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Low level TGF-β1-treated Umbilical mesenchymal stem cells attenuates microgliosis and neuropathic pain in chronic constriction injury by exosomes/lncRNA UCA1 / miR-96-5p / FOXO3a

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ABSTRACT

Neuropathic pain is a chronic pain state that usually caused by injuries in peripheral or central nerve. Inhibition of spinal microglial response is a promising treatment of neuropathic pain caused by peripheral nerve injury. In recent years, mesenchymal stem cells (MSCs) that characterized with multipotent ability have been widely studied for disease treatment. TGF-β1 is a well-known regulatory cytokine that participate in the response to cell stress and is closely correlated with the function of nerve system as well as MSC differentiation. This work aimed to determine the effects of exosomes that extracted from TGF-β1-induced umbilical mesenchymal stem cells (hUCSMCs) on the neuropathic pain. In this work, we established a rat model of chronic constriction injury (CCI) of the sciatic nerve and LPS-induced microglia cell model. The hUCSMCs cell surface biomarker was identified by flow cytometry. Exosomes that extracted from TGF-β1-treated hUCSMCs were characterized by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA) and used for treatment. We observed that TGF-β1 upregulates the level of lncRNA UCA1 (UCA1) in hUCMSC-derived exosomes. Treatment with exosomal lncRNA UCA1 (UCA1) alleviated the neuropathic pain, microgliosis, and production of inflammatory mediator both in vivo and in vitro. UCA1 directly interact with the miR-96-5p, and the miR-96-5p acts as sponge of FOXO3a. Knockdown of UCA1 upregulated the level of miR-96-5p and downregulated the FOXO3a expression, which could be recovered by inhibition of miR-96-5p. In summary, the TGF-β1-stimulated exosomal UCA1 from hUCMSCs alleviates the neuropathic pain and microgliosis. These findings may provide novel evidence for treatment of neuropathic pain caused by chronic constriction injury.

1. Introduction

Neuropathic pain is a chronic pain state that usually caused by injuries in peripheral or central nerve or other systemic diseases and lacks effective management approaches [1,2]. Microgliosis is the representative characteristic of the central nervous system (CNS) upon nerve injuries that caused by ischemic and inflammatory diseases or trauma [3]. When peripheral or central nerve injury occurs, microglia are activated and proliferative, simultaneously produce multiple inflammatory factors such as interleukin (IL)-1α, IL-6, and tumor necrosis factor alpha (TNFα), which exacerbate the neuroinflammation and tissues injuries [4]. Accumulating evidence have proved that inhibiting the spinal microglial response may be a promising therapeutic approach for peripheral nerve injury-induced neuropathic pain [5,6].

Mesenchymal stem cells (MSCs) are multipotent cells that are capable of self-renewal and differentiation into different cell lineages, such as chondrocytes, tenocytes, osteoblasts, myoblasts, and adipocytes [7]. The lineage commitment and differentiation processes of MSCs are modulated by various chemokines and cytokines, including the members of the transforming growth factor-β (TGF-β) family [8]. For example, TGF-β/SMAD signaling regulates the adipocyte commitment of MSCs [9]. Moreover, stimulation with TGF-β regulates the production of cytokines and various signaling factors of MSCs and the subsequent regulation on phenotypes and functions of adjacent and distant cells [10,11]. MSCs can be isolated from multiple tissues, such as bone marrow, connective tissues, adipose tissue, peripheral blood, and umbilical cord [12]. Studies have revealed that MSCs contribute to tissue homeostasis, differentiation and repairment, via direct or indirect regulation [13].

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MSCs also affects the population and function of microglia in multiple diseases [14–16]. One of the manners that MSCs participate in disease pathology is through secreting exosomes that deliver various regulators [17].

Among the cargos delivered by exosomes, noncoding RNAs, especially the long noncoding RNAs (lncRNAs) have stood out in numerous research [18,19]. LncRNAs mainly function through binding with miRNAs to impede the mRNA targeting ability of miRNAs and have been reported to be involved in numerous cellular processes, including differentiation, metabolism, proliferation, apoptosis, metastasis, and stress response [20]. LncRNA urothelial carcinoma-associated 1 (UCA1) is widely reported to participate in the progression of multiple carcinomas and other diseases and modulates cell behaviors and inflammation response [21–25]. For examples, lncUCA1 participates in the apoptosis, oxidative stress, and inflammation of dopaminergic neurons in Parkinson's disease via modulating the PI3K/Akt signaling pathway [26]. The expression of lncUCA1 is correlated with the levels of IL-6, IL-17, and intracellular adhesion molecule-1 (ICAM1) in acute ischemic stroke patients, as well as the prognosis [27].

In this study, we aimed to explore the effects of exosomes that extracted from TGF-β1-induced hUCSMCs on the neuropathic pain and the underlying mechanisms. We analyzed the level of lncUCA1 in TGF-β-induced hUMSC-exosomes, and their function during CCI. We determined that lncUCA1 suppressed the production of inflammatory factors in microglia of CCI rat model via acting as the sponge of miR-96-5p to upregulate the expression of FOXO3a. Our data presented novel mechanism of TGF-β-induced hUMSC-exosomes for treatment for CCI-induced microgliosis and neuropathic pain.

