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NLRP3 inflammasome inhibition of OP9 cells enhance therapy for inflammatory bowel disease

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ABSTRACT

Mesenchymal stem cells (MSCs) are becoming more popular in therapy. Therefore, in-depth studies on mesenchymal stem cells in therapy are urgently needed. However, the difficulty in culturing and propagating MSCs *in vitro* complicates potential studies on MSCs in a murine model. OP9 cells are a stromal cell line from mouse bone marrow, which have similar characteristics and functions to MSCs and can maintain their original characteristics. Because of these properties, OP9 cells have become a suitable substitute for research on MSCs. Previously, we have found that MSCs can cure inflammatory bowel disease in mice. In this study, we aimed to investigate whether OP9 cells can functionally regulate and alleviate inflammatory diseases. We evaluated the therapeutic effect of OP9 cells in the mouse model of inflammatory bowel disease and found OP9 cells were able to ameliorate inflammatory bowel disease. We explored the existence of NLRP3 inflammasome in OP9 cells, and showed better therapeutic effects when the NLRP3 inflammasome was suppressed. Thus, OP9 cell line is similar to MSCs in characteristic and function, and is an ideal substitute for MSCs research. The preliminary exploration of the inflammasome system in OP9 cells lays a theoretical and methodological foundation for further study of MSCs.

1. Introduction

Due to the immunomodulation and differentiation potential of mesenchymal stem cells (MSCs), they have been considered a promising therapeutic strategy to treat various diseases [1]. In many inflammatory diseases, such as colon inflammation [2], acute or chronic liver injury [3], lung inflammatory diseases [4], neuroinflammatory diseases [5], acute kidney injury (AKI) or chronic kidney disease (CKD) [6], autoimmune [7] and ocular inflammatory disease [8], MSCs exhibit anti-inflammatory activity and could be

Abbreviations: MSCs, mesenchymal stem cells; IBD, inflammatory bowel disease; DSS, dextran sodium sulfate; LPS, lipopolysaccharide; NIG, nigericin.

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applied to attenuate specific organ and systemic inflammation [9].

Although the administration of MSCs can effectively suppress immune responses and promote the repair of injured tissues, there are still many challenges in expanding MSCs *in vitro* for further research [10]. The proliferative ability of MSCs is crucial for therapy because the number of MSCs available from biological sources is limited. However, the application of MSCs in clinical settings often requires a large quantity of cells [11]. The homing/migration and trophic ability, as well as the proliferative, multipotent, and immunosuppressive functions of MSCs are key in exerting a curative effect in therapy [1]. Furthermore, accumulating evidence suggests that MSC-derived extracellular vesicles (MSC-EVs) are enriched with messenger RNAs (mRNAs), microRNAs (miRNAs), cytokines, chemokines, and immunomodulatory factors, which exert major immunosuppressive effects in the treatment of MSC-based inflammatory diseases [12]. Significant research progress has been made in understanding the mechanisms employed by MSCs to regulate immune function and promote the repair of damaged tissues, but further exploration remains a major focus. Therefore, it is of great significance to obtain a continuous and stable source of MSCs or MSC substitutes for studies in animal models.

To date, MSCs have been successfully isolated, identified, and expanded from various human organs. In contrast, it is quite difficult to achieve a large number of murine MSC amplification, and the reason is unknown yet [13]. It may be related to the decrease of stem cell characteristics of MSCs with increasing number of cellular divisions. Since mice are one of the most important animal models, it is necessary to develop effective expansion strategies and establish homogeneous MSC lines. Due to their osteogenic, chondrogenic, and adipogenic differentiation potential, MSCs *in vitro* cannot proliferate in large quantities while maintaining the characteristics of stem cells, greatly hampering potential research and clinical applications of MSCs [14].

