research, CCI treatment increased the expression of JAG1 and Notch1 and HES1, indicating the activation of Notch signaling. Later, the injection of propofol downregulated the expression of North family proteins. Consistently, another research reported that the activation of the Notch or Hes1 signaling plays a role in NP by strengthening excitatory synaptic transmission (Duan 2021). The Notch signaling influences the choice between the excitatory or inhibitory cell fatesin the developing spinal cord to ensure the proper

generation of excitatory neurons from the sensory interneuron progenitors (Xie 2015a). Therefore, propofol inhibits Notch signaling pathway to relieve NP in CCI rats.

In summary, propofol upregulates the expression of miR-140-3p which targets and inhibits JAG1 in CCI rat model and JAG1 is closely linked with activation of Notch signaling. This research analyzed the analgesic mechanism of propofol to some extent and may hint a promising therapeutic target of NP. However, since JAG1 is just one of the abundant target genes of miR-140-3p, the research failed to further investigate JAG1 in the clinic. In the future, the topics of whether propofol could be new medicine for NP treatment in the clinic and whether propofol could regulate other target genes via regulating miR-140-3p need to be further discussed.

Declarations

Ethics approval and consent to participate

This study was conducted with an approval of the ethic committee of the Affiliated Lianyungang Oriental Hospital of Bengbu Medical College. Adequate measures were taken to minimize the number of rats and to ensure minimal pain or discomfort.

Consent for publication

All authors agreed to publish.

Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

Competing interests

None.

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Authors' contributions

Conceptualization: FC and WQ; validation, research, resources, data reviewing, and writing: AXY, FFY and YC; review and editing: JXM. All authors read and approved the final manuscript.