2. Materials and methods

2.1. Cell lines and treatment

Human umbilical mesenchymal stem cells (hUMSCs) and rat microglia HAPI (High Aggressively Proliferating Immortalized) cells were bought from ZhongQiaoXinZhou (China), maintained in 1640 or microglia medium that contains 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin and streptomycin (Thermo, USA) at 37 °C incubator with 5% CO_2. To induce inflammatory response, microglia were treated with 5 μg/ml lipopolysaccharides (LPS; Sigma, USA) for 24 h. The cell surface biomarkers (CD34, CD45, CD44, CD73, CD105, CD90, CD151, and CD133) were stained with antibodies (eBioScience, USA) and analyzed by flow cytometry.

2.2. Cell transfection

The shRNAs that target lncUCA1 (shUCA1), UCA1 overexpression vectors, FOXO3a overexpression vectors, miR-96-5p mimics and inhibitors, and negative control (NC) were designed and synthesized by RiboBio (China). The oligonucleotides were mixed with Lipofectamine 2000 (Invitrogen, USA) and incubated with cells for 48 h. Cells were then collected for following study.

2.3. Exosomes isolation and identification

The hUMSCs were transfected with shUCA1 or NC for 48 h and treated with TGF-β1 (10 ng/ml) for 24 h. The exosomes isolated from TGF-β1-stimulated hUMSCs that transfected with shUCA1 or NC were short as TGF-β1-exo shUCA1 and TGF-β1-exo shNC, respectively. Cell culture medium were collected, and exosomes were extracted using ultracentrifugation following the standard procedure. In brief, the cell medium was sequentially centrifuged at 2000 g and 4 °C for 20 min to remove dead cells and were further centrifuged at 10,000 g and 4 °C for 30 min to discard debris. The supernatants were then transferred to another clean ultra-tube and ultracentrifuged at 100,000 g and 4 °C for 70 min. After discarding the supernatants, the deposited exosomes were resuspended in PBS and ultracentrifuged at 100,000 g and 4 °C for another 70 min. Extracted exosomes were resuspended in PBS and stored at −80 °C. Transmission electron microscopy (TEM; Hitachi, Japan) and nanoparticle tracking analysis (NTA; Malvin, Germany) were performed to measure the morphology and particle size of exosomes. The expression of exosome biomarkers, including the CD9, CD81, CD63, and TSG101, was detected by western blotting assay. For in vitro study, the exosomes were administrated at 10 μg/mL for 24 h [28].

2.4. Exosomes internalization experiment

To check the uptake of exosomes by microglia, we labeled the extracted exosomes with PKH-26 (Sigma, USA) as per manufacturer's description. HAPI cells were seeded into confocal dishes at a density of 5×10^4 cells per well and incubated with the exosomes (10 μg/mL) for 24 h. The cell nuclei were then stained with DAPI. Images were taken by a SP8 confocal microscope (Leica, Germany).

2.5. Chronic constriction injury (CCI) model

Male Sprague–Dawley (SD) rats that aged 6–8 weeks old and weighed 220–250 g were purchased from Vital River Laboratory (China) and housed in the specific pathogen free environment. All experiments were approved by the Institutional Ethics Committee of Technology Department, Everunion Biotechnology Co. LTD (Approval No. 202200103). All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Rats were randomly assigned to different experimental groups: the sham group, CCI model group, exosomal-shNC treatment group, and exosomal-shUCA1sh treatment group. Each group contains 9 rats. CCI was established following the reported procedure [29]. In short, the rats were anesthetized, and the left sciatic nerve was separated from surrounding tissue. Subsequently, the nerve was tied loosely by 4-0 suture along 5 mm-length at 1-mm intervals. All ligations were of the same tightness. Then the nerve was placed back to the intramuscular spaces and the skin wound was closed. For the rats in the sham group, the nerve was exposed and placed back but not ligated. Two weeks after injury, the rats were anesthetized with isoflurane, and the spinal dorsal horn of rats were dissected for subsequent experiments. For treatment, rats received injection of exosomes that derived from TGF-β1-stimulated hUMSC that transfected with shUCA1 (TGF-β1-exo shUCA1) or NC (TGF-β1-exo shNC).

2.6. Intrathecal catheter implantation of exosomes

The exosome treatment was performed using intrathecal catheter implantation following the previous studies [30]. In short, the rat back was opened by a longitudinal incision, then a PE-10 catheter was placed into subarachnoid space crossing the intervertebral space between L4 and L5. To examine the successful posing of the catheter, lidocaine test was performed three days after the implantation. The catheter was considered placed in the right place if the hind limbs were paralyzed 30 s after lidocaine injection and recovered within 30 min. Those rats with catheter implantation were used for study. The exosomes were injected through the catheter 3 days before the CCI operation at a dose of 10 μg in 10 μl PBS. The rats in sham group were treated with PBS.

