Interleukin-1β attenuates the proliferation and differentiation of oligodendrocyte precursor cells through regulation of the microRNA-202-3p/β-catenin/Gli1 axis

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Received February 13, 2020; Accepted May 12, 2020

DOI: 10.3892/ijmm.2020.4648

Abstract. The inflammatory cytokine interleukin (IL)-1β has been implicated in demyelinating diseases, such as multiple sclerosis and experimental autoimmune encephalomyelitis, and brain degenerative diseases, such as Alzheimer's disease. However, the cellular and molecular mechanisms underlying the damaging effects of IL-1β on myelination are poorly understood. Therefore, the present study was designed to investigate whether IL-1β modifies the proliferation and differentiation of oligodendrocyte precursor cells (OPCs) through regulating the miR-202-3p/β-catenin/glioma-associated oncogene homolog 1 (Gli1) axis. It was observed that IL-1β significantly attenuated the proliferation and differentiation of OPCs, as evidenced by a decrease in bromodeoxyuridine incorporation and reduced percentage of myelin basic protein-positive cells among the total number of oligodendrocyte transcription factor 2-positive cells. In addition, IL-1β markedly decreased the expression of miR-202-3p and increased the protein expression of β-catenin and Gli1, all of which were reversed by the IL-1β inhibitor, IL-1Ra. Treatment with the β-catenin inhibitor XAV939, Gli1 siRNA, or miR-202-3p mimic transfection, attenuated the IL-1β-induced suppression of OPC proliferation and differentiation. Treatment with XAV939 decreased the expression of Gli1. Transfection of miR-202-3p mimic attenuated the expression of β-catenin and Gli1. As demonstrated by the findings of the present study, IL-1β suppressed the proliferation and differentiation of OPCs through regulation of the miR-202-3p/β-catenin/Gli1 axis. Therefore, the miR-202-3p/β-catenin/Gli1 axis may be of value as a therapeutic target in multiple sclerosis.

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Key words: interleukin-1β, oligodendrocyte precursor cells, β-catenin, glioma-associated oncogene homolog 1, miR-202-3p

Introduction

Myelin is a membrane structure surrounding the axon of nerve cells that acts as an insulating sheath to prevent the mutual interference between nerve impulses conducted by various nerve fibers, but also acts by increasing the impulse conduction velocity of nerve fibers (1). Therefore, myelin plays an important role in the function of the nervous system. Demyelinating diseases of the central nervous system, such as multiple sclerosis, may destroy the myelin sheath, leading to its rupture, with subsequent clearance of myelin debris by phagocytes, such as microglia, a process referred to as demyelination. These changes markedly affect the function of axons and neurons in diseased tissues, and promote the development of central nervous system (CNS) diseases (2). The myelin sheath of the CNS is mainly composed of oligodendrocytes derived from oligodendrocyte precursor cells (OPCs). OPCs are widely distributed in the gray and white matter of the adult brain, and play an important role in the regeneration and repair of myelin in chronic demyelinating diseases (3,4). However, the inflammatory reaction and oxidative stress occurring in these diseases often adversely affect the proliferation and differentiation of OPCs and affect the regeneration and repair of the myelin sheath (4-6). Furthermore, when these diseases occur, some inflammatory cells are activated and accumulate in the lesion sites, releasing a large number of inflammatory factors, including interleukin (IL)-1β, tumor necrosis factor-α and IL-11. These inflammatory factors can affect the formation of the myelin sheath by regulating the functional activities of oligodendrocytes (7-9). Among those, IL-1β can promote oligodendrocyte apoptosis (8). Additionally, IL-1β has been reported to impede OPC recruitment during chronic cerebral hypoperfusion (10). However, the molecular mechanisms underlying the damaging effects of IL-1β on OPCs remain unclear.

A possible mechanism underlying endogenous OPC injury is the dysregulation of various microRNAs. MicroRNA(miR)-219 has been reported to play a critical role in coupling differentiation to proliferation arrest in the oligodendrocyte lineage, enabling the rapid transition from proliferating OPCs to myelinating oligodendrocytes (11). Stroke can downregulate the expression of miR-9 and miR-200 to stimulate the upregulation of serum response factor and, thus, regulate OPC differentiation (12). Overexpression

of miR-146a promotes oligodendrogenesis in stroke (13). miR-202-3p, an intracellular target of IL-1β, has been reported to be expressed in glioma (14). However, the functional role of miR-202-3p in the regulation of OPC proliferation and differentiation remains unclear.

In light of these observations, it was hypothesized that IL-1β modulates the expression of miR-202-3p in OPCs, and that this modulation, if present, is responsible for the damaging effects of IL-1β on OPC proliferation and differentiation.

