Reversing Microglial Polarization by High Intensity Interval Training: A Novel Approach to Mitigate Inflammatory Responses in Osteoarthritis via Jak2/Stat3 Pathway

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December 16, 2024

Abstract

Osteoarthritis (OA) is associated with inflammatory responses linked to microglial polarization within the central nervous system. However, exploring therapeutic approaches and their underlying mechanisms remains a direction for future research. The present study investigates the potential of high-intensity interval training (HIIT) to alleviate inflammation and facilitate the shift from M1 to M2 microglial polarization via the Jak2/Stat3 pathway in an OA rat model. Wistar rats were induced with OA via intra-articular injection of monosodium iodoacetate and subsequently underwent HIIT for six consecutive weeks after a four-week establishment period. Pain thresholds were measured using the von Frey test. Immunofluorescence detected Tmem119, SP, Vglut2, c-Fos, and IL6, while flow cytometry analyzed CD68 and CD163 levels. Proteomics compared the protein differences between the OA and HIIT groups. The Jak2/Stat3 pathway was activated in OA rats with C-A1 injections, followed by HIIT and subsequent Western blot analysis of inflammatory cytokines.The results indicated a significant decrease in pain threshold from the third to the tenth week in OA rats, while HIIT was found to increase pain thresholds. HIIT was found to promote M1 to M2 microglial polarization and downregulate the expression of Tmem119, SP, Vglut2, c-Fos, and IL6. Additionally, HIIT was more effective in suppressing Jak2 and Stat3 expression levels compared to OA rats. Activation of the Jak2/Stat3 pathway significantly increased the expression of Glu, c-fos, SP, and IL-6, but HIIT reversed these OA-induced increases. Compared to the OA+C-A1 group, the expression levels of Glu, c-fos, SP, and IL-6 were significantly reduced in the OA+C-A1+HIIT group. In conclusion, HIIT effectively mitigates OA-induced inflammatory responses by reversing microglial polarization through the Jak2/Stat3 pathway.

Introduction

Osteoarthritis (OA) is the most common musculoskeletal disease among middle-aged and elderly people. With the increase in susceptible factors (such as aging and obesity) over time, the prevalence of OA has reached 7% [1]. Pain is the main clinical manifestation of OA, often accompanied by varying degrees of limited joint mobility, deformity, and peripheral muscle weakness [2]. Pain is mainly related to the infiltration of proinflammatory factors in the intra-articular environment. The posterior horn of the spinal cord releases inflammatory factors and pain-causing neurotransmitters, both of which in turn lead to increased neuronal excitability and aggravated pain [3, 4]. OA pain is positively associated with persistent knee synovitis, marked by recurrent effusion and bone marrow edema. This inflammatory condition, in turn, triggers the release of neurotransmitters by the central nervous system (CNS) [5]. Moreover, as the disease progresses, patients not only develop an increased pain sensitivity but also exhibit resistance to pain-relieving medications [6]. High-intensity interval training (HIIT), as a form of physical therapy, not only mitigates pain but also enhances the muscular support around the affected joints, leading to a delay in the progression of OA [7]. Nonetheless,

it is imperative to elucidate the underlying mechanisms by which HIIT alleviates pain associated with OA, in order to provide robust evidence supporting the clinical implementation of HIIT in the management of OA.

Microglia, as glial cells, serve as crucial components of the innate immune system within the nervous tissues, acting as the primary line of defense in the CNS [8]. Neurotransmitter factors activate microglia in the CNS, manifesting as proliferation and migration, mainly divided into M1- and M2-type microglia. M1 microglia contribute to the release of pro-inflammatory factors, including TNF-α, IL-1β, and IL-6, leading to cellular death and tissue damage. Conversely, M2 microglia exhibit a neuroprotective role and releases anti-inflammatory factors, such as IL-10, IL-4, and TGF-β, which effectively inhibit neuroinflammation and promote tissue repair [9-11]. Recent studies have highlighted the pivotal role of promoting phenotypic shifts from M1 to M2 microglia as a key concept for alleviating chronic pain and exerting neuroprotective effects [12-14].

The Janus kinase 2 (Jak2)/ signal transducer and activator of transcription 3 (Stat3) pathway is involved in microglia activation and regulation of pain [15]. In addition, the Jak2/Stat3 pathway is also involved in various pathophysiological processes, such as inflammation regulation, and apoptosis, and has become a hotspot in the field of cytokine research [16]. When the Jak2/Stat3 pathway was inhibited, the inflammatory response mediated by microglia was attenuated and thus inflammatory pain would be alleviated [17]. Some studies have shown that exercise training can modulate the Jak2/Stat3 pathway. For example, in a rat model of myocardial infarction, exercise improved myocardial infarct size and fibrosis via Jak2/Stat3 [18]. In a mouse model of asthma, aerobic exercise participated in the regulation of airway inflammation through the Jak2/Stat3 signaling pathway, remodeling the effect of airway hyperresponsiveness [19]. Endurance exercise also increased the expression of Jak2 and Stat3 in the hypothalamus, thereby activating leptin signaling [20]. However, whether HIIT could promotes pheno-typic transformations of microglia through Jak2/Stat3 has not been elucidated.

Osteoarthritis (OA)-associated inflammatory responses are mediated not just by cartilage degeneration but also by the activation of microglia within the CNS, as an increasing body of evidence suggests [4, 21-23]. In our study, we developed an OA model to investigate whether HIIT could mitigate inflammation by inducing phenotypic shifts in microglia through the Jak2/Stat3 signaling pathway.

Materials and methods

Experimental animals

Adult male Wistar rats, weighing 250–300 g and 8 weeks old, were sourced from the Animal Experiment Center of Nanjing Medical University's Affiliated Nanjing Hospital. They were housed under the constant temperature and humidity (24 ± 2 , and 50 +- 5%). All animals were housed in a group of three per cage (cage type: 46cmx35cmx20cm ventilated cage). All rats were euthanized by intraperitoneal injection of 10% chloral hydrate after the behavioural experiments. All animal experiments were approved by the experimental Animal Welfare and Ethics Committee.

Experimental design

A total of 84 rats were included in the two experiments of this study. The experimental timeline and the sample size of rats are illustrated in Figure 1. Experiment 1 included 36 rats and were randomly divided into the control group, OA group and OA+HIIT group (N = 12/group). Experiment 2 included 48 OA rats and were randomly divided into the OA group, OA+HIIT group, OA+C-A1 group, and OA+C-A1+HIIT group (N= 12/group). Jak2/Stat3 pathway activator (C-A1, 100ug/kg/d, MedChemExpress, New Jersey, USA) was administered by intraperitoneal injection in the third week for 28 days [24]. HIIT treatments were conducted in the fourth week and continued for six weeks. After six weeks of exercise, the L3-L5 segments of the spinal cord tissue were harvested and analyzed by proteomics sequencing, quantitative real-time polymerase chain reaction (qRT-PCR), flow cytometry, Western blot (WB) and immunofluorescence (IF). Immunohistochemistry (IHC) was used to detect the matrix metalloproteinase 13 (MMP-13) and IL-6

in the extracellular matrix of articular cartilage.