2.7. Behavioral assessment

The mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) were examined on the ipsilateral paw using von Frey filaments (North Coast Medical, USA) and thermal pain test instrument (Ugo Basile, Italy), separately. For MWT measurement, rats were placed in separated chambers with wire mesh floor and left to acclimate for 30 min. After that the plantar surface of paw was

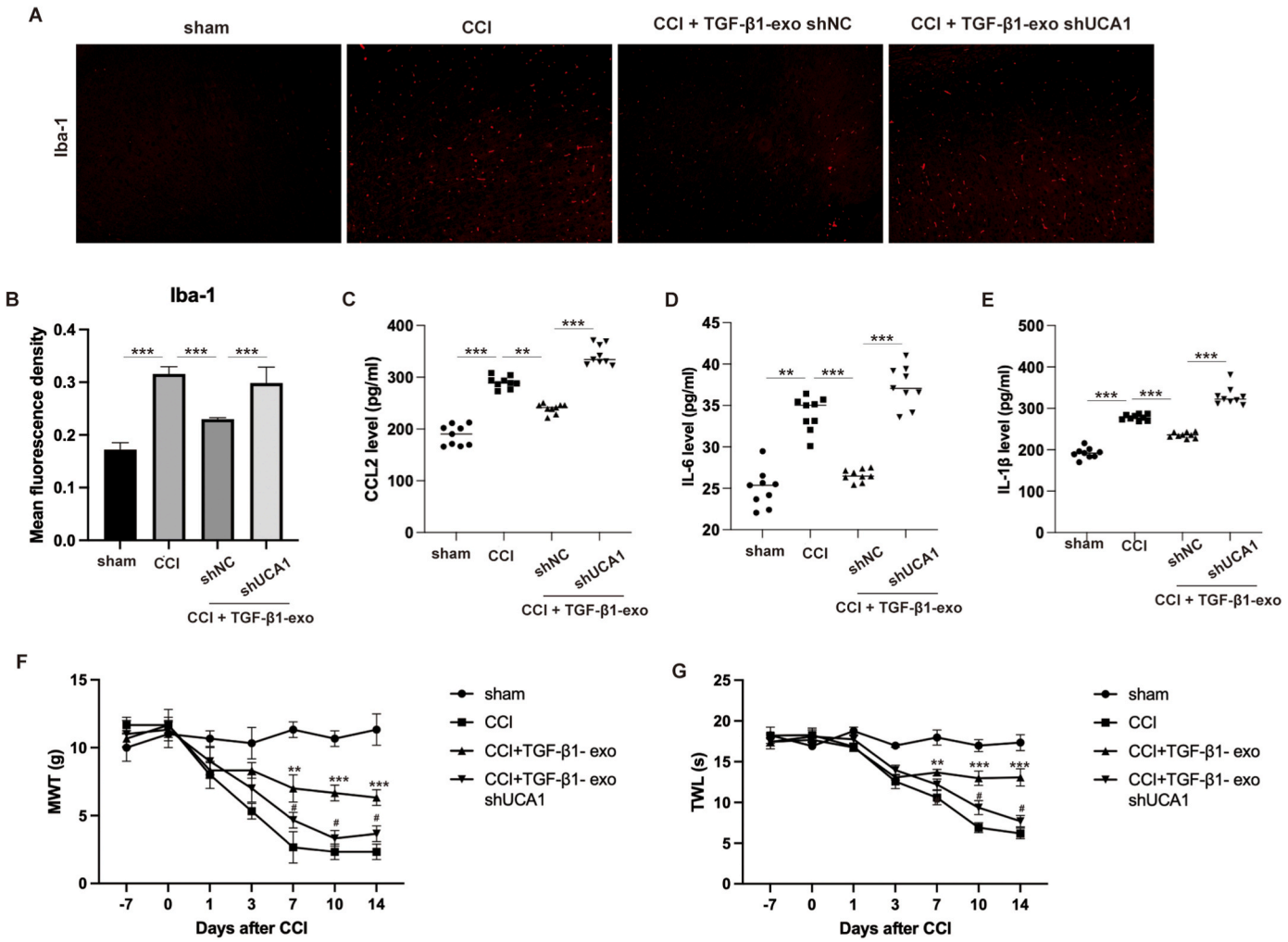

Fig. 1. TGF-β1- hUCMSCs-derived exosomal UCA1 alleviates microgliosis and neuropathic pain in vivo. (A) The representative immunofluorescence image of Iba expression in ipsilateral spinal dorsal horn. (B) Quantification of fluorescence density of Iba1 (n = 3). (C–E) The levels of CCL2, IL-6 and IL-1β were checked by enzyme-linked immunosorbent assay (ELISA). (F and G) The mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) on the ipsilateral paw were checked on day −7, day 0, day 1, day 3, day 7, day 10, and day 14 from CCI therapy. Each group contains 9 rats (n = 9). **p < 0.01, ***p < 0.001. Scale bar, 50 μm.

stimulated by von Frey filaments (0.4–15 g). The MWT was analyzed using the up and down method. The lowest force value that evokes three consistent withdrawal responses was recorded as MWT.

For TWL detection, rats were placed in separated chambers with heat conductive glass and left for 30 min to habituate. Heat stimuli was administrated on the plantar surface of paw three times in a 5-min interval. The average latency was calculated as TWL.

2.8. Immunofluorescence (IF) assay

Iba1 is the biomarker of microglia and macrophages in brain and other tissues. The presence of microglia and inflammation in brain tissues was determined by Iba1 expression. For Iba1 staining, microglia were placed in confocal wells, incubated with exosomes, and transfected with oligonucleotides for 24 h. After that, the cells were washed with PBS, fixed with 4% PFA, blocked with goat serum, and incubated with anti-Iba1 antibody (Abcam, USA) overnight at 4 °C. Next day, the Iba was probed with AlexaFluor 633 for 1 h. Nuclei were labeled with DAPI. Images were taken by a SP8 confocal microscope (Leica, Germany).