Materials and methods

Reagents. Recombinant rat IL-1β (cat. no. 501-RL) and the IL-1β receptor inhibitor IL-1Ra (cat. no. 1545-RA) were purchased from R&D Systems, Inc.; the β-catenin inhibitor XAV939 (cat. no. X3004) and bromodeoxyuridine (BrdU) incorporation ELISA kit (cat. no. 11647229001) for cell proliferation detection were purchased from Sigma-Aldrich; Merck KGaA. Anti-platelet-derived growth factor receptor (PDGFR)-α (cat. no. ab203491, dilution 1:500), anti-β-catenin (cat. no. ab32572, dilution 1:5,000), anti-glioma-related oncogene homolog (Gli)-1 (cat. no. ab49314, dilution 1:1,000), anti-GAPDH (cat. no. ab9485, dilution 1:2,500), anti-myelin basic protein (MBP, cat. no. ab7349, dilution 1:200) and anti-oligodendrocyte transcription factor (Olig)2 (cat. no. ab42453, dilution 1:200) antibodies were purchased from Abcam. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (cat. no. A16116, dilution 1:2,000) was purchased from Thermo Fisher Scientific, Inc. miR-202-3p mimic, negative scramble control (NC), blank siRNA and Gli1 siRNA were synthesized by Guangzhou RiboBio Co., Ltd. The transfection reagent Lipofectamine 2000, Alexa Fluor 488 and Alexa Fluor 568 were purchased from Invitrogen; Thermo Fisher Scientific, Inc. The BCA kit for protein detection, DAPI and RIPA lysis buffer were purchased from Shanghai Beyotime Biotechnology Co., Ltd. Primer sequences were synthesized by Shanghai Sangon Biotech Co., Ltd.

Isolation and culture of OPCs. As described previously (10), primary OPCs were isolated from 20 Sprague-Dawley rats (1-2 days old) of SPF grade, provided by the Laboratory Animal Center of Chengdu Medical College. All animal procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee at Chengdu Medical College (Chengdu, China). Briefly, the rats were anesthetized by hypothermia (chilling on ice for ~5 min) and sacrificed by decapitation. After the animals were sacrificed, their brains were removed and cerebral cortices were harvested, minced and digested in 0.25% trypsin solution for 15 min at 37˚C. Dissociated cells were adjusted to $1.2x10^9$ cells/l, plated in the flasks, and cultured for ~10 days in an incubator with 5% CO_2 at 37˚C. After the OPCs were >70% confluent, the flasks were placed in an orbital laboratory shaker (model SHKE2000; Thermo Fisher Scientific, Inc.) at 37˚C and shaken for 1 h at a speed of 1.03 x g to remove microglia. After the medium was replaced by fresh medium, the flasks continued to be shaken overnight (20 h). The medium was collected, plated on a 10-cm culture dish, and cultured for 1 h at 37˚C for adherence of astrocytes and microglia. To eliminate contaminating astrocytes and microglia, the non-adherent cells were collected to obtain the purified OPCs. Purified OPCs were identified by immunofluorescence detection of PDGFR-α. When the purity of PDGFR-α-positive cells was >95%, cultured OPCs were selected for drug treatment.

For the observation of OPC proliferation, purified OPCs were replated in DMEM containing 2% B27, 10 ng/ml PDGF-AA and 10 ng/ml basic fibroblast growth factor (OPC proliferation medium) on 96-well plates. For the observation of OPC differentiation, purified OPCs were replated in DMEM containing 10 ng/ml ciliary neurotrophic factor and 15 nmol/l T3 thyroid hormone (OPC differentiation medium) on a 6-well plate coated with poly-L-lysine.

Drug treatment of OPCs. Before the proliferation or differentiation experiments, OPCs were randomly divided into the normal control, IL-1β, IL-1Ra, XAV939, blank siRNA, Gli1 siRNA, negative control (NC) transfection and miR-202-3p transfection groups. The normal control group cells were cultured in medium without any drugs for 4 days. Cells in the other groups were cultured in medium containing 30 ng/ml recombinant rat IL-1β, with or without IL-1Ra (10 ng/ml for the IL-1Ra group), XAV939 (0.05 μmol/l for the XAV939 group), Gli1 siRNA (50 nmol/l for the Gli1 siRNA group), or miR-202-3p mimics (30 nmol/l for the miR-202-3p transfection group) for 4 days. The blank siRNA and NC transfection groups were cultured in medium containing 30 ng/ml IL-1β with blank siRNA or Lipofectamine 2000, respectively. After 4 days of drug treatment, OPCs were collected for analysis. The doses of IL-1Ra and XAV939 were selected according to previous reports (15,16).

Detection of OPC proliferation. The BrdU incorporation method was used to detect OPC proliferation. One day before the completion of drug treatment, BrdU was added into each well at a final concentration of 10 μmol/l, and the culture was continued for 24 h at 37˚C. After being fixed with FixDenat for 30 min at room temperature, OPCs were incubated with the anti-BrdU-POD antibody at room temperature for 90 min, followed by substrate reaction solution for 30 min. After the reaction was terminated with a termination reaction solution, the absorbance value at 450 nm (A450) of each well was detected using a Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments, Inc.).