Rat model of OA

After one week of adaptive feeding, all rats were anesthetized with 10% chloral hydrate (Sigma-Aldrich, St. Louis, Missouri, USA). OA rats have injected monosodium iodoacetate (MIA, 1 mg/50μL, 50μL, Sigma-Aldrich) into the right knee intraarticularly. The control group received an intraarticular injection of an equal volume of 0.9% normal saline (NS, 50μL) [25].

Pain threshold testing

Pain threshold was measured by the Von Frey testing (NC12775-99, North Coast, USA). The mechanical withdrawal threshold (MWT) was assessed by independent

observer blind to different groups rats. Briefly, we placed the rats in a chamber with a wire mesh floor ($22 \times 12 \times 12$ cm^3) and acclimated to the environment for 20 min. The plantar surface of the right hind paw was stimulated by Von Frey filaments ranging from 2.0 g to 15.0 g in sequence. The MWT value was recorded while withdrawing the paw. The results were presented as the following formula: 50% threshold (g) = $(10^{[\Xi\varphi+ K\delta]})$ /10000. Where X_f referred to as the value of the final von Frey filaments, k as coefficient value for the pattern of positive/negative responses, and δ was equal to 0.224 [26].

Exercise prescription

The exercise intensity of the HIIT exercise prescription for OA rats was set according to VO_{2max}, which was 38.6±8.8mL/ (kg.min) [27]. The rats underwent familiarization training for one week. The training program was as follows: the rats first moved freely on the turned-off treadmill (YS101, Tianjin, China) for 10min, then moved at 5m/min˜10m/min for 10min. A total of eleven rats were removed during the exercise, including seven that did not cooperate with the training and four with severe infections in their paw and tails. The number of missing rats in each group was supplemented until all the rats acclimated to the treadmill environment and speed change. HIIT was received as follows: First, warm-up at 10 m/min (30% VO2max) for 3min, and then high-intensity running at 24 m/min (80% VO2max) for 1 min (five cycles), moderate-intensity running at 15 m/min (50% VO2max) for 3min (five cycles), and finally cool down at 10 m/min for 2 min. The interval ratio of high-intensity to moderate-intensity exercise was 1:3, and each exercise was 25 min, three days a week (10:00 am every Tuesday, Thursday and Saturday). The total duration of HIIT exercise training was 75 min/week (Table 1). The other groups were given exercise intervention at a speed of 10 m/min, and the exercise duration and frequency were the same as HIIT.

Table 1 HIIT training protocol for OA rats

Session	Intensity(% VO_{2max})	Time(min)	cycle
Warm-up	30%	3	1
High-intensity running	80%	1	5
Moderate-intensity running	50%	3	5
Cool down	30%	2	1

Enzymelinked immunosorbent assay (ELISA)

Blood samples were collected from the tail vein of the rats, centrifuged at 3000 rpm for 10 min, and serum was collected. The concentration gradient of the standard was diluted according to the instructions of the IL-6 ELISA kit and the SP ELISA kit. IL-6 standard concentration dilution gradient: 160, 80, 40, 20, 10, 5 pg/mL; SP standard concentration dilution gradient: 8, 4, 2, 1, 0.5, 0.25 ng/mL. 50 μL of different concentrations of standards were added to each standard well, and 50 μL of the tested sample was added to the sample well to be tested. Except for blank wells, 100 μL of horseradish peroxidase labelled detection antibody was added to each well of standard wells and sample wells to be tested, gently shaken to mix evenly, and incubated at 37 for 1 h. After repeated washing five times, 50 μL of each substrate A and B were added

to each well and incubated at 37 ° C in the dark for 15 min. Finally, 50μL of termination solution was added to each well, and the liquid changed from blue to yellow to terminate the reaction. The absorbance values of each well were measured at 450 nm using a microplate reader.

IHC

After rats were euthanized, cartilage was harvested, and the articular part of the right knee of rats was isolated and then dehydrated, decalcified, and embedded in paraffin. The knee articular tissue was placed in EDTA decalcification (Servicebio, Wuhan, China) solution and shaken at 120 rpm at a constant temperature (25 ˜ 30). Longitudinal-oriented sections (3 μm) of the knee joint were cut using a paraffin microtome (RM2265, Leica, Germany). The primary antibodies used were anti-IL-6 (1:500, Sigma-Aldrich) and anti-MMP-13 (1:50, Absin, Shanghai, China). IHC labelling was applied as routine technology. In brief, after being blocked with 10% goat serum for 20 min at room temperature, sections were incubated with primary antibodies for 1 h at 37 . Horseradish peroxidase-labelled secondary antibodies were added and stood at room temperature for 1 h. The nucleus was then stained by mounting medium with DAPI and observed with a digital pathology image scanner (version 12.3.3.5048, Leica, Wetzlar, Germany).

IF

The L3-L5 segments of the spinal cord tissues of rats were fixed for 6 h in 4% paraformaldehyde and then embedded in paraffin. The spinal cord cross-section was cut using a paraffin microtome (3 μm). Primary antibodies used were anti-transmembrane protein 119 (Tmem119, 1:100, Novus Biologicals, New York, USA). The primary antibodies were incubated at 4 overnight, and subsequently, secondary antibodies were incubated at room temperature for 1 h. Nucleus were then stained by mounting medium with DAPI fluorescent sealing reagent (Elabscience, Wuhan, China) for 3-5 min. The images were observed with a digital pathology image scanner (DM5000B, Leica, Germany).

Flow Cytometry

The expression levels of the M1 (anti-CD68) and M2 (anti-CD163) in the microglial cell of the L3-L5 segments of the spinal cord tissues were analyzed by the flow cytometry (Cytomics FC 500, Beckman, USA). The pancreatin (Beyotime, Shanghai, China) digested the spinal cord tissues at 37 for 5 min. The cell suspension was centrifuged at 1,000 rpm for 5 min and then transferred to a 1.5 mL EP tube. The total number of cells was 1×10^6/tube. The anti-CD68 (1:500, Abcam) and anti-CD163 (1:60, Abcam) were incubated at room temperature for 30 min in the dark. Finally, the expression levels of the anti-CD68 and anti-CD163 were detected by flow cytometer (Cytomics FC 500, Beckman).