OP9 is a mouse bone marrow stromal cell line derived from newborn calvaria of the (C57BL/6 × C3H)F2-*op/op* mouse, which is functionally deficient in macrophage colony-stimulating factor (M-CSF) due to a mutation in the M-CSF gene [15]. Similar to MSCs, OP9 cells have hematopoietic supportive ability. When used as a feeder layer for *in vitro* cultures, OP9 cells can support the differentiation of embryonic stem cells into a variety of hematopoietic lineage cells, including granulocytes, erythrocytes, lymphocytes, and megakaryocytes [16]. Previous studies have shown that OP9 cells have the same immune phenotype (FLK-1^{-}, CD11b^{-}, CD45^{-}, CD31^{-}, CD34^{-}, CD86^{-}, MHCII^{-}, CD44^{+}, CD29^{+}, and Sca-1^{+}) as the canonical mouse MSCs. In terms of function, OP9 cells exhibit a strong clonogenic ability and can be induced to form osteocytes, adipocytes, and chondrocytes [17]. In short, OP9 cells could serve as a replacement for MSCs, helping to elucidate the biological characteristics of MSCs at the stem cell level.

In our previous study, we demonstrated NLRP3 inflammasome existed in bone-associated MSCs [18]. Since OP9 cells can be used as a substitute for MSCs, we want to explore whether OP9 cells contain NLRP3 inflammasome and assess its therapeutic effects in inflammatory diseases. Studying the therapy of OP9 cells will further expand their potential in basic research application and may open new prospects for MSC-based therapy research. Most importantly, continuous exploration of the properties of OP9 cells would lay a foundation for our study on the characteristics and functions of MSCs, eliminate the difficulty in obtaining stable cell sources, and provide a new perspective and convenient conditions for efficient study of MSCs.

2. Materials and methods

2.1. Cell culture

OP9 cells (ATCC CRL-2749) was purchased from Procell Company, Wuhan, China. Cells were incubated in MEM-alpha medium (Gibco) containing 10% fetal bovine serum (Gibco) under an atmosphere of 5% CO_2 at 37 °C.

2.2. Animal management

C57BL/6 female mice, 6–8 weeks, were purchased from the Laboratory Animal Center of Fourth Military Medical University (Xi'an, China). All mice were fed in a specific pathogen free animal house with a standard management. All experiments on mice were reviewed and approved by the Welfare and Ethics Committee of the Laboratory Animal Center of the Fourth Military Medical University.

2.3. Reagents and kits

Lipopolysaccharides (LPS) (*E. coli* O111:B4) and nigericin (14K05-MM) used to induce pyroptosis were purchased from Invitrogen. Dextran sodium sulfate (DSS) (#118K7374V) used to induce inflammatory bowel disease (IBD) mouse model were obtained from Sigma-aldrich. Anti-mouse antibodies like NLRP3 (#AG-20B-0014, Adipogen), Caspase1-p20 (#AG-20B-0042, Adipogen), IL-1β (#12426, Cell Signaling Technology), GSDMDC1 (#sc-393656, Santa Cruz Biotechnology), alpha Tubulin (#66031-1-1 g, Proteintech), HRP goat anti-mouse IgG antibody (#EK010, Zhuangzhibio), and HRP goat anti-rabbit IgG antibody (#EK020, Zhuangzhibio) were used for immunoblot analysis. The anti-mouse antibodies used for flow cytometry analysis were as follows: APC-CD105 (#MJ7/18, Biolegend), APC-CD90 (#OX-7, BD), PE-CD44 (#IM7, BD, USA), PE-CD51 (#RMV-7, eBioscience), APC-CD3 (#17A2, Biolegend), APC-CD45R/B220 (#RA3-6B2, Biolegend), FITC-CD14 (#Sa14-2, Biolegend), FITC-CD11c (#117306, Biolegend), APC-CD11b (#101212, Biolegend) and APC-Gr1 (#17–5931, eBioscience). The IL-1β Enzyme-Linked Immunosorbent Assay (ELISA) kit was purchased from R & D Systems (#P16807).

2.4. The stimulation of OP9 cells

OP9 cells were stimulated by 500 ng/ml LPS for 4 h, followed by 5 μM nigericin for 1 h. OP9 cells and supernatants were collected and subjected to ELISA and Western blot detection. OP9 cells were pretreated with 10 μM MCC950 and 500 ng/ml LPS for 4 h and 5 μM nigericin for 1 h, after which the cells were harvested and subjected to Western blot.

2.5. The establishment of IBD mouse model

The IBD mouse model was established by feeding C57BL/6 mouse with 3% DSS for 7 days. Activity, body weight and feces of IBD mice and control mice were monitored and recorded daily. Experimental mice were randomly assigned to each group.