Detection of the proportion of MBP-positive cells. The double immunofluorescence staining method was used to detect the expression of MBP and Olig2. The proportion of MBP-positive cells among cultured cells was used as the differentiation index of OPCs based on a previous study (17). After the completion of drug treatment, the OPCs attached to the coverglass were fixed with 10% formalin at room temperature for 30 min, treated with 0.1% Triton-X for 20 min, and blocked with 1.5% goat serum for 1 h. OPCs were then incubated with primary antibodies (anti-MBP and anti-Olig2) at 4˚C overnight followed by the fluorescent secondary antibodies (Alexa Fluor 568-labeled anti-rabbit IgG and Alexa Fluor 488-labeled anti-mouse IgG) at room temperature for 1 h. After the nuclei were counterstained with DAPI, the images were collected

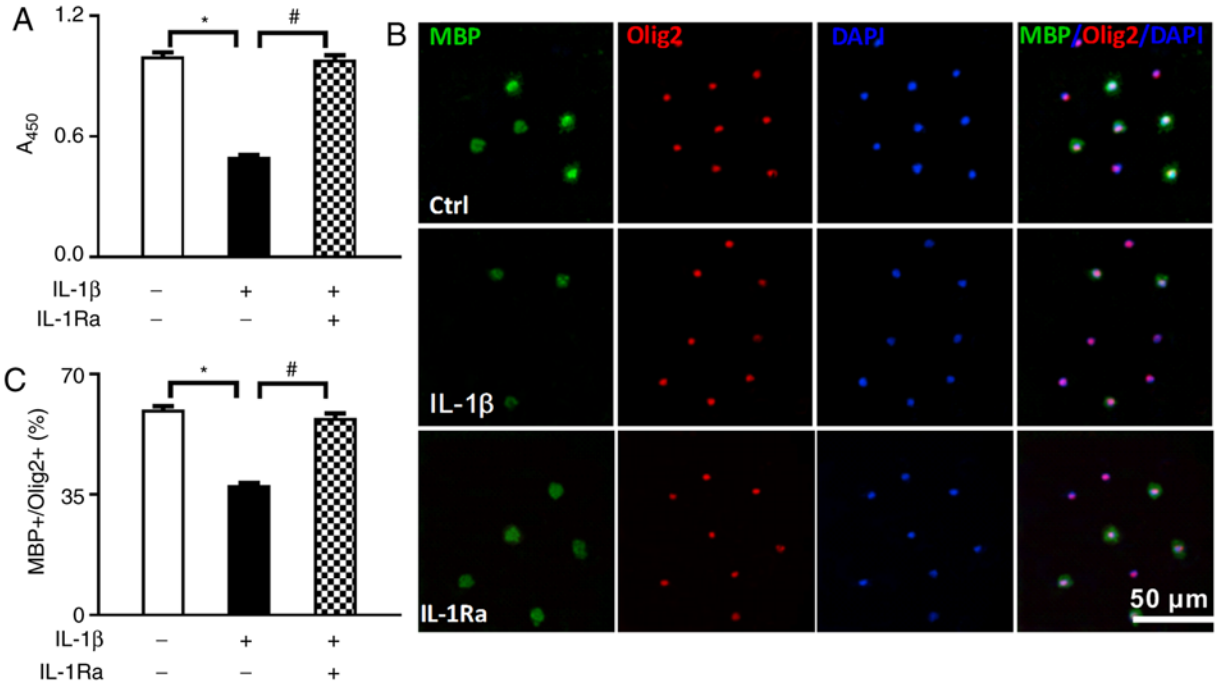

Figure 1. Inhibitory effects of IL-1β on the proliferation and differentiation of OPCs. (A) Bromodeoxyuridine incorporation in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of the IL-1β receptor inhibitor IL-1Ra (10 ng/ml). (B) Representative images of double immunofluorescence staining for MBP and Olig2. (C) Percentage of MBP-positive cells in the total number of Olig2-positive cells [(MBP$^+$/Olig2$^+$)%] in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of IL-1Ra (10 ng/ml). Values are presented as mean ± standard error of the mean of 8-10 independent trials and are expressed as folds of control. *P<0.05 vs. normal control group; $^\#$P<0.05 vs. IL-1β alone. IL, interleukin; OPCs, oligodendrocyte precursor cells; MBP, myelin basic protein; Olig2, oligodendrocyte transcription factor 2.

and analyzed using a Nikon ECLIPSE Ti-S fluorescent microscope at a magnification of x200 (Nikon Corporation). At least 20 fields were counted for each sample. As MBP is expressed in mature oligodendrocytes and Olig2 is expressed in all developmental stages from OPCs to mature oligodendrocytes, the percentage of MBP-positive cells in the total number of Olig2-positive cells [(MBP$^+$/Olig2$^+$)%] was considered as the indicator of OPC differentiation in the present study.