2.9. Quantitative real time PCR (qPCR) assay

Total RNA was extracted from cells or spinal dorsal horn tissue using Trizol lysis buffer. A total of 1 μg RNA was transcribed to cDNA using PrimeScript RT reagent Kit (Takara, Japan), following the manufacturer's protocol. The qPCR was performed using SYBR Green PCR Kit (Takara, Japan). Relative RNA expression of CCL2, IL-1β, IL-6, lncUCA1, miR-96-5p was measured following the 2-ΔΔCt method and normalized to endogenous reference gene β-actin or U6.

2.10. Western blotting assay

Protein lysis of tissues and cells were obtained using RIPA buffer following the manufacturer's protocol and quantified using BCA kit. A total of 30 μg protein was separated using 8%–12% SDS-PAGE and transferred to PVDF membranes. Following block in 5% non-fat milk, the targeted proteins were probed using primary antibodies against CD9, CD63, CD 81, TSG101, and Calnexin overnight at 4 °C. Next day, the protein bands were incubated with HRP-conjugated secondary antibodies and reacted with ECL solution (Millipore, Germany) for visualization.

2.11. Enzyme-linked immunosorbent assay (ELISA)

The production of inflammatory factors in ipsilateral spinal dorsal horn tissues and microglia was checked by ELISA assay. In brief, tissues

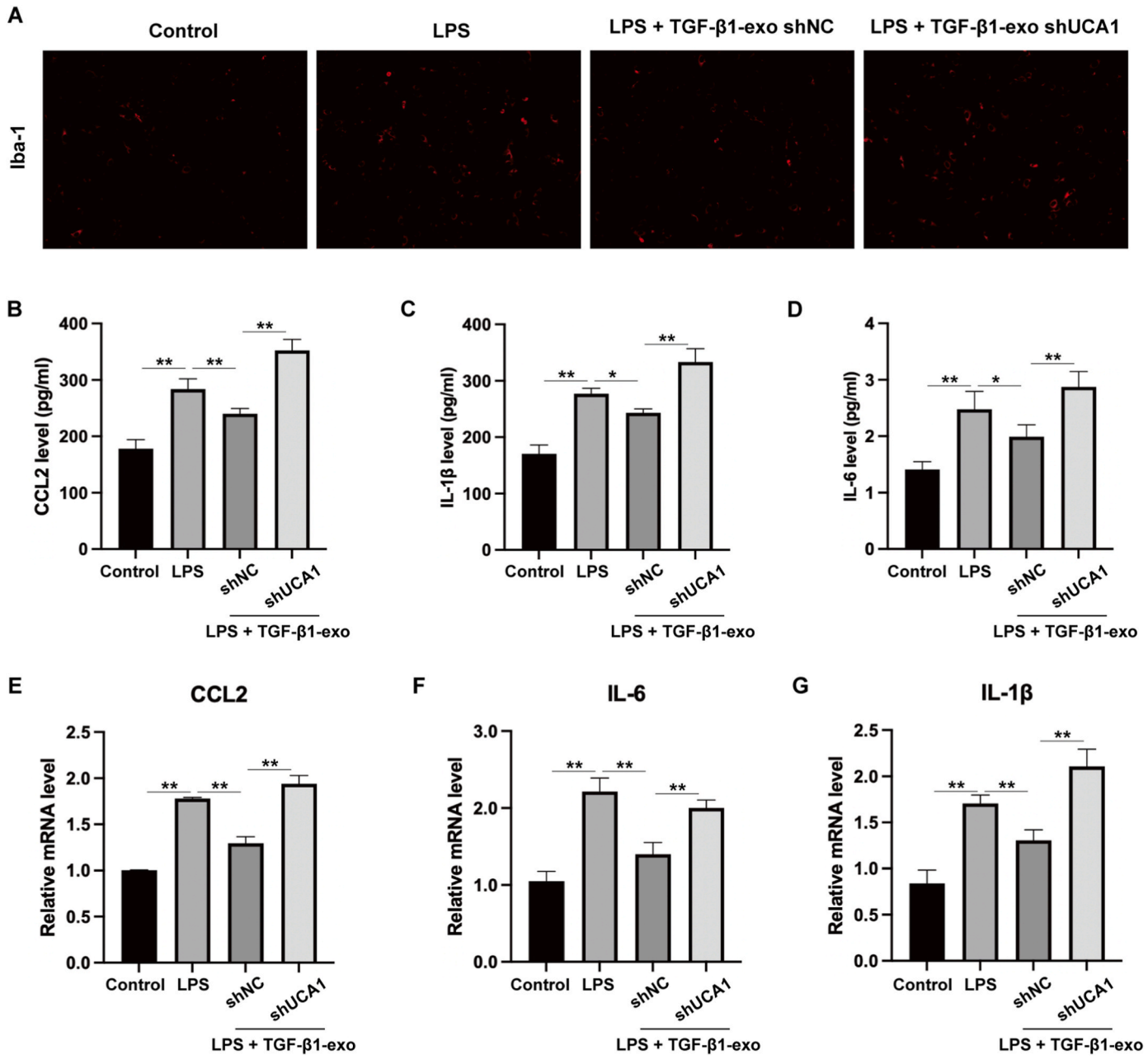

Fig. 2. TGF-β1- hUCMSCs -derived exosomal UCA1 alleviates microgliosis and inflammation in LPS-induced primary microglia. Primary microglia were stimulated with LPS and treated with exosomes from TGF-β1-stimulated hUCMSCs after transfection with shNC or shUCA1. (A) Iba expression was checked by immunofluorescence staining. (B–D) The levels of CCL2, IL-6 and IL-1β were checked by enzyme-linked immunosorbent assay (ELISA). (E–G) The RNA levels of CCL2, IL-6 and IL-1β were checked by qPCR. LPS: stimulation with LPS; LPS + TGF-β1-exo: treatment with LPS and exosomes extracted from TGF-β1-stimulated MSCs; shNC: transfection with shNC; shUCA1: transfection with shUCA1. Each experiment was conducted three independent times (n = 3). **p < 0.01. Scale bar, 50 μm.