2.6. Treatment of IBD mice

After IBD was successfully established, OP9 cells and OP9 cells pretreated with MCC950 (the specific NLRP3 inhibitor) for half an hour were transplanted intravenously into mice. 2×10^6 cells per mouse were injected to treated group, and the control group was injected with PBS. After treatment for 3 days, C57BL/6 mice were sacrificed by cervical dislocation. The length and weight of colon were measured. And then histopathological changes of colon were analyzed by H&E staining.

2.7. Flow cytometry analysis

Cells were cultured *in vitro*, harvested and resuspended in PBS containing 5% FBS and 0.4% NaN3. Then, the cells were stained with specific antibodies for 20 min at 4 °C in dark. After the cells were washed with PBS for 3 times, the stained OP9 cells were detected by BD FACSCanto Plus flow cytometry (BD Biosystems). The data was analyzed by FlowJo software. OP9 cells were identified using antibodies against CD105, CD90, CD44 and CD51. In addition, OP9 cells were evaluated by using CD3, B220, CD14, CD11c, CD11b and Gr-1 antibodies.

2.8. Immunoblot analysis

OP9 cells were seeded in 6-well plates and after stimulated by LPS for 4 h and nigericin for 1 h in serum-free MEM-α medium, cell proteins were extracted respectively for the detection of Caspase1-p20 and NLRP3. To collect the OP9 cells lysis protein, cells were lysed with RIPA (#P0013C, Beyotime). After centrifuging in 13,000 rpm for 20 min, the protein concentration was detected by BCA Protein Assay Kit (#P0010, Beyotime). 5 × SDS loading buffer (#P0015, Beyotime) were mixed with protein and heated in 100 °C to denature protein. The same amount of protein was separated by SDS-PAGE and then transferred onto PVDF membrane. The PVDF membranes were sealed and incubated overnight in a 4 °C shaker with antibody as described above (Caspase1-p20, NLRP3 and α-Tubulin). After that, they were washed by PBST for 3 times. Then PVDF membrane were incubated with goat anti-mouse and goat anti-rabbit HRP-conjugated secondary antibodies respectively at room temperature for 1 h. After the PVDF membranes were washed, the bands were visualized using a ChemiDoc XRS + Imaging System (BioRad). The procedures described above were repeated to detect the expression levels of IL-Iβ, Caspase1-p20, and Cleaved GSDMD in OP9 cells pretreated with MCC950 followed by LPS and nigericin stimulation.

2.9. Hematoxylin-eosin staining and analysis

After treated with OP9 cells and PBS for 3 days, mice were sacrificed for collecting colons and paraffin sections were prepared. After that, paraffin sections were stained by hematoxylin-eosin (H&E) standard protocol. Semi-quantitative histological scoring was performed independently by two individuals using a blind manner, according to a previous study [19]. The scoring criteria were composed of inflammatory cell infiltration (score 0–4) and intestinal structural damage (score 0–4). And the pathological changes of colon were scored according to the following criteria: inflammatory cells occasionally appear (0); the number of inflammatory cells increases (1); inflammatory cells reach the mucosa and submucosa (2); inflammatory cells extend to the mucosa and submucosa, and sometimes infiltrate across the intestinal wall (3); inflammatory cells infiltrate severely and extend across the intestinal wall (4); no mucosal damage (0); local erosion of mucosa (1); mild crypt loss and focal ulcer (2); moderate crypt loss and severe ulcer (3); extensive crypt loss, mucosal damage, and deeper structural damage to the intestinal wall (4). The combined histological score ranges from 0 (no injury) to 8 (extensive inflammatory cells infiltration and tissue damage).

2.10. Scanning electron microscope

OP9 cells were seeded on glass slides in 24-well plates with 5×10^4 cells per well and cultured overnight to make them adhered properly. The cells were then treated as described before. After removing the cell supernatant, and the samples were fixed with 2.5% glutaraldehyde for 24 h. The samples were sent to the Electron Microscopy Center of Fourth Military Medical University for detection. The samples were imaged with a scanning electron microscope (Olympus N300 M, Shinjuku-ku, Tokyo, Japan). After the images were taken, two individuals independently counted the pores in the cell membrane in a blind manner. Five photos were randomly selected from each group and the number of pores was recorded.