Western blot analysis. The protein expression of β-catenin and Gli1 was detected by western blotting. After drug treatment, the cells of each group were collected and total protein in OPCs was extracted with RIPA lysis buffer. The protein concentration was detected by the BCA protein detection kit. Proteins (40 μg) were separated by 10% SDS-PAGE and transferred to a PVDF membrane (Invitrogen; Thermo Fisher Scientific, Inc.). The membrane was blocked with 5% skimmed milk for 1 h. Subsequently, the membranes were incubated overnight at 4˚C with anti-β-catenin, anti-Gli1 and anti-GAPDH. The membranes were incubated for 1 h at room temperature with HRP-conjugated anti-IgG. Enhanced chemiluminescence reagent was added to display the band of target proteins. Images of the protein bands were digitally captured and analyzed with ImageJ software (version 1.52v, National Institutes of Health). The relative expression of target proteins was expressed as the ratio of target proteins to the GAPDH protein.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. RT-qPCR analysis was used to detect the expression of miR-202-3p. After drug treatment, total RNA was extracted from OPCs with TRIzol reagent kit (Invitrogen; Thermo Fisher Scientific, Inc.). RNA concentration was measured by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). Subsequently, cDNA was synthesized by the TaqMan microRNA Reverse Transcription kit (Invitrogen; Thermo Fisher Scientific, Inc.), and the amplification reaction was completed in the MX3005p PCR instrument (Agilent Technologies, Inc.). For miR-202-3p amplification, the amplification conditions included denaturation at 95˚C for 3 min, followed by 35 cycles at 95˚C for 10 sec, and 60˚C for 30 sec. Primer sequences were as follows: miR-202-3p sense, 5'-CTCCAGAGAUAGUAGAG CCT-3' and antisense, 5'-CTCAACCACCATCACCTGAC AGA-3'; and internal reference sense, 5'-CTCGCTTCGGAG CACA-3' and antisense, 5'-AACGCTTCACGAATTGCGT-3'. The relative expression of miR-202-3p was expressed as miR-202-3p/U6.

Statistical analysis. All quantitative data are expressed as mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test. P<0.05 was considered to indicate statistically significant differences.

Results

IL-1β inhibits the proliferation and differentiation of OPCs. To observe the effects of IL-1β on OPC proliferation, the BrdU incorporation method was applied and cell proliferation was expressed as A450 of each well. As shown in Fig. 1A, when compared with the normal control group, IL-1β treatment significantly inhibited the proliferation and viability of OPCs

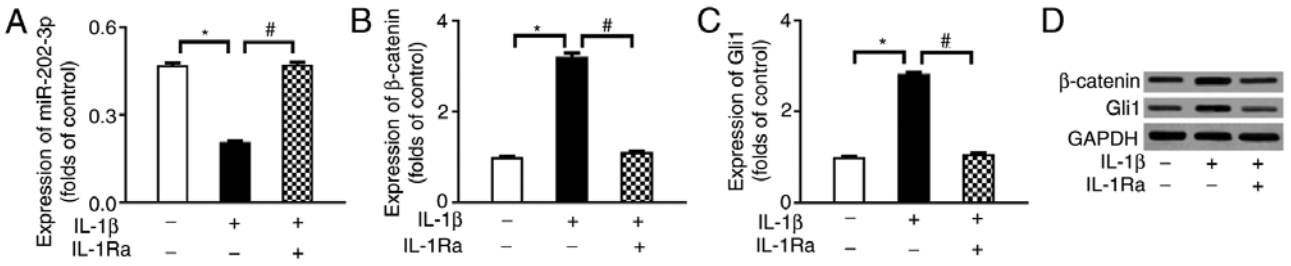

Figure 2. Effects of IL-1β on the expression of the miR-202-3p/β-catenin/Gli1 axis in OPCs. Relative expression of (A) miR-202-3p or (B) β-catenin or (C) Gli1 in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of the IL-1β receptor inhibitor IL-1Ra (10 ng/ml). (D) Representative images of β-catenin and Gli1 protein bands. Scale bar, 50 μm. Values are presented as mean ± standard error of the mean of 6-8 independent trials and are expressed as folds of control. *P<0.05 vs. normal control group; #P<0.05 vs. IL-1β alone. IL, interleukin; OPCs, oligodendrocyte precursor cells; Gli1, glioma-related oncogene homolog 1.