were collected and homogenized, then centrifuged to collect the supernatant, and the culture medium of microglia was centrifuged and collected. The levels of CCL2, IL-1β, and IL-6 were measured by ELISA assay kit (Thermo, USA) following manufacturer's description.

2.12. Luciferase reporter gene assay

The UCA1 and 3′UTR sequence of FOXO3a were cloned into the pmirGLO vectors to obtain the wild-type (WT) reporter gene vectors. Similarly, the sequences with mutated miR-96-5p binding site were cloned into pmirGLO vectors to obtain MUT vectors. The WT or MUT vectors were transected into microglia together with miR-96-5p or NC for 48 h. Cells were then homogenized, and luciferase activity was measured by using a dual luciferase reporter gene assay system (Promega, USA).

2.13. RNA pulldown assay

The biotin-labeled wild type (WT) and mutated (MUT) miR-96-5p mimics were brought from RiboBio (Guangzhou, China). Cells lysis were incubated with magnetic beads and then hatched with the miRNA mimics overnight at 4 °C. After elution and washing with PBS, the level of UCA1 pulled down by miR-96-5p was measured by qPCR.

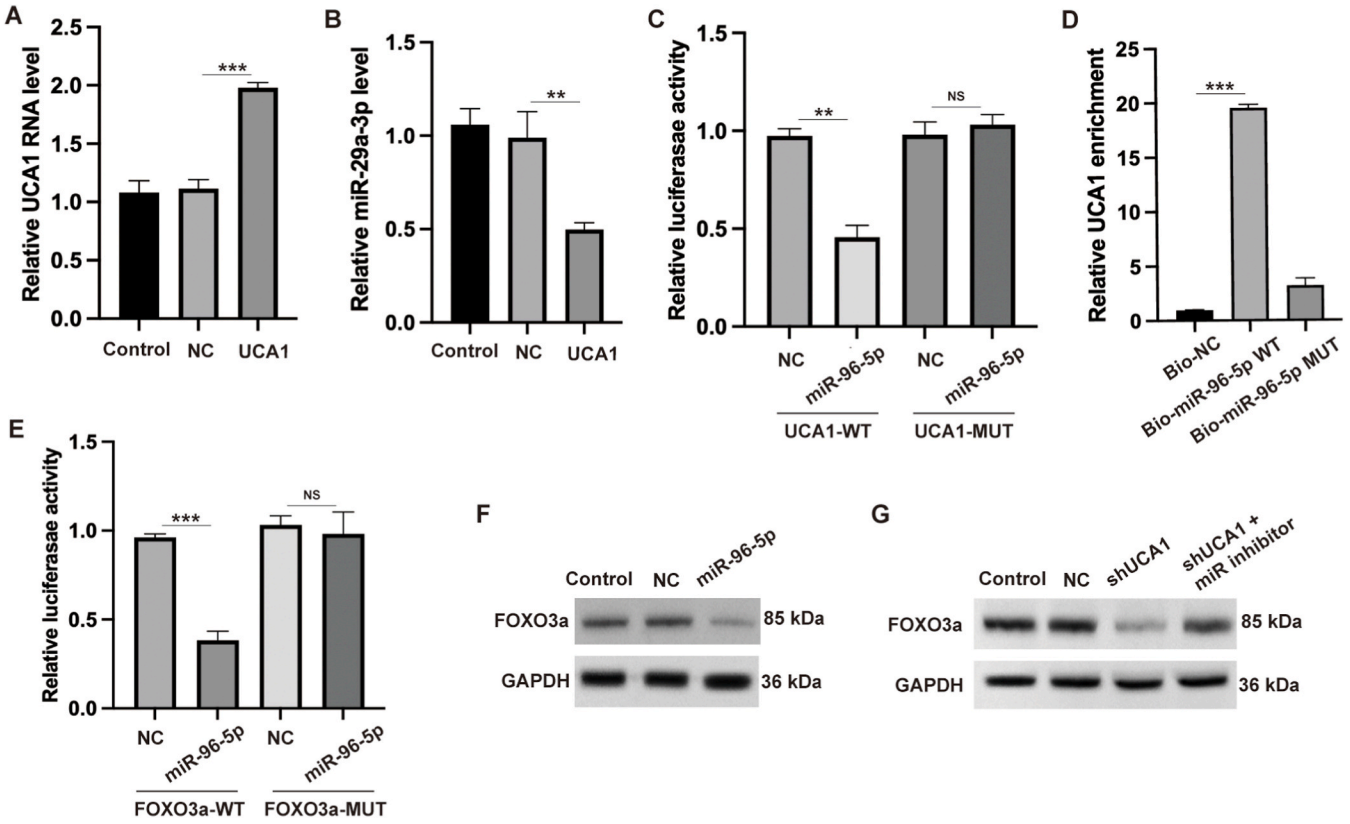

Fig. 3. UCA1 upregulates FOXO3a expression by targeting miR-96-5p in microglia. (A and B) The level of (A) UCA1 and (B) miR-96-5p in microglia that transfected with UCA1 overexpression vectors (UCA1). (C) Luciferase reporter gene activity of wild type (WT) and mutated (MUT) vectors of UCA1. (D) RNA pulldown assay to examine the UCA1 level that bind with biotin-labeled wild type (WT) and mutated (MUT) miR-96-5p. (E) Luciferase reporter gene activity of wild type (WT) and mutated (MUT) vectors of FOXO3a 3′UTR. (F and G) The protein level of FOXO3a was measured by western blotting. NC: transfection with negative control; miR-96-5p: transfection with miR-96-5p mimics; miR inhibitor: transfection with miR-96-5p inhibitors; shUCA1: transfection with shUCA1. Each experiment was conducted three independent times (n = 3). **p < 0.01, ***p < 0.001.

2.14. Statistics

The data were shown as means ± SD and analyzed by SPSS software (Version 19.0) and GraphPad Prism 7.0. The comparison between two groups and multiple groups was conducted by two-tailed unpaired Student's *t*-test and one-way or two-way ANOVA, respectively. $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. TGF-β1 upregulates lncRNA UCA1 expression in hUCMSC-derived exosomes