Quantitative technique of protein mass spectrometry and KEGG pathway analysis

The protein sequencing and quantification were performed at Guangzhou Omicsmart Corp. Guangzhou, China. The L3-L5 segments of the spinal cord tissues were treated with lysis buffer (KeyGEN) for 30 min on ice, and then were homogenized using an ultrasonic homogenizer (Voshin, Wuxi, Jiangsu, China) for 2 min. The homogenate was centrifuged at 12000 rpm for 15 min at 4 and the supernatant was collected. The protein mass spectrometry was determined by direct data-independent acquisition (dDIA). To explore the biological functions of the different genes involved, gene ontology (GO) terms (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the cluster profiler method. The calculated p value was carried out through FDR correction, taking FDR [?] 0.05 as a threshold. Protein-protein interaction (PPI) network was identified using string, which determined genes as nodes and interaction as lines in a network. The network file was visualized using cytoscape software (version 3.6.1) to present a core and hub gene biological interaction.

qRT-PCR

Total RNA from L3-L5 segments of the spinal cord**tissues was extracted with Trizol (** KeyGEN**). The cDNA was first synthesized with a reverse transcription reagent kit (Vazyme Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. The reagent mixes were**

prepared with the ChamQ Universal SYBR QPCR Master Mix (Vazyme Biotech), and cDNA was amplified using specific oligonucleotide primers (Table 2). The qRT-PCR was conducted with the PCR System (QTOWER 2.2, Analytik Jena, Germany) using settings of 95 degC for 30 s, followed by 40 cycles of 95 degC for 10 s, 60 degC for 20 s, and a final 72 degC for 1 min. The relative expression of target genes was calculated using the 2 $^{-CT}$ method and normalized to GAPDH**.**

Table 2. Primer sequences for quantitative real-time polymerase chain reaction in rats.

Gene		Primer sequence (5'-3')
GAPDH	**F**	**CAAGTTCAACGGCACAGTCA**
	R	**CCCCATTTGATGTTAGCGGG**
Stat3	**F**	AAGGAGGAGGCATTCGGAAA
	R	ACATCGGCAGGTCAATGGTA
Stat1	**F**	TTGCCTCTGGAATGATGGGT
	R	GTGAATGTGATGGCCCCTTC
Grb2	**F**	ACCAGCAGATATTCCTCCGG
	R	ATGAAGTCTCCTCGGCGAAA
Jak2	**F**	AACTGTCACGGCCCAATTTC
	R	ACGGCAAAGGTCAGGAAGTA

F, forward; R, reverse.

WB

The protein in the L3-L5 segments of the spinal cord tissues was extracted with lysis buffer (KeyGEN, Nanjing, China) and protein concentrations were measured using the BCA assay kit (KeyGEN). An SDS loading buffer (5 ×) was added to the protein samples and denatured at 95 for 15 min. Protein samples were separated on the 4% ˜ 20% SDS-PAGE gel and then transferred onto PVDF membranes (Immobilon, Shanghai, China). The transferred membranes were blocked with blocking buffer (NCM Biotech, Suzhou, China) for 10 min at ambient temperature. The anti-SP (1:1000, Santa Cruz Biotechnology, Dallas, Texas, USA), anti-Glu (1:1000, Abcam, Cambridge, UK), anti-IL6 (1:1000, Absin, Shanghai, China), anti-cellular oncogene fos (c-fos, 1:500, Santa Cruz Biotechnology), and anti-GAPDH (1:1000, Abcam) were incubated overnight at 4 . Glyceraldehyde-3phosphate dehydrogenase (GAPDH) was used as a loading control. The secondary antibodies were incubated for 2 h at room temperature. The proteins were visualized using an enhanced chemiluminescence reagent (ThermoFisher) and quantified using the Image 4000 system (Tanon 6600, Beijing, China).

Statistical analysis

The data were presented as mean +- SD and statistical graphs were performed by the GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). The normality of the data in each group was measured by the Shapiro-Wilk test. Statistical comparisons were performed by the one-way analysis of variance (ANOVA). Pain threshold data satisfied the assumptions of normality, variance, and sphericity, and repetitive measures ANOVA was used. LSD method was used as a post-hoc test.$P < 0.05$ was considered statistically significant.

Results

General state and pain threshold of rats

During the experiment, no rats in any of the groups died. The control rats had well-grown fur that was white and shiny. The fur of the rats with OA appeared haggard and yellow, and they exhibited signs of irritability. Within one week of the MIA injection, the OA rats showed a limp, but their movement gradually improved after three weeks. The OA+HIIT rats also exhibited limp movement and irritability one week after the MIA

injection. After six weeks of HIIT training, it was expected that the OA+HIIT rats would show improved movement in their right knee joint, but four rats suffered from serious infections in their paws and tails. The OA+C-A1 and OA+C-A1+HIIT rats showed a limp during the first week after the MIA injection, but their condition gradually improved after the third week. After six weeks of HIIT training, the fur of the OA+C-A1+HIIT rats became shiny, but three rats had severe infections in their paws and tails. The body weight of the rats in each group increased significantly over the feeding period, with no significant difference in the rate of weight gain ($P > 0.05$, Figure 2 A, C).

OA rats manifested a lower threshold when the von Frey test stimulated the hind paw. OA rats exhibited significantly decreased MWT from the third and tenth week compared with control rats, but HIIT treatment reversed the OA-induced trend ($P < 0.001$, Figure 3 B). There was no significant difference in the MWT of rats in each group before MIA injection ($P > 0.05$). From the seventh to tenth week, the MWT of OA+HIIT rats was significantly higher than that of OA rats (seventh week: $P < 0.01$; eighth to tenth week: $P < 0.001$). The MWT of OA+C-A1 rats was significantly lower than that of OA rats ($P < 0.05$). At the eighth to tenth weeks, the MWT of OA+C-A1+HIIT rats was significantly lower than that of OA+HIIT rats ($P < 0.05$, Figure 2 B, D).

HIIT reversed the OA-induced inflammatory response

Compared with control rats, the contents of SP and IL-6 in the serum of OA rats were significantly increased. However, HIIT can reduce the contents of SP and IL-6 in the serum of OA rats ($P < 0.05$, Figure 3A). IHC analyzed the inflammatory factors of the cartilage extracellular matrix. Compared with control rats, the expression of MMP-13 and IL-6 displayed significantly increased in OA rats. However, HIIT decreased MMP-13 and IL-6 expression of OA rats ($P < 0.05$, Figure 3 B-C).

HIIT reversed microglial polarization and mitigated inflammatory responses in OA rats

TMEM119 was a key indicator of microgliosis. We respectively detected TMEM119 in the microglia by immunofluorescence in control, OA, and OA+HIIT rats. The expression level of Tmem119 was significantly higher in OA rats than in control rats, and HIIT decreased its expression level ($P < 0.001$, Figure 4A). In addition, we detected the expression of CD68 and CD163 to distinguish pheno-typic transformations of microglia. The flow cytometry results showed that the expression level of CD68 was higher in the OA group than in the control group. However, CD163 was significantly lower in the OA group compared with the control group, and the expression level of CD163 was higher in the OA+HIIT group than in the OA group ($P < 0.01$, Figure 4B). Moreover, OA+HIIT exhibited decreased expression levels of Glu, c-fos, SP and IL-6 compared with OA rats ($P < 0.05$, Figure 4C).