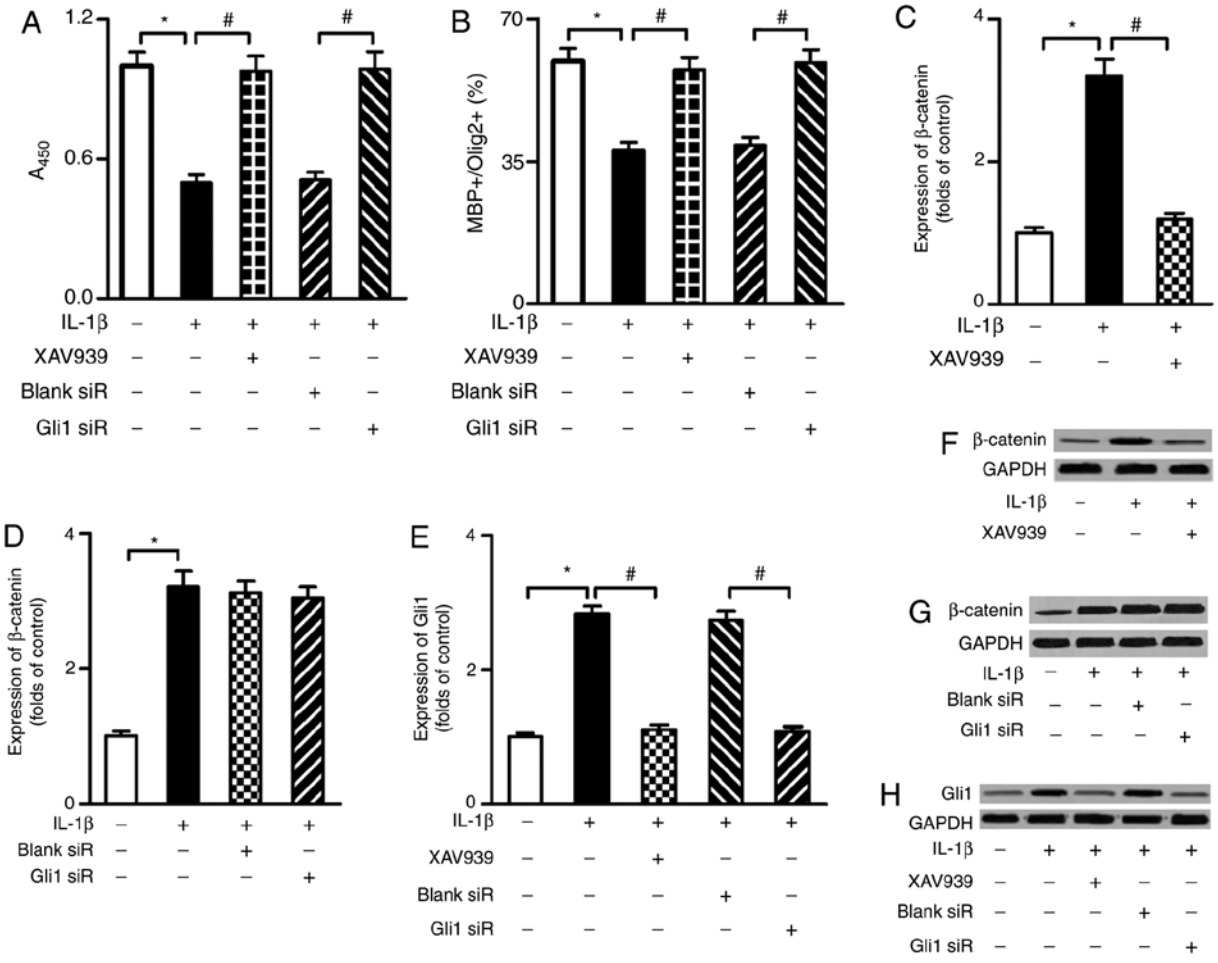

Figure 3. Effects of inhibition of the β-catenin/Gli1 pathway on the proliferation and differentiation of OPCs. (A and B) Bromodeoxyuridine incorporation and the percentage of MBP-positive cells in the total number of Olig2-positive cells [(MBP+/Olig2+)%] in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of the β-catenin inhibitor XAV939 (0.05 μmol/l) or Gli1 siRNA (50 nmol/l). (C-E) Relative expression of β-catenin and Gli1 in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of the β-catenin inhibitor XAV939 (0.05 μmol/l), blank siRNA (50 nmol/l), or Gli1 siRNA (50 nmol/l). (F-H) Representative images of β-catenin and Gli1 protein bands. Values are presented as mean ± standard error of the mean of 6-8 independent trials and are expressed as folds of control. *P<0.05 vs. normal control group; #P<0.05 vs. IL-1β alone or blank siRNA. IL, interleukin; OPCs, oligodendrocyte precursor cells; MBP, myelin basic protein; Olig2, oligodendrocyte transcription factor 2; Gli1, glioma-related oncogene homolog 1.

(P<0.05), which was reversed by the IL-1β receptor inhibitor IL-1Ra (P<0.05).

To observe the effects of IL-1β on OPC differentiation, double immunofluorescence staining was applied to detect the expression of MBP and Olig2. The percentage of MBP-positive cells in the total number of Olig2-positive cells [(MBP+/Olig2+)%] was considered as the index of OPC differentiation. As shown in Fig. 1B, the cytoplasm in MBP-positive cells appeared as bright green, and the nuclei in Olig2-positive cells were red. As shown in Fig. 1C, when compared with the normal control group, IL-1β treatment significantly decreased the differentiation index of OPCs (P<0.05), which was reversed by the IL-1β receptor inhibitor IL-1Ra (P<0.05).