We first identified the obtained hUCMSCs by flow cytometry. The cell surface biomarkers of MSCs, including CD90, CD73, CD44, CD105, CD151, were highly expressed in hUCMSCs, whereas the level of CD34, CD45, CD133 were low (Fig. S1A). Then exosomes were extracted from the hUCMSCs and results from TEM and NTA presented the morphology (Fig. S1B) and diameter around 100 nm (Fig. S1C). The high levels of CD9, CD63, CD81, and TSG101 and low expression of Calnexin further confirmed the successful extraction of exosomes (Fig. S1D). Moreover, stimulation with TGF-β1 notably enhanced the expression of exosome biomarkers in exosomes that extracted from hUCMSCs compared with those from non-stimulated cells, indicating the elevated content of exosomes (Fig. S1D). After incubation with HAPI cells, the PKH-26-labeled exosomes were observed in cytoplasm, suggesting the successful internalization of exosomes by HAPI cells (Fig. S1E). Noteworthy, the level of UCA1 in exosomes extracted from TGF-β1-stimulated hUCMSCs was significantly higher than that of non-stimulated cells, and depletion of UCA1 in hUCMSCs led to decreased level of UCA1 in extracted exosomes ($p < 0.01$; Fig. S1F). Furthermore, the incubation with TGF-β1-induced exosomes notably elevated the level of UCA1 in HAPI cells, and knockdown of UCA1 also decreased its level in HAPI cells (Fig. S1G).

3.2. TGF-β1- hUCMSCs-derived exosomal UCA1 alleviates microgliosis and neuropathic pain in vivo

To investigate the role of TGF-β1-stimulated exosomal UCA1 for neuropathic pain and spinal microgliosis after CCI, we used exosomes from TGF-β1-stimulated hUCMSCs that transfected with shNC or shUCA1 for treatment of CCI rats. Two weeks after surgery, the ipsilateral spinal dorsal horns were collected for examination. As shown in Fig. 1A and B, the CCI rats exhibited notably elevated the portion of activated microglia, as indicated by the increased expression of Iba1, compared with the sham group ($p < 0.001$). The treatment with exosomes from TGF-β1-stimulated hUCMSCs significantly suppressed this activation, whereas UCA1 depletion abolished this suppression ($p < 0.001$; Fig. 1A and B). Moreover, TGF-β1-stimulated exosomes remarkably attenuated the CCI-induced production of inflammatory mediators, including IL-1β ($p < 0.001$), CCL2 ($p < 0.01$), and IL-6 ($p < 0.001$), whereas knockdown of UCA1 suppressed this effect ($p < 0.001$; Fig. 1C–E). We further evaluated the changes on neuropathic pain by detecting the MWT and TWL. As shown in Fig. 1F and G, the CCI rats that treated with TGF-β1-stimulated exosomes displayed a notable increase the MWT and TWL from day 7, compared with that of the CCI group ($p < 0.001$). Treatment with exosomes from UCA1-depleted hUCMSCs abolished the therapeutic effects of hUCMSCs-exosomes ($p < 0.05$; Fig. 1F and G). These data indicated that TGF-β1-stimulated exosomal