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Figures

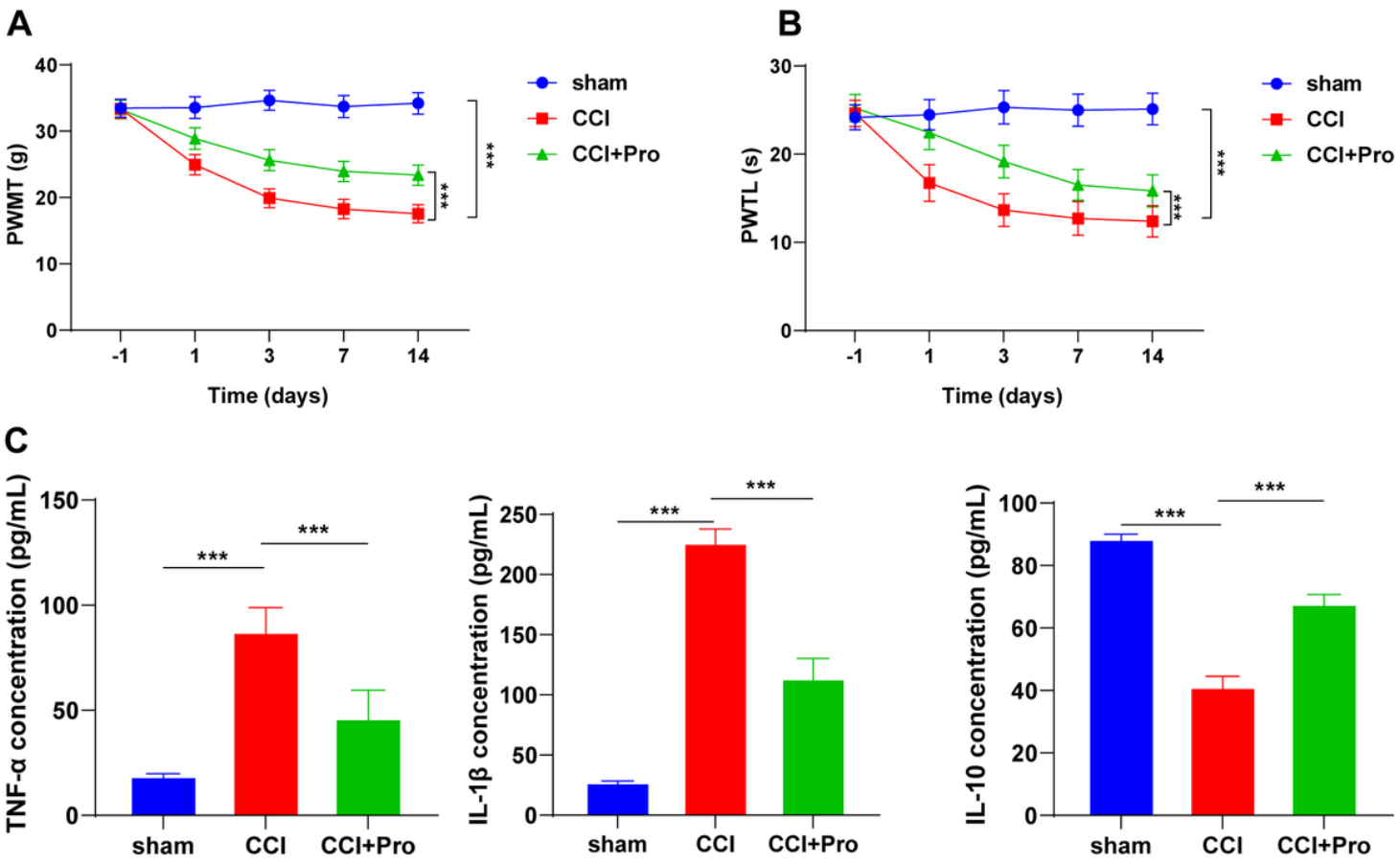

Figure 1

Protective effect of propofol on rats with neuropathic pain. CCI model was established, followed by the treatment of propofol. Rats were allocated into sham group, CCI group and CCI+Pro group. A: Von Frey test was conducted to detect PWMT of rats in different groups at 1 d before and 1, 3, 7, 14 d after modeling; B: PWTL of rats in different groups was measured at 1 d before and 1, 3, 7, 14 d after modelling; C: ELISA kits were used to detect the level of TNF-α, IL-1β and IL-10, N=6. The data were represented as mean ± SD. The data in Figure A, B were analyzed by Two-Way ANOVA and the data in

Figure C were analyzed by One-Way ANOVA. All data were checked by Tukey's multiple comparisons test, ***P < 0.001.