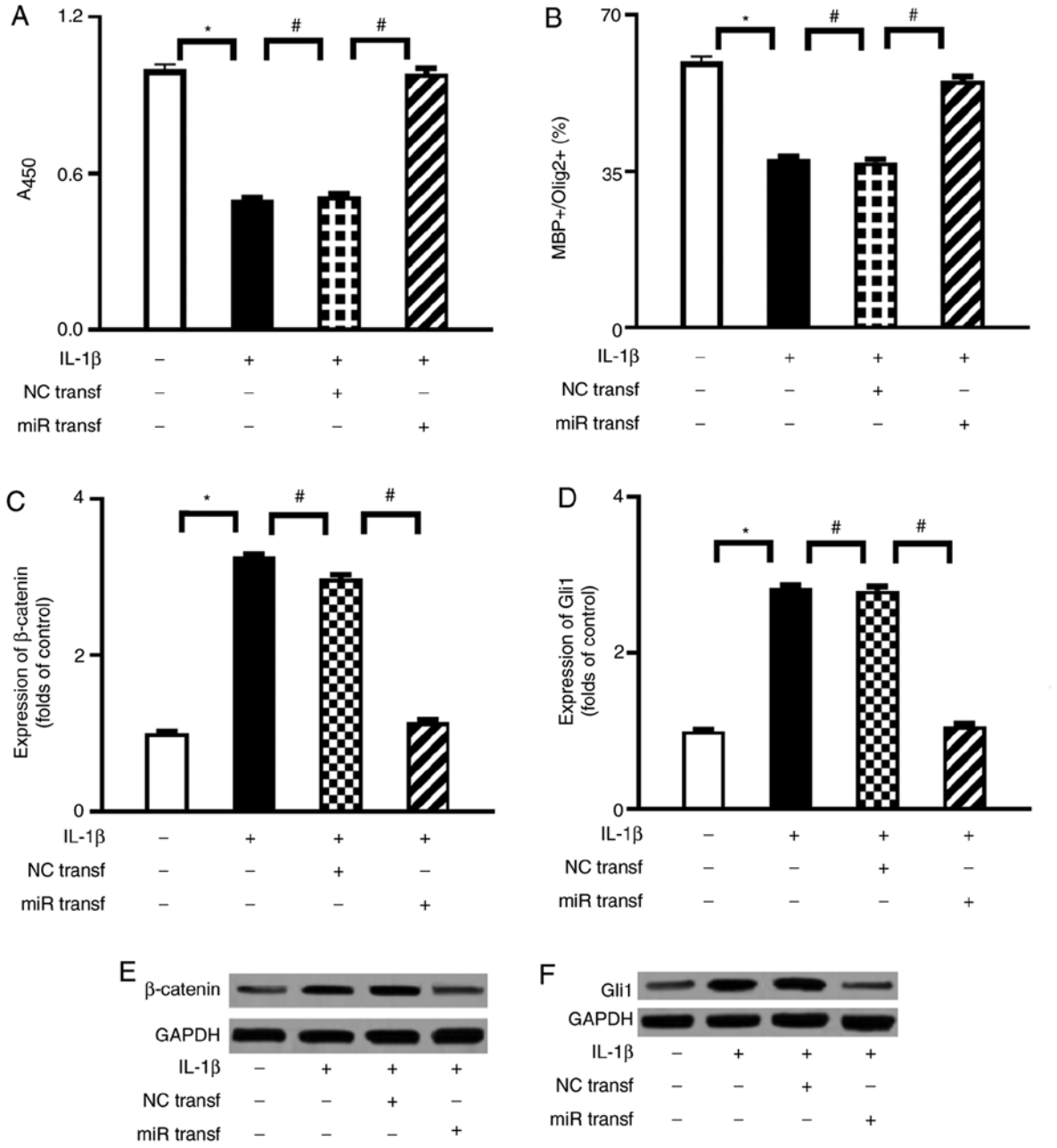

Figure 4. Effects of miR-202-3p mimic transfection on the proliferation and differentiation of OPCs and the expression of the β-catenin/Gli1 pathway. (A and B) Bromodeoxyuridine incorporation and the percentage of MBP-positive cells in the total number of Olig2-positive cells [(MBP$^+$/Olig2$^+$)%)] in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of miR-202-3p mimic transfection (30 nmol/l). (C and D) Relative expression of β-catenin and Gli1 in cultured OPCs treated with 30 ng/ml IL-1β in the presence or absence of miR-202-3p mimic transfection (30 nmol/l). (E and F) Representative images of β-catenin and Gli1 protein bands. Values are mean ± standard error of the mean of 6-8 independent trials and are expressed as folds of control. *P<0.05 vs. normal control group; $^\#$P<0.05 vs. NC transfection. IL, interleukin; OPCs, oligodendrocyte precursor cells; MBP, myelin basic protein; Olig2, oligodendrocyte transcription factor 2; Gli1, glioma-related oncogene homolog 1.

IL-1β modulates the expression of the miR-202-3p/β-catenin/Gli1 axis in OPCs. To investigate the possible role of the miR-202-3p/β-catenin/Gli1 axis in IL-1β suppression of OPC proliferation and differentiation, the expression of the miR-202-3p/β-catenin/Gli1 axis was determined by western blotting and RT-qPCR analysis. As shown in Fig. 2, when compared with the normal control group, IL-1β treatment significantly decreased the expression of miR-202-3p (P<0.05) and increased the protein expression of β-catenin and Gli1 (P<0.05), all of which were reversed by the IL-1β inhibitor IL-1Ra (P<0.05).