Proteomic analysis of HIIT-regulated activation of spinal microglia in OA rats

The protein sequencing approach was used to analyse the gene transcriptome between OA and OA+HIIT rats to elucidate the mechanisms of action by which HIIT would relieve OA-induced pain. A total of 32 genes showed significant differences, of which 10 genes were up-regulated and 22 genes were down-regulated when compared OA+HIIT with OA group (Figure 5A, E). PPI network shows the interactions between different proteins (Figure 5B). KEGG pathway analysis was shown in Figure 5C-D, in which Jak2/Stat3 signaling pathway is closer to HIIT treatment. The qRT-PCR analysis verified down-regulation of genes related to HIIT, with the expression levels of Stat3, Stat1, Jak2, and Grb2 significantly down-regulated in the OA+HIIT group compared with the OA group ($P < 0.05$, Figure 5F).

HIIT reversed microglial polarization via the Jak2/Stat3 pathway in OA rats

To examine the mechanisms by which HIIT would alleviate OA-induced pain through the Jak2/Stat3 pathway, we activated the Jak2/Stat3 signalling pathway by intraperitoneal injection of C-A1 in OA rats. Flow cytometry results confirmed that the CD68 expression of the OA+C-A1 rats was higher than that of OA rats, but CD163 was lower than that of OA rats ($P < 0.001$). The expression levels of CD68 were higher in the OA+C-A1+HIIT rats than in OA+ HIIT rats, but CD163 was lower than that of OA+HIIT rats ($P < 0.001$, Figure 6 A-B). Subsequently, pain neurotransmitters were measured by WB to determine whether

Jak2/Stat3 pathway could affect their release. Activation of the Jak2/Stat3 pathway significantly increased the expression of Glu, c-fos, SP, and IL-6, but HIIT reversed the OA-induced increase. The expression levels of Glu, c-fos, SP, and IL-6 were significantly reduced in the OA+C-A1+HIIT group compared to the OA+C-A1 group ($P < 0.01$, Figure 6 C-D).

Discussion

In the present study, we found a decreased pain threshold with the progression of OA, increased levels of SP and IL-6 in the serum of OA rats, activated M1-type microglia in the spinal cord, and increased expression of pain neurotransmitters. We also demonstrated that HIIT remarkably down-regulated pain neurotransmitters and proinflammatory cytokines in OA rats, driven by the M2-type transformation of microglia. Moreover, the Jak2/Stat3 pathway plays a critical role in microgliosis and pain. C-A1 can overexpress Jak2 and Stat3, and in rats treated with OA+C-A1+HIIT, the downregulation of pain neurotransmitters was significantly less compared to rats treated with OA+HIIT alone. Therefore, HIIT promotes the phenotypic transformation of microglia through the Jak2/Stat3 signaling pathway and mitigates inflammatory responses.

Microglia activation plays a key role in the development of pain. At the early stage of injury, microglia usually differentiate into M1-type and release proinflammatory cytokines. While M2-type microglia can inhibit inflammation and increase antiinflammatory mediators, promoting tissue repair and regulating the neuronal release of pain-causing substances [28-30]. In this study, we found that there was an M1-type of microglia in the CNS of OA rats and that HIIT promoted the pheno-typic transformations of microglia, thereby alleviating the OA-induced neuroinflammatory response and suppressing pain. These findings suggest that HIIT exerts a pain-relieving effect by regulating the pheno-typic transformations of microglia and balancing anti-inflammatory and pro-inflammatory cytokines.

We found the differential proteins, such as Stat1, Stat2, Stat5b, Stat6, Sos1, and Pdgfrb, through the PPI method in OA and OA+HIIT rats, and most genes were related to the Stat family. Then we analyzed the top 20 pathways between the two groups by KEGG enrichment, among which the pathway Jak2/Stat3 was screened. Previous studies have shown that microglial activation was affected by multiple pathways, and activation of the Jak2/Stat3 pathway may be the main pathway leading to microglial transformation [31, 32]. We found that HIIT can reduce the expression of Jak2 and Stat3. To investigate that HIIT relieves chronic pain by driving the pheno-typic transformations of microglia via the Jak2/Stat3 pathway in OA rats. We overexpressed the Jak/Stat pathway by intraperitoneal injection of C-A1 and then provided HIIT intervention. Our results suggest that HIIT promoted the transformation of microglia from the M1 to the M2 type and that the expression levels of inflammatory factors and pain neurotransmitters were down-regulated. The pain neurotransmitters in OA+C-A1+HIIT rats showed a significant decrease compared to those in OA+HIIT rats. Although C-A1 might exacerbate the inflammatory response in the short term, HIIT could potentially counteract these effects over a longer period or with increased exercise intensity, thereby yielding significant anti-inflammatory outcomes. Taken together, HIIT promotes the pheno-typic transformations of microglia through the Jak2/Stat3 signalling pathway and affects the release of pain-causing neurotransmitters and proinflammatory cytokines.

A major limitation of this study is the lack of effects of proinflammatory cytokines in the joint synovium of OA rats on pain. Pain caused by OA is mediated by a variety of factors, including changes in joint structure [33], secretion of proinflammatory cytokines in articular cartilage, synovium, and muscles [34, 35], and release of neurotransmitters from peripheral and central neurons [36], microglia phenotype transformation, and ion channel changes [37]. In the future study, we will continue to explore the effects of HIIT on the muscle, synovial membrane and synovial fluid of OA rats and the underlying mechanisms. Furthermore, male rats were chosen for this study due to their greater behavioral and physiological stability compared to female rats, as well as their lesser vulnerability to hormonal fluctuations. As a simple and non-invasive treatment method, HIIT can not only reduce levels of inflammatory cytokines and pain neurotransmitters in the CNS, but also improve muscle strength and further delay the degeneration of articular cartilage [38, 39]. Compared with regular exercise, HIIT has the highest level of exercise efficiency and exercise performance [40], providing new insights into the molecular mechanisms and diagnosis and treatment for OA. It is worth

noting that weight-bearing running is not suitable for patients with severe deformities of OA. Therefore, we could prescribe HIIT exercise on a anti-gravity treadmill or power bicycle in future clinical trials.

In conclusion, our findings provide substantial evidence that HIIT improves OA-associated inflammation by regulating the phenotypic transformations of microglia via the Jak2/Stat3 signaling pathway.