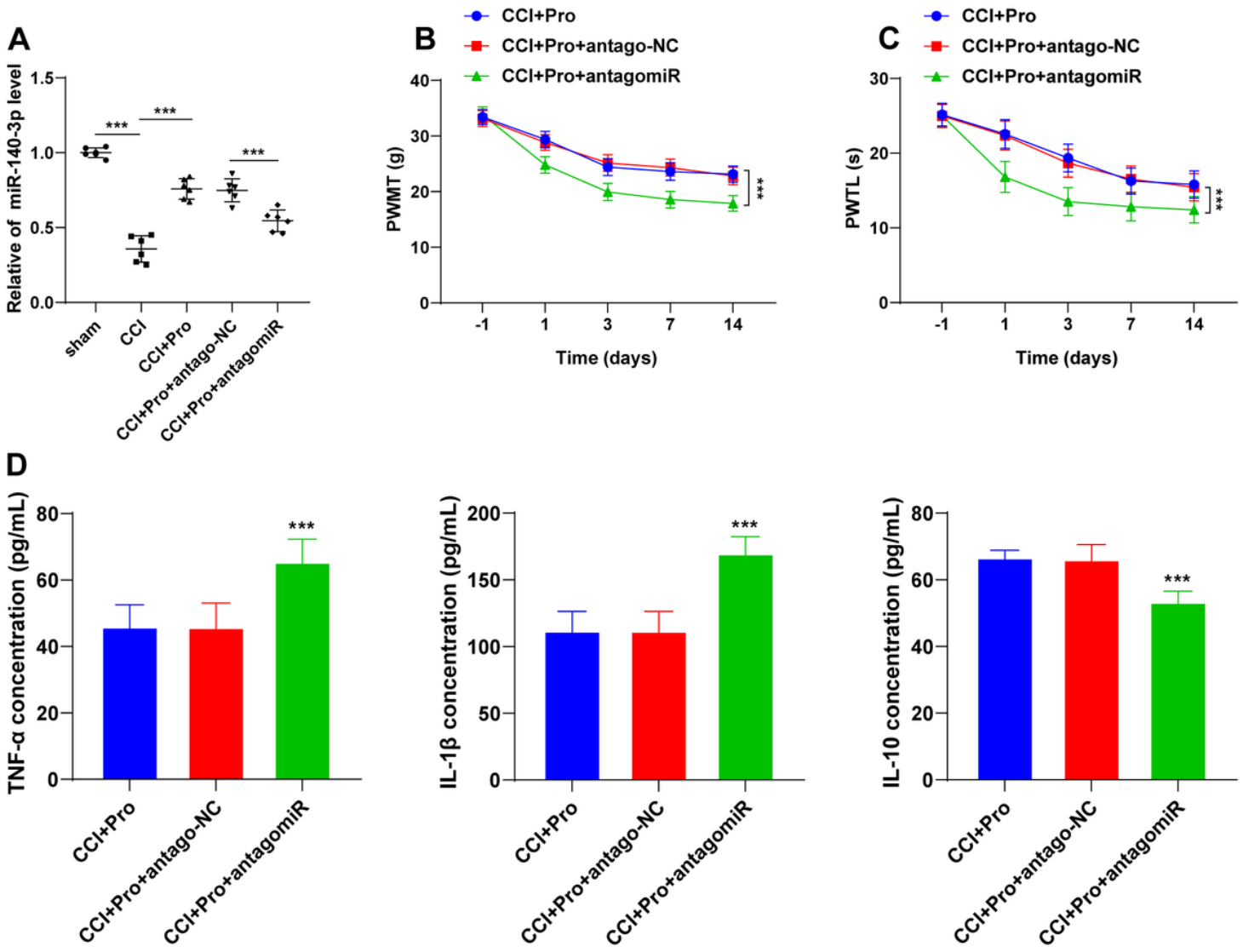

Figure 2

Propofol exerts protective effect on CCI rats via miR-140-3p. AntagoiR-140-3p was used to be transfected with CCI rats treated with propofol. Rats were allocated into sham group, CCI group, CCI+Pro group, CCI+Pro+ antago-NC group. A: qRT-PCR detected the level of miR-140-3p in different groups. B: Von Frey test was conducted to detect PWMT of rats in different groups at 1 d before and 1, 3, 7, 14 d after modelling; C: PWTL of rats in different groups was measured at 1 d before and 1, 3, 7, 14 d after modelling; D: ELISA kits detected the levels of TNF-α, IL-1β and IL-10 among CCI+Pro, CCI+Pro+ antago-NC and CCI+Pro+ antagomir, N=6. Data in Figure A were enumeration data, and data in Figure B, C and D were measurement data. All data were presented as mean ± SD. The data in Figure A, D were analyzed by One-Way ANOVA, and the data in Figure B, C were analyzed by Two-Way ANOVA. All data were checked by Tukey's multiple comparisons test, ***P< 0.001.