Inhibition of the β-catenin/Gli1 pathway improves the proliferation and differentiation of OPCs. To evaluate the role of the β-catenin/Gli1 pathway in IL-1β-mediated suppression of OPC proliferation and differentiation, the β-catenin inhibitor XAV939 and Gli1 siRNA were used in the present study. Knockdown of Gli1 protein was validated by western blot analysis (Fig. S1). As shown in Fig. 3, when compared with the IL-1β group, the β-catenin inhibitor XAV939 markedly improved the proliferation and differentiation of OPCs (P<0.05) and decreased the expression of Gli1 (P<0.05). Furthermore, when compared with the blank siRNA group,

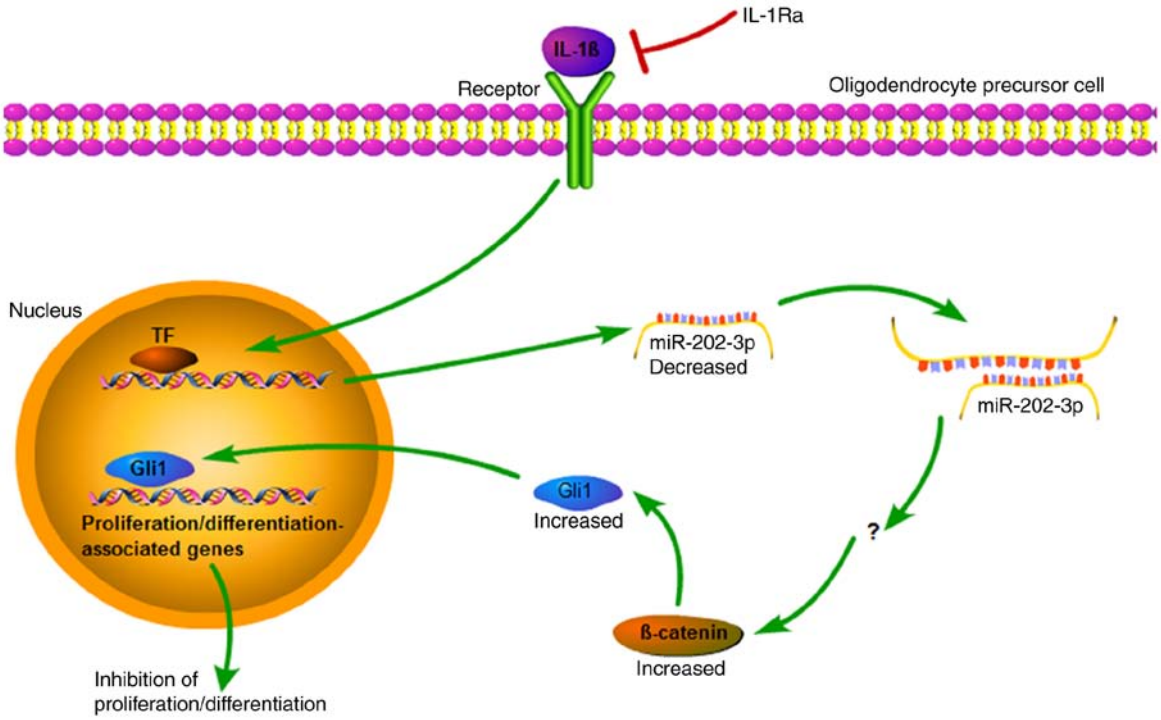

Figure 5. Proposed mechanisms underlying the damaging effects of IL-1β on OPCs. IL, interleukin; OPCs, oligodendrocyte precursor cells; TF, transcription factor; Gli1, glioma-related oncogene homolog 1.

the Gli1 siRNA group exhibited enhanced proliferation activity and higher differentiation index of OPCs ($P<0.05$). However, the transfection of Gli1 siRNA into OPCs did not affect β-catenin expression in response to IL-1β stimulation.

Activation of miR-202-3p improves the proliferation and differentiation of OPCs through downregulation of the β-catenin/Gli1 pathway. To evaluate a possible interaction between miR-202-3p and the β-catenin/Gli1 pathway in IL-1β-mediated suppression of OPC proliferation and differentiation, miR-202-3p mimic transfection was performed in the present study. Successful transfection of miR-202-3p mimic was validated by RT-qPCR analysis (Fig. S1). As shown in Fig. 4, when compared with the NC transfection group, miR-202-3p mimic transfection significantly enhanced the proliferation activity and differentiation index of OPCs and attenuated the expression of β-catenin and Gli1 under IL-1β stimulation.

Discussion

The present study demonstrated that the inflammatory cytokine IL-1β modulated the expression of the miR-202-3p/β-catenin/Gli1 axis in cultured OPCs via its specific receptor. In addition, modulation of the miR-202-3p/β-catenin/Gli1 axis mediated IL-1β suppression of OPC proliferation and differentiation. A schematic illustration of the proposed mechanisms underlying the damaging effects of IL-1β on OPCs is presented in Fig. 5.