Declaration

Consent for publication

Consent for publication was obtained from the participants.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

We thank the National Natural Science Foundation of China (No.82072541; 82272596) for financial support. Thanks to MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript

Ethics approval and consent to participate

All animal experiments were approved by the experimental Animal Welfare and Ethics Committee, Nanjing First Hospital (Nanjing, Jiangsu, China).

Availability of data and material

The datasets generated/analysed during the current study are available.

Funding

National Natural Science Foundation of China (No.82072541; 82272596).

Author contributions

Xinwei Wang and Xueping Li conceived and designed the study. Xinwei Wang and Mingxia Gao performed the experiments, analyzed data. Ziqi Ye designed the HIIT exercise prescription for rats. Peng Xia and Fanghui Li reviewed and edited the manuscript. Fanghui Li and Xueping Li provided technical and logistic support. All authors read and approved the final version of the manuscript.

References [1]. Leifer VP, Katz JN, Losina E. The burden of OA-health services and economics. Osteoarthritis Cartilage, 2021,30(1):10-16. [2]. Driban JB, Harkey MS, Barbe MF, et al. Risk factors and the natural history of accelerated knee osteoarthritis: a narrative review. BMC Musculoskelet Disord, 2020. 21(1): 332. [3]. Dimitroulas T, Duarte RV, Behura A, et al. Neuropathic pain in osteoarthritis: a review of pathophysiological mechanisms and implications for treatment. Semin Arthritis Rheum, 2014. 44(2):145-54. [4]. Mease PJ, Hanna S, Frakes EP, et al. Pain Mechanisms in Osteoarthritis: Understanding the Role of Central Pain. J Rheumatol, 2011. 38(8):1546-1551. [5]. Zhang Y, Nevitt M, Niu J, et al. Fluctuation of knee pain and changes in bone marrow lesions, effusions, and synovitis on magnetic resonance imaging. Arthritis Rheum, 2011. 63(3): 691-699. [6]. Staud, R. Peripheral and central mechanisms of fatigue in inflammatory and noninflammatory rheumatic diseases. Curr Rheumatol Rep, 2012. 14(6): 539-48. [7]. Townsend K, Imbert I, Eaton V, et al. Voluntary exercise blocks ongoing pain and diminishes bone remodeling while sparing protective mechanical pain in a rat model of advanced osteoarthritis pain. Pain, 2022,163(3):476-487. [8]. Hanani M, Verkhratsky A. Satellite Glial Cells and Astrocytes, a Comparative Review. Neurochem Res., 2021. 46(10): 2525-2537. [9]. Ma L, Peng S, Wei J, et al. Spinal microglial β-endorphin signaling mediates IL-10 and exenatide-induced inhibition of synaptic plasticity in neuropathic pain. CNS Neurosci Ther, 2021. 27(10): 1157-1172.[10]. Guo A, Li J, Luo L, et al. Valproic acid mitigates spinal nerve ligation-induced neuropathic pain in rats by modulating microglial function and inhibiting neuroinflammatory response. International Immunopharmacology, 2021. 92: 107332.[11]. Pan TT, Pan F, Gao W, et al. Involvement

of Macrophages and Spinal Microglia in Osteoarthritis Pain. Curr Rheumatol Rep, 2021. 23(5): 29.[12]. Sun ZQ, Liu JF, Luo W, et al. Lycium barbarum extract promotes M2 polarization and reduces oligomeric amyloid-β-induced inflammatory reactions in microglial cells. Neural Regen Res, 2022, 17(1): 203-209.[13]. Borgonetti V, Galeotti N. Intranasal delivery of an antisense oligonucleotide to the RNA-binding protein HuR relieves nerve injury-induced neuropathic pain. Pain, 2021, 162(5): 1500-1510.[14]. Zhong Z, Chen A, Fa Z, et al. Bone marrow mesenchymal stem cells upregulate PI3K/AKT pathway and down-regulate NF-ϰB pathway by secreting glial cell-derived neurotrophic factors to regulate microglial polarization and alleviate deafferentation pain in rats. Neurobiol Dis, 2020, 143: 104945.[15]. Li D, Yan Y, Yu L, et al. Procaine Attenuates Pain Behaviors of Neuropathic Pain Model Rats Possibly via Inhibiting JAK2/STAT3. Biomol Ther (Seoul), 2016, 24(5): 489-94.[16]. Hao Y, Yang X, Chen C, et al. STAT3 signalling pathway is involved in the activation of microglia induced by 2.45 GHz electromagnetic fields. Int J Radiat Biol, 2010, 86(1): 27-36.[17]. Yu S, Zhao G, Han F, et al. Muscone relieves inflammatory pain by inhibiting microglial activation-mediated inflammatory response via abrogation of the NOX4/JAK2-STAT3 pathway and NLRP3 inflammasome. International Immunopharmacology, 2020, 82: 106355.[18]. Zhang L, Wang X, Zhang H, et al. Exercise-induced peptide EIP-22 protect myocardial from ischaemia/reperfusion injury via activating JAK2/STAT3 signalling pathway. Cell Mol Med, 2021, 25(7): 3560-3572.[19]. Almeida-Oliveira AR, Aquino-Junior J, Abbasi A, et al. Effects of aerobic exercise on molecular aspects of asthma: involvement of SOCS-JAK-STAT. Exerc Immunol Rev, 2019, 25: 50-62.[20]. Zhao J, Tian Y, Xu J, et al. Endurance exercise is a leptin signaling mimetic in hypothalamus of Wistar rats. Lipids Health Dis., 2011,10: 225.[21]. Dimitroulas T, Duarte RV, Behura A, et al. Neuropathic pain in osteoarthritis: A review of pathophysiological mechanisms and implications for treatment. Seminars in arthritis and rheumatism, 2014, 44(2): 145-154.[22]. Balbinot G, Schuch CP, Nascimento PSD, et al. Photobiomodulation therapy partially restores cartilage integrity and reduces chronic pain behavior in a rat model of osteoarthritis: involvement of spinal glial modulation. Cartilage, 2021,13(2):1309-1321.[23]. Hahm SC, Lee JS, Yoon YW, et al. Analgesic tolerance development during repetitive electric stimulations is associated with changes in the expression of activated microglia in rats with osteoarthritis. Biomedicines, 2020, 8(12).[24]. Liu GL, Liu J, Wang JS, et al. Bulleyaconitine a attenuates oxaliplatin-induced neuropathic pain by down-regulating Nav1.6 expression through JAK2/STAT3 signaling pathway. Chinese Journal of Pathophysiology, 2021, 37(9): 1628-1635.[25]. Amodeo G, Niada S, Moschetti G, et al. Secretome of human adipose-derived mesenchymal stem cell relieves pain and neuroinflammation independently of the route of administration in experimental osteoarthritis. Brain, Behavior, and Immunity, 2021. 94: 29-40.[26]. Mazzitelli M, akhnitsa V, Neugebauer B, et al. Optogenetic manipulations of CeA-CRF neurons modulate pain- and anxiety-like behaviors in neuropathic pain and control rats. Neuropharmacology, 2022, 210: 109031.[27]. Li F, Sun L, Wu DS, et al. Proteomics-based identification of different training adaptations of aged skeletal muscle following long-term high-intensity interval and moderate-intensity continuous training in aged rats. Aging (Albany NY), 2019, 11(22): 10781-10782.[28]. Scholz J, Woolf C. The neuropathic pain triad: neurons, immune cells and glia. Nat Neurosci, 2007, 10(11): 1361-8.[29]. Wang X, Jiang Y, Li J, et al. DUSP1 promotes microglial polarization toward M2 phenotype in the medial prefrontal cortex of neuropathic pain rats via inhibition of MAPK Pathway. ACS Chem Neurosci, 2021, 12(6): 966-978.[30]. Lively S, Schlichter L. Microglia responses to pro-inflammatory stimuli (LPS, IFNγ+TNFα) and reprogramming by resolving cytokines (IL-4, IL-10). Front Cell Neurosci., 2018, 12: 215.[31]. Pisanu A, Lecca D, Mulas G, et al. Dynamic changes in pro- and anti-inflammatory cytokines in microglia after PPAR-γ agonist neuroprotective treatment in the MPTPp mouse model of progressive Parkinson's disease. Neurobiol Dis, 2014, 71: 280-91.[32]. Dominguez E, Rivat C, Pommier B, et al. JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat. Neurochem, 2008, 107(1): 50-60.[33]. Allen K, Walsh D. Modelling pathology: pain relationships in osteoarthritis. Osteoarthritis Cartilage, 2021, 29(10): 1386-1388.[34]. Xia P, Wang X, Wang Q, et al. Low-intensity pulsed ultrasound promotes autophagy-mediated migration of mesenchymal stem cells and cartilage repair. Cell Transplantation, 2021, 30: 963689720986142.[35]. Chiu YS, Bamodu OA, Fong IH, et al. The JAK inhibitor tofacitinib inhibits structural damage in osteoarthritis by modulating JAK1/TNF-alpha/IL-6 signaling through Mir-149-5p. Bone, 2021, 151: 116024.[36]. Soni A, Wanigasekera V, Mezue M, et al. Central sensitization in knee osteoarthritis: relating presurgical brainstem