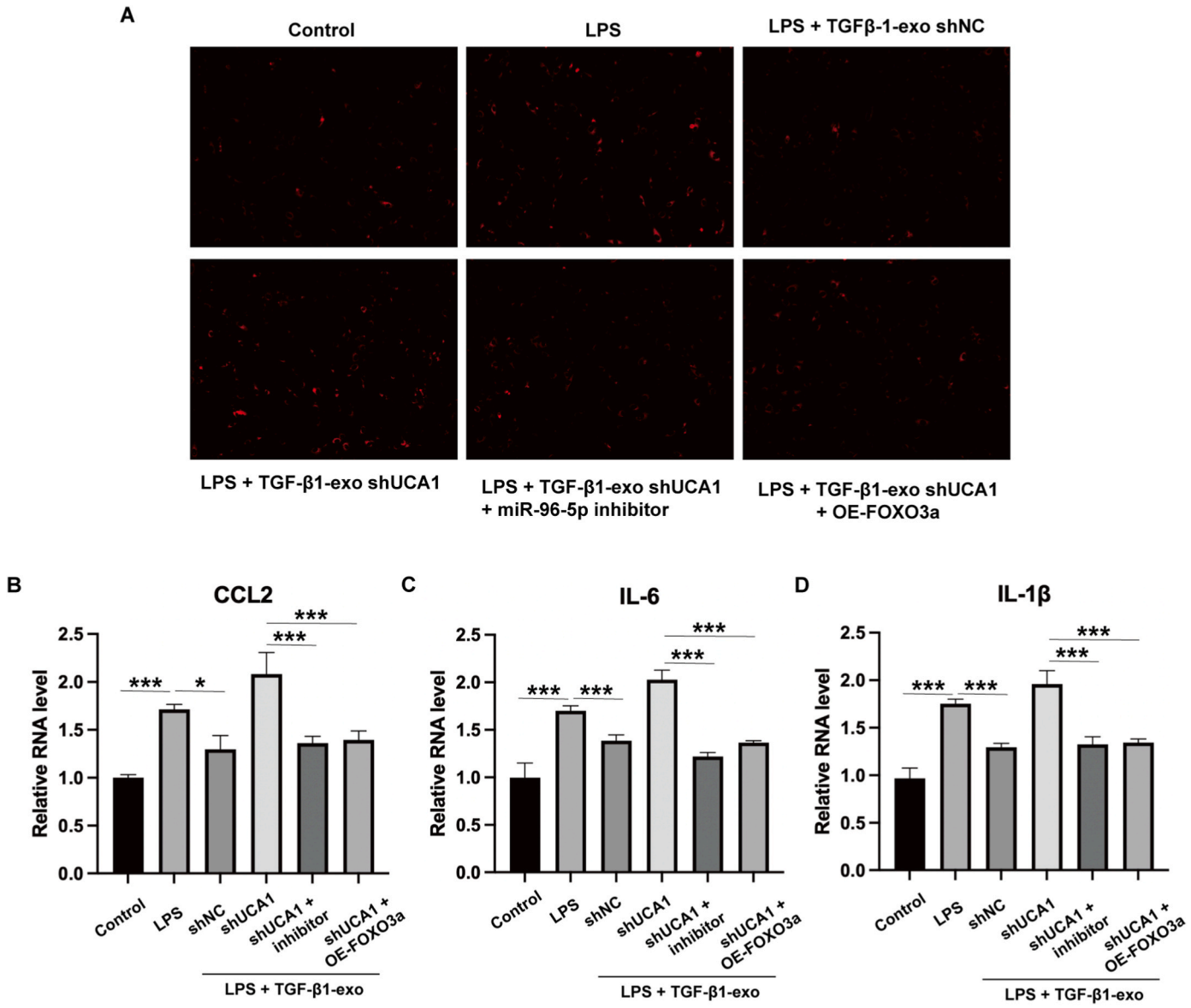

Fig. 4. TGF-β1-hUCMSCs-derived exosomal UCA1 alleviates microgliosis and inflammation in LPS-induced primary microglia by miR-96-5p/FOXO3a. (A) Iba expression was checked by immunofluorescence staining. (B–D) The RNA levels of CCL2, IL-6 and IL-1β were checked by qPCR. LPS: stimulation with LPS; LPS + TGF-β1-exo: treatment with LPS and exosomes extracted from TGF-β1-stimulated MSCs; shNC: transfection with shNC; shUCA1: transfection with shUCA1; inhibitor: transfection with miR-96-5p inhibitors; OE-FOXO3a: transfection with FOXO3a overexpression vectors. Each experiment was conducted three independent times (n = 3). *$p < 0.05$, ***$p < 0.001$. Scale bar, 50 μm.

UCA1 alleviated CCI-induced inflammation and neuropathic pain.

3.3. TGF-β1- hUCMSCs -derived exosomal UCA1 alleviates microgliosis and inflammation in LPS-induced primary microglia

The protective effects of exosomal UCA1 against CCI-induced damage were further checked by in vitro model. The primary microglia were stimulated with LPS to mimic CCI-induced inflammatory response. The LPS-induced activation of microglia was suppressed by TGF-β1-hUCMSCs-derived exosomes, which was then notably elevated by knockdown of UCA1 in hUCMSCs (Fig. 2A). The secretion (Fig. 2B–D) and RNA expression (Fig. 2E–G) of LPS-induced inflammation factors, CCL2 ($p < 0.01$), Il-6 ($p < 0.05$) and Il-1β ($p < 0.05$), were remarkably suppressed by exosomes from TGF-β1-stimulated hUCMSCs, whereas knockdown of UCA1 abolished this effect ($p < 0.01$). These data indicated that the exosomal UCA1 suppresses production of inflammatory factors in microglia in vitro.