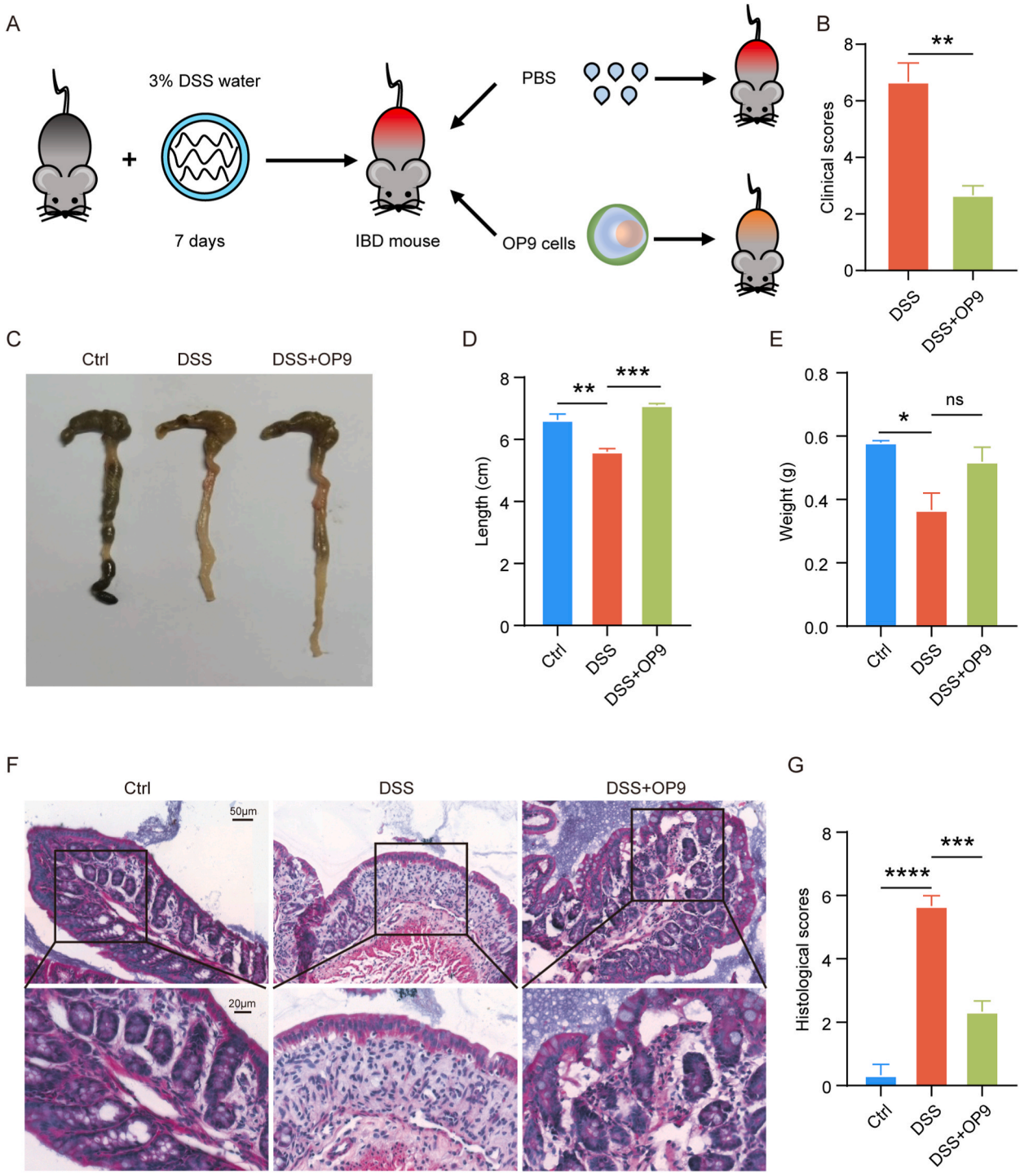


Fig. 1. OP9 cells were administrated in the murine model of IBD. (A) Schematic diagram of IBD mice treated with PBS and OP9 cells. (B) Clinical scores of IBD mice treated with PBS and OP9 cells (n = 3). (C) Colonic morphology of control mice, PBS-treated IBD mice, and OP9-treated IBD mice. (D) The colon length of control mice, PBS-treated IBD mice, and OP9-treated IBD mice (n = 3). (E) The weight of control mice