neuroimaging and painDETECT-based patient stratification to arthroplasty outcome. Arthritis & Rheumatology, 2019, 71(4): 550-560. [37]. Pitake S, Middleton LJ, Abdus-Saboor I, et al. Inflammation Induced Sensory Nerve Growth and Pain Hypersensitivity Requires the N-Type Calcium Channel Cav2.2. Frontiers in Neuroscience, 2019, 13:1009.[38]. Holm PM, Petersen KK, Wernbom M, et al. Strength training in addition to neuromuscular exercise and education in individuals with knee osteoarthritis-the effects on pain and sensitization. Eur J Pain, 2021, 25(9): 1898-1911.[39]. Hall M, Dobson F, Van Ginckel A, et al. Comparative effectiveness of exercise programs for psychological well-being in knee osteoarthritis: A systematic review and network meta-analysis. Semin Arthritis Rheum, 2021, 51(5): 1023-1032.[40]. Keogh JW, Grigg J, Vertullo CJ. Is high-intensity interval cycling feasible and more beneficial than continuous cycling for knee osteoarthritic patients? Results of a randomised control feasibility trial. Peer J, 2018, 6: 4738.

Figure legend

Figure 1 Study design and timeline. The rats were injected with monosodium iodoacetate to induce an osteoarthritis (OA) model. In experiment 1: The rats were randomly divided into a control group, an OA group and an OA+HIIT group (N= 12/group). Control rats received an intraarticular injection of an equal volume of normal saline. HIIT was conducted in the fourth week and consecutively exercised for six weeks. In experiment 2: OA rats were randomly divided into the OA group, OA+HIIT group, OA+ C-A1 group and OA+ C-A1+HIIT group (N= 12/group). In the third week of the OA model, rats were given an intraperitoneal injection of C-A1 to activate the Jak2/Stat3 pathway, followed by HIIT intervention in the fourth week.

Figure 2 Body weight and pain threshold of rats in each group. A: Changes in body weight after MIA injection into the right knee of control rats, OA rats, and OA+HIIT rats. N=6/ group, two-way repeated measures ANOVA with Tukey and Bonferroni tests as post hoc tests. B: The von Frey method measured the Mechanical withdrawal threshold (MWT). N=6/ group, repeated measures ANOVA with Tukey and Bonferroni tests as post hoc tests. $^{**}P < 0.01$, $^{***}P < 0.001$. C: Changes in body weight after MIA injection into the right knee of OA rats, OA+HIIT rats, OA+ C-A1 rats, and OA+ C-A1+HIIT rats. N=6/ group, two-way repeated measures ANOVA with Tukey and Bonferroni tests as post hoc tests. D: The von Frey method measured the Mechanical withdrawal threshold (MWT). N=6/ group, repeated measures ANOVA with Tukey and Bonferroni tests as post hoc tests. $^{*}P < 0.05$, $^{***}P < 0.001$.

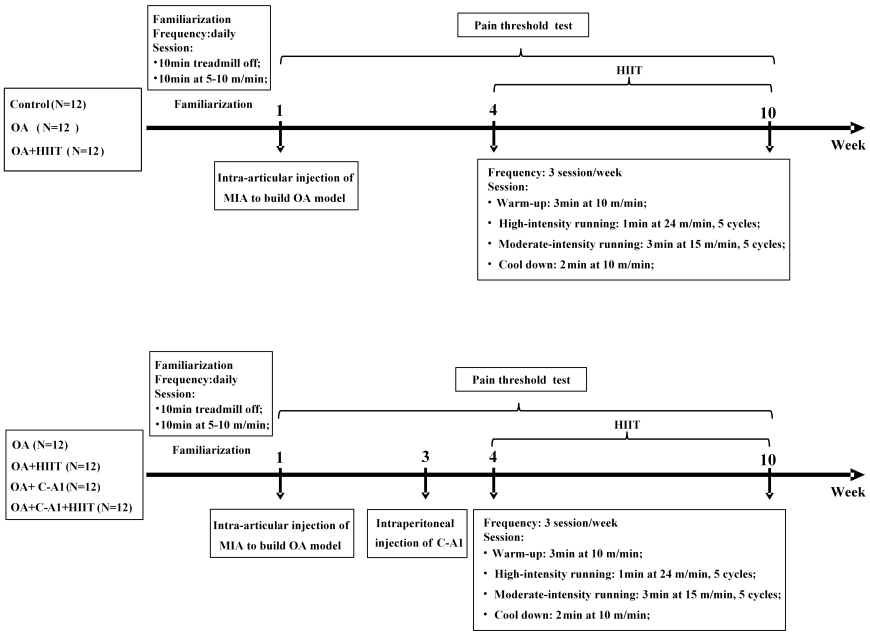
Figure 3 HIIT reversed the osteoarthritis (OA)-induced inflammatory response. A: The content levels of SP and IL-6 in the serum of rats. N=6, one-way ANOVA, $^{*}P < 0.05$, $^{***}P < 0.001$. B-C: Representative immunohistochemical images and quantitative analysis of MMP-13 and IL-6 in the extracellular matrix, blue is the nucleus, brown is target protein expression, scale =100 μm. One-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