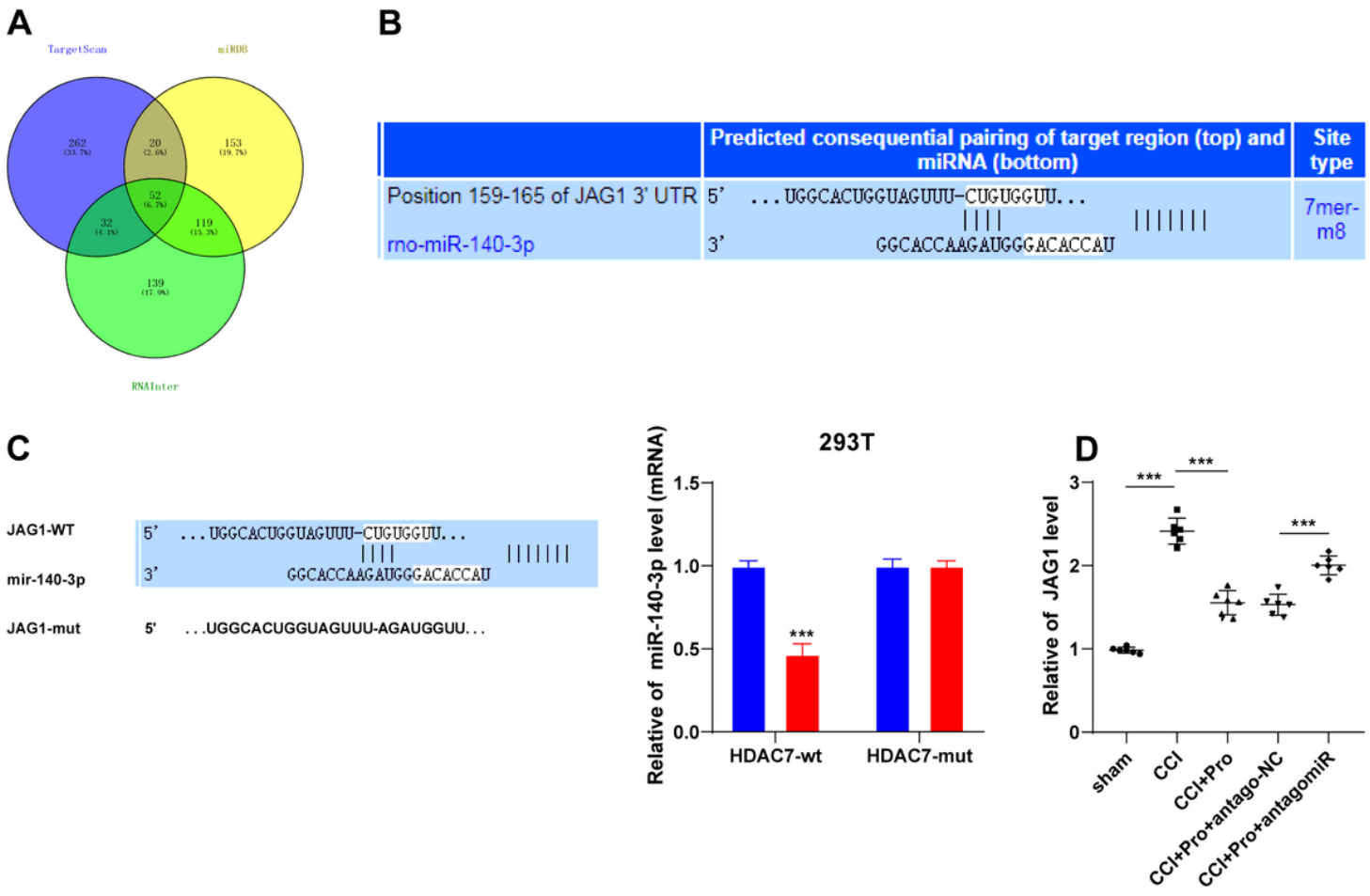

Figure 3

miR-140-3p targeted JAG1. TargetScan, miRDB, RNAInter predicted downstream target genes. A: Target genes were predicted and the intersection was obtained. B: TargetScan predicted the binding sites of miR-140-3p and JAG1. C: Dual-luciferase assay detected the target binding of miR-140-3p and JAG1. D: qRT-PCR assay detected the level of JAG1 among different groups (N=6). Each cell experiment repeated 3 times independently. The data in Figure C were measurement data and the data in Figure D were enumeration data. All data were presented as mean ± SD. The data in Figure D were analyzed by One-Way ANOVA and checked by Tukey's multiple comparisons test, ***P< 0.001.