3.4. UCA1 upregulates FOXO3a expression by targeting miR-96-5p in microglia

Subsequently, we examined the molecular mechanisms underlying UCA1 regulated microglia phenotype. We observed that transfection with UCA1 notably elevated the level of UCA1 in primary microglia ($p < 0.001$; Fig. 3A) and downregulated the level of miR-96-5p ($p < 0.01$; Fig. 3B). Moreover, we observed that miR-96-5p notably suppressed the luciferase activity of wild type reporter (WT) gene vectors of UCA1 (UCA1-WT) ($p < 0.01$) but did not affect the activity of mutated vectors (MUT) (Fig. 3C). The biotin-labeled miR-96-5p could significantly pull down the UCA1 RNA ($p < 0.001$; Fig. 3D). These data indicated that UCA1 directly interact with the miR-96-5p. We next determined whether miR-96-5p directly target the FOXO3a RNA. The results from luciferase reporter gene assay indicated that miR-96-5p significantly suppressed the activity of wild type FOXO3a vector ($p < 0.01$), rather than the mutated type (Fig. 3E). Besides, the miR-96-5p mimics notably

suppressed the protein level of FOXO3a in microglia (Fig. 3F). Furthermore, knockdown of UCA1 suppressed FOXO3a expression and inhibition of miR-96-5p recovered the level of FOXO3a (Fig. 3G). These data demonstrated that UCA1 modulates the expression of FOXO3a via targeting miR-96-5p.

3.5. TGF-β1-hUCMSCs-derived exosomal UCA1 alleviates microgliosis and inflammation in LPS-induced primary microglia by miR-96-5p/FOXO3a

Subsequently, we verified the UCA1 regulated miR-96-5p/FOXO3a axis in microglia inflammation. The exosomal UCA1 decreased the level of Iba-positive microglia (Fig. 4A) and production of inflammatory cytokines (Fig. 4B–D), and UCA1 knockdown inhibited this effect. However, the inhibition of miR-96-5p and overexpression of FOXO3a repressed the effects of UCA1 depletion on microglia activation and inflammatory response. These data confirmed that the TGF-β1-hUCMSCs-derived exosomal UCA1 functions through miR-96-5p/FOXO3a axis in LPS-stimulated microglia.

4. Discussion

In present work, we explored the function and underlying mechanisms of TGF-β1-stimulated hUCMSCs-derived exosomal UCA1 in microgliosis and neuropathic pain using a rat CCI model and LPS-induced microglia model. We observed that TGF-β1-stimulated hUCMSCs presented elevated level of UCA1 in exosomes, which could be internalized by microglia. The study on effects of exosomal lncRNA UCA1 in diseases is rare and is absent in studies on neuropathic pain. Previous study indicated that exosomal lncUCA1 that secreted by hypoxia-induced pancreatic stellate cells was delivered to pancreatic cells and promoted pancreatic cancer resistance to gemcitabine via recruiting EZH2 to regulate the methylation level in SOCS3 gene [31]. The UCA1 produced by cervical cancer stem cells can augment the self-renewal ability and differentiation ability of cancer stem cells by targeting the miR-122-5p and upregulating the SOX2 expression [32]. Besides, the lncUCA1 could promote the repair of hyperglycemic vascular smooth muscle cells to alleviate diabetic angiopathy by targeting the miR-582-5p [33]. Here, we proved that exosomal UCA1 from TGF-β1-stimulated hUCMSCs alleviated the neuropathic pain, as manifested by elevated MWT and TWL, and suppressed the activation of microglia and production of inflammatory cytokines in CCI rats and LPS-induced microglia model.

Microglia are the resident immune cells in CNS and act as the first active immune defense [34]. Peripheral nerve injury induces microgliosis in the spinal dorsal horn and is regarded as the major factor of neuropathic pain that resulted from peripheral nerve injury [35,36]. Accumulating evidence has demonstrated that inhibiting the microglial response could effectively alleviate the neuropathic pain [37]. A great number of signaling factors in microglia has been identified to participate in the microgliosis, such as the MCP-1, CX3CL1, and LPS [38]. Besides, the development of microgliosis also relies on the inflammatory cytokines [39]. The activated microglia produce various inflammatory factors, such as IL-1β, IL-6, and TNF, which sensitize the spinal neurons and contributes to neuropathic pain following peripheral nerve injury [35]. Further study on molecular mechanisms of TGF-β1-stimulated hUCMSCs exosomal UCA1 revealed that UCA1 targets miR-96-5p to upregulate the expression of FOXO3a in microglia. The miR-96-5p has been reported to participate in development of several diseases. For example, miR-96-5p targets the MAPK signaling to promote the migration of breast cancer cells [40]. The miR-96-5p promotes the production of inflammatory cytokines and the activation of mTOR/NF-κB pathway in the pathophysiological process of allergic rhinitis [41]. Previous study has reported that FOXO3a exhibits neuroprotective effect on microglia by alleviating pyroptosis and mitophagy [42]. Consistently, our findings proved that the FOXO3a expression in microglia was elevated by exosomal UCA1.

5. Conclusion

To conclude, the TGF-β1-stimulated hUCMSCs contain high level of UCA1, which could be delivered to microglia to suppress the microgliosis and alleviate the neuropathic pain. The molecular mechanism study demonstrated that UCA1 directly interact with miR-95-6p to release the expression of FOXO3a. Our findings presented TGF-β1-stimulated hUCMSCs exosomal UCA1 as potential therapeutic manner to alleviate the neuropathic pain.

Funding

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Declaration of competing interest

There are no conflicts of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101477.

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