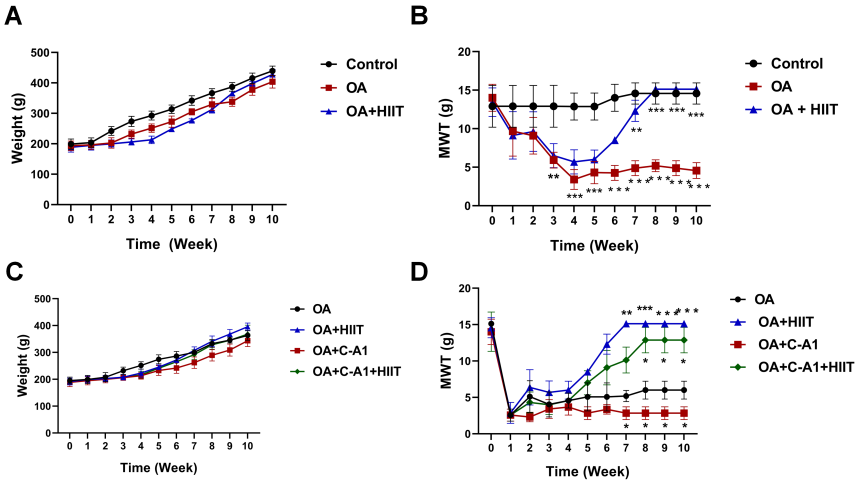
Figure 4 HIIT drove the pheno-typic transformations of microglia and down-regulated pain factors. A: The representative images of immunofluorescence in the microglia (blue is denoted for the nucleus and gold is for Tmem119 protein; scale bar = 100 μm) and quantitative analysis of Tmem119. N = 6 each group, $^{***}P < 0.001$. B: Flow cytometry analysis and quantification of CD68 and CD163 in microglia. N= 6 each group, $^{**}P < 0.01$. C: Representative western blotting images of Glu, c-fos, SP, and IL-6, and quantification. N= 6 each group. One-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

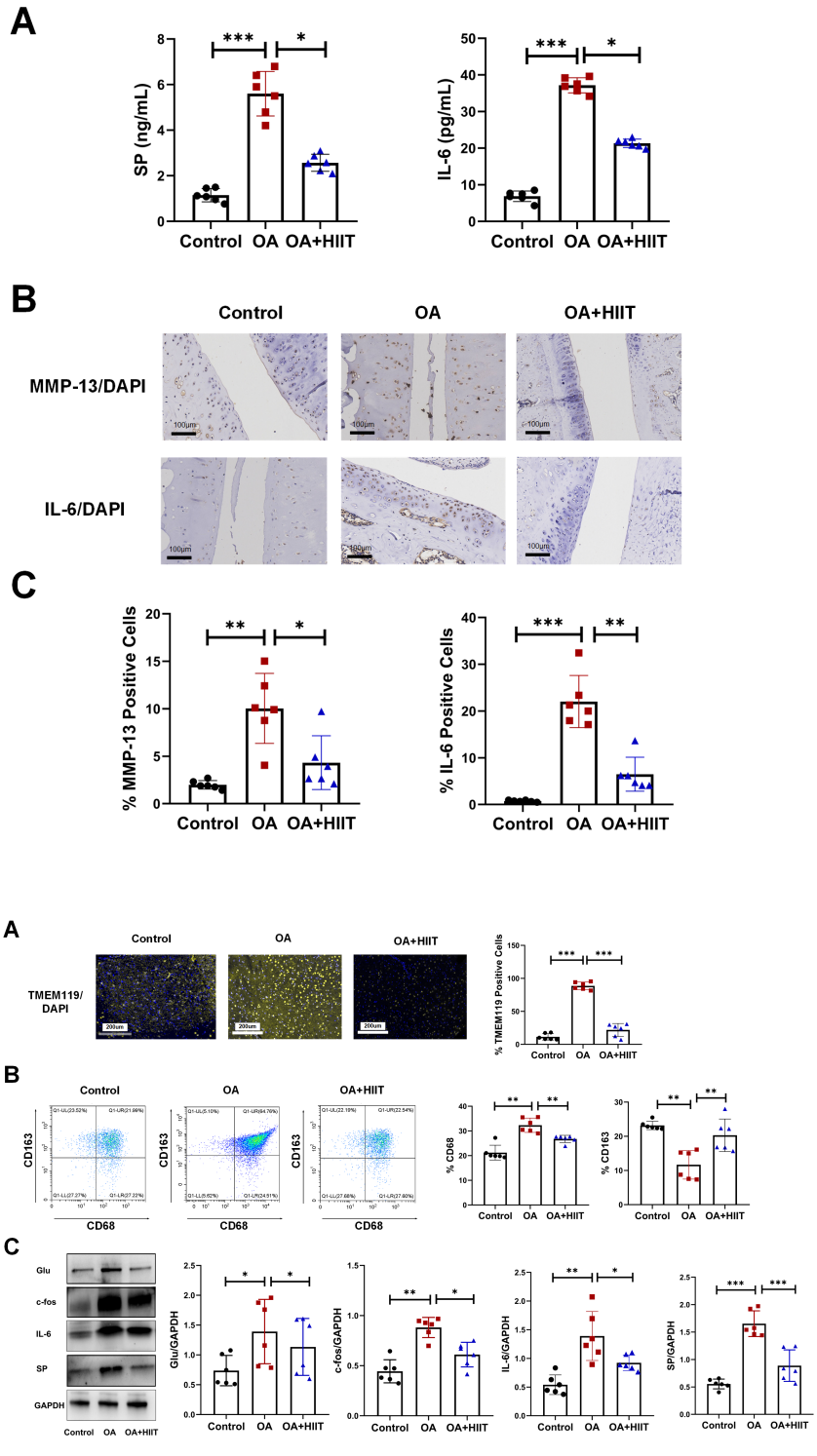
Figure 5 The quantitative technique of protein mass spectrometry and KEGG pathway analysis. The protein sequencing approach was used to map the transcriptome of OA and OA+HIIT rats. (A) Heat map of the differentially expressed genes. (B) Protein-protein interaction (PPI) network shows the interactions among different proteins. (C-D) The top 20 KEGG pathways were enriched between OA and OA-HIIT groups. (E) The differentially expressed genes were shown in the volcano plot, with red denoted for up-regulation and blue for down-regulation. (F) The main proteins involved in Jak2/Stat3 signalling pathway were analyzed by qRT-PCR. N= 6 each group. $^{*}P < 0.05$ vs. OA.