One of the main findings of the present study was that IL-1β attenuated the proliferation and differentiation of OPCs via activation of the β-catenin/Gli1 pathway. Canonical Wnt/β-catenin signaling has been implicated in the regulation of cell proliferation, differentiation, migration, and the development of axon, dendrites and synapses in the CNS (18,19). Dysregulation of Wnt/β-catenin signaling has been linked to demyelinating diseases of the CNS, such as multiple sclerosis (20-22). However, the results from different scientific groups are controversial. Some demonstrated that activation of Wnt/β-catenin signaling may mediate failure of remyelination in chronic demyelinating diseases (20,21), whereas others demonstrated that the downregulation of Wnt/β-catenin signaling inhibits the myelination process (21,22). A possible explanation for these contradictory observations is that canonical Wnt signaling may interact with other pathways, such as the Hedgehog pathway. Gli1, a key factor of the Hedgehog pathway (23), can be expressed by OPCs (24). γ-catenin (also referred to as plakoglobin), a partial functional homolog of β-catenin, has been reported to be upregulated by Gli1 in gastric cancer cells (25). Contrary to γ-catenin, β-catenin has been demonstrated to upregulate the expression of Gli1 (26,27). Although a high degree of sequence homology exists in the central armadillo repeat regions between the two catenins, there is little homology in their N- and C-termini (28). The differences in the N- and C-termini between the catenins are considered to be linked to the failure of γ-catenin to fully make up for β-catenin loss in mediating Wnt signaling (29). Therefore, structural differences between them may explain why the two catenins can be differentially regulated by Gli1. However, whether the β-catenin/Gli1 pathway in OPCs is regulated in response to IL-1β stimulation remains unknown. As shown in Fig. 2, the expression of β-catenin and Gli1 in OPCs were upregulated in response to IL-1β treatment. Conversely, the β-catenin inhibitor XAV939 markedly decreased the expression of Gli1. Therefore, these findings suggest that β-catenin acts as an upstream regulator of Gli1. Subsequently,

it was hypothesized that IL-1β modulates the proliferation and differentiation of OPCs via the β-catenin/Gli1 pathway. The results of the present study demonstrated that IL-1β suppressed the proliferation and differentiation of OPC and upregulated the expression of the β-catenin/Gli1 pathway, both of which were reversed by IL-1β receptor inhibition. Treatment with the β-catenin inhibitor XAV939 or Gli1 siRNA attenuated IL-1β-induced suppression of the proliferation and differentiation of OPCs. Treatment with XAV939 downregulated the expression of Gli1. Thus, activation of the β-catenin/Gli1 pathway may mediate the adverse effects of IL-1β on OPC proliferation and differentiation.

miRNAs are small non-coding RNA molecules that negatively control the expression of target genes and play key roles in animal development, cell proliferation, differentiation and metabolism. Recent researches have proposed that several miRNAs regulate the function of OPCs. miR-219 and miR-146a promote OPC differentiation and enhance remyelination in demyelinating diseases (30,31). By contrast, miR-26b, miR-9 and miR-200 inhibit OPC differentiation (12,32). IL-1β has been reported to regulate miR-372 in human neural stem cells (33), miR-147b in human astrocytes (34) and miR-202-3p in human nucleus pulposus cells (35). However, whether miR-202-3p mediates the damaging effects of IL-1β on OPCs remains unknown. The present study was therefore designed to investigate the possible role of miR-202-3p in IL-1β-induced suppression of OPC proliferation and differentiation. The results demonstrated that IL-1β markedly decreased the expression of miR-202-3p, increased the expression of β-catenin and Gli1, and inhibited the proliferation and differentiation of OPCs. By contrast, miR-202-3p mimic transfection significantly attenuated the expression of β-catenin and Gli1 and enhanced the proliferation and differentiation of OPCs under IL-1β stimulation. Thus, the downregulation of miR-202-3p may mediate the effects of IL-1β on OPCs. Additionally, a previous study demonstrated that miR-202-3p directly suppresses the expression of Gli1 to regulate the proliferation and apoptosis of gastric cancer cells (25). In the present study, miR-202-3p mimic transfection significantly enhanced the proliferation activity and differentiation index of OPCs and attenuated the expression of β-catenin and Gli1 under IL-1β stimulation. However, these data do not rule out the possibility that miR-202-3p directly suppresses the expression of Gli1 to regulate the proliferation and differentiation of OPCs. Interestingly, lipoprotein receptor-related protein 6 (LRP6) and cyclin D1 of the Wnt/β-catenin signaling pathway have been also identified as direct targets of miR-202-3p (36). Both LRP6 and cyclin D1 have been implicated in the differentiation of OPCs (37). Thus, the results of the present and other studies suggest that miR-202-3p regulates OPCs by targeting the β-catenin/Gli1 pathway at multiple levels. The precise mechanism underlying miR-202-3p regulation of the β-catenin/Gli1 pathway remains elusive and must be further elucidated in the future.

In conclusion, the present study investigated the possible role of the miR-202-3p/β-catenin/Gli1 axis in IL-1β-induced effects on OPCs. To the best of our knowledge, the present study is the first to demonstrate that IL-1β can prevent the proliferation and differentiation of OPCs through modulation of the miR-202-3p/β-catenin/Gli1 axis. As the failure of endogenous OPCs to differentiate into myelinating oligodendrocytes is considered to be implicated in the demyelinating diseases of the CNS, such as multiple sclerosis, these findings raise the possibility that the miR-202-3p/β-catenin/Gli1 axis may be of value as a therapeutic target for multiple sclerosis.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Research Fund of Chengdu Medical College (grant no. CYZ19-21).

Availability of data and materials

The datasets generated and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YaL and XD conceived and designed the research. YaL, LL, YoL, QY and BR performed the experiments. YoL and LL analyzed the data. YaL, LL and XD wrote the paper. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All animal procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee at Chengdu Medical College (Chengdu, China).

Patient consent for publication

Not applicable.

Competing interests

All the authors declare that they have no competing interests.

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