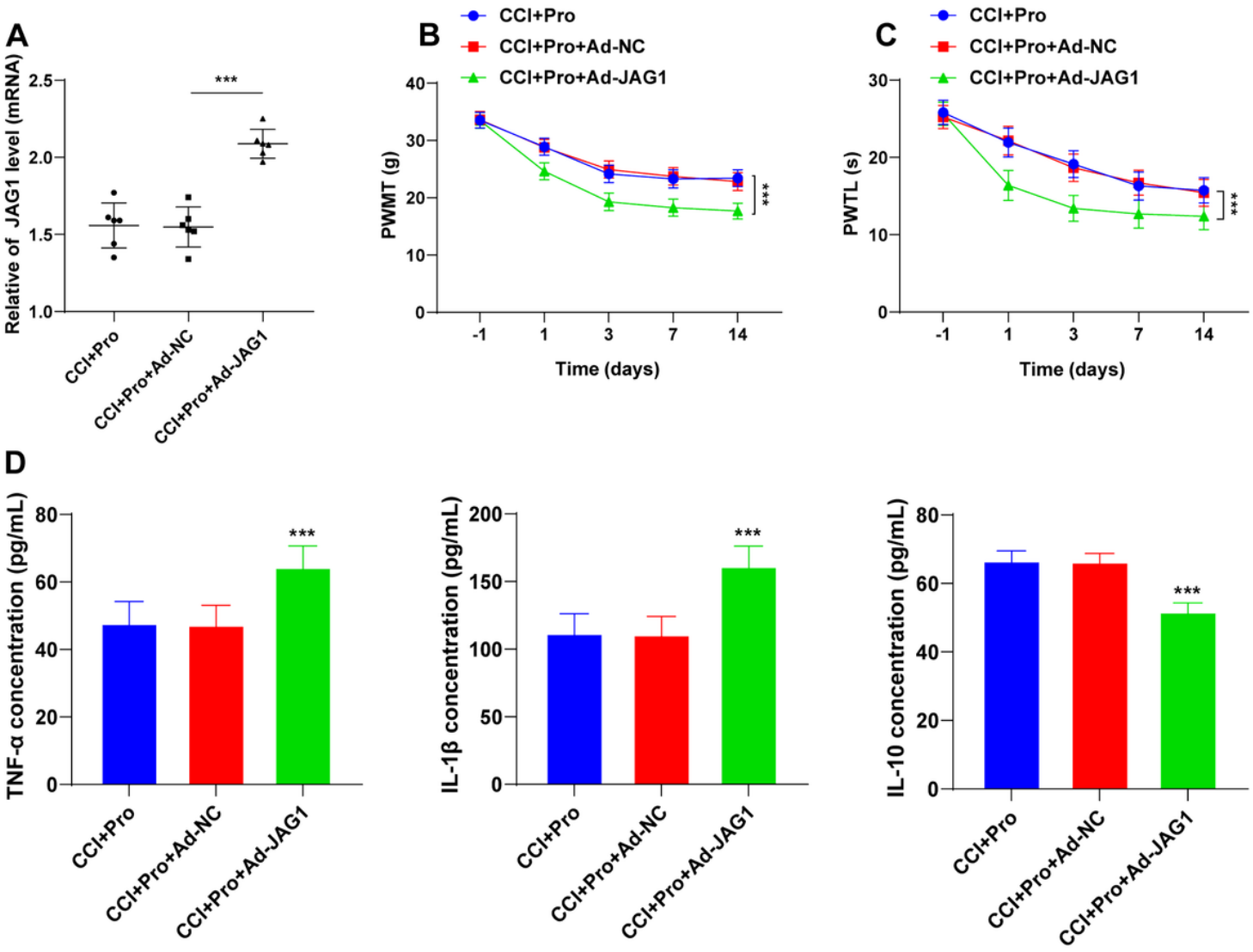

Figure 4

Upregulation of JAG1 reversed the protective effect of propofol. CCI rats were injected with Ad-JAG1 by caudal veins. Rats were allocated into CCI+Pro group, CCI+Pro+ antago-NC group and CCI+Pro+ antagomir group. A: qRT-PCR assay detected the level of JAG1 in different groups; B: Von Frey test was conducted to detect PWMT of different groups at 1 d before and 1, 3, 7, 14 d after modelling; C: PWTL of different groups was measured at 1 d before and 1, 3, 7, 14 d after modelling; D: ELISA kits detected the level of TNF-α, IL-1β and IL-10 14 d after modelling, N=6. Data in Figure A were enumeration data and data in Figure B, C, and D were measurement data. The data were presented as mean ± SD. The data in Figure A, D were analyzed by One-Way ANOVA and the data in Figure B, C were analyzed by Two-Way ANOVA. All data were checked by Tukey's multiple comparisons test, ***P< 0.001.

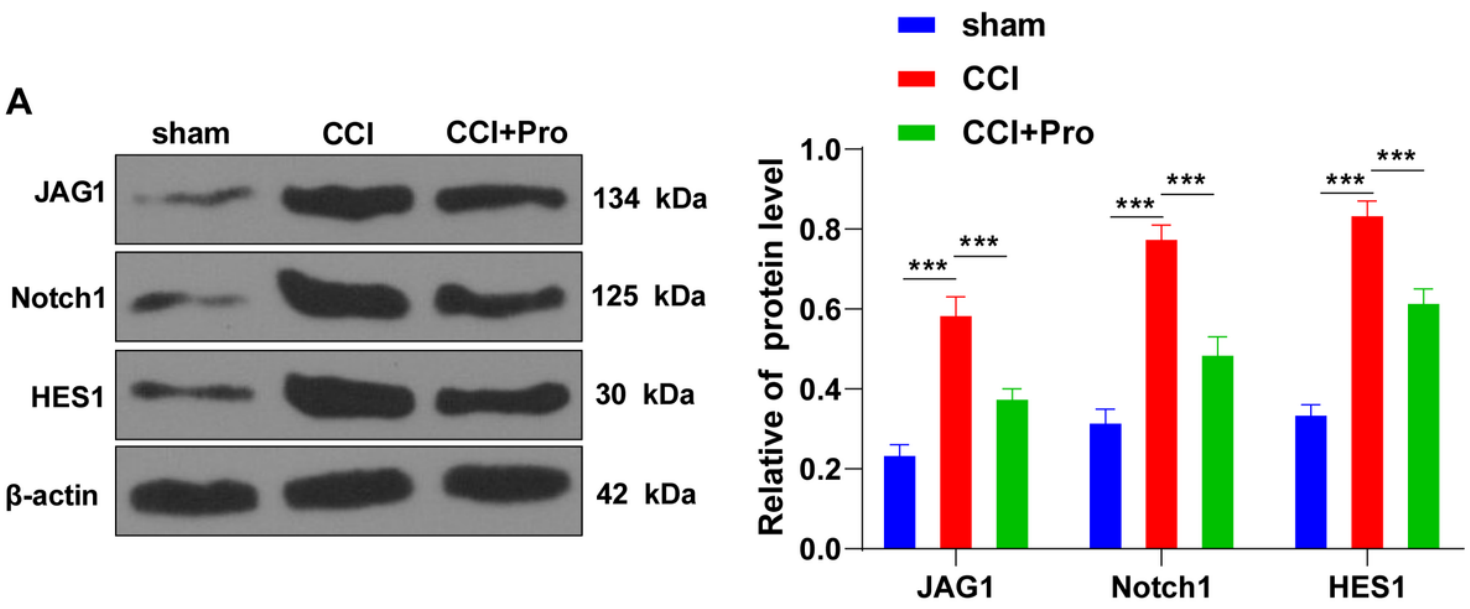

Figure 5

Propofol exerts protective effect on CCI rats via the Notch signaling pathway. A comparison was made among sham group, CCI group and CCI+pro. A: Western blot detected the levels of JAG1, Notch1 and HES1 proteins. The data were measurement data and presented as mean ± SD. The data were analyzed by One-Way ANOVA and checked by Tukey's multiple comparisons test, ***P< 0.001.