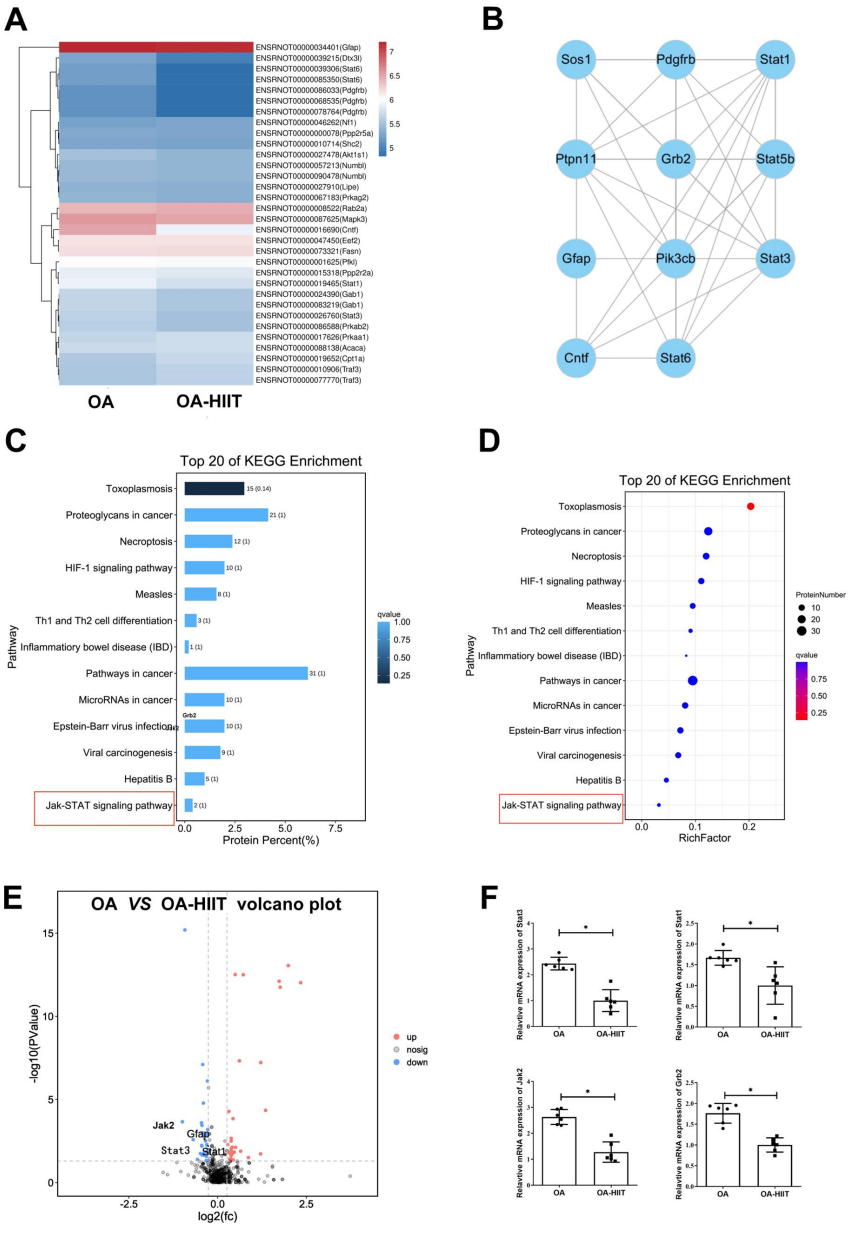
Figure 6 HIIT promoted the pheno-typic transformations of microglia and relieved pain in OA rats through Jak2/STAT3 pathway. A-B: Flow cytometry analysis and quantification of CD68 and CD163 in microglia.

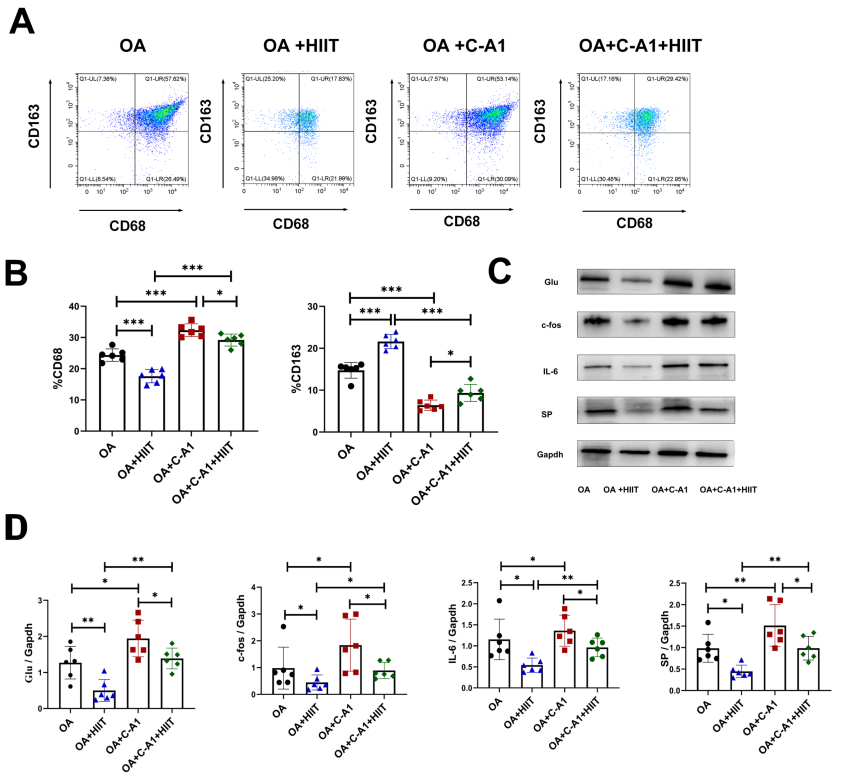
N = 6 each group. $^{***}P < 0.001$. C: Representative western blotting images of Glu, c-fos, SP, and IL-6, and quantification. N = 6 each group. $^{*}P < 0.05$, $^{**}P < 0.01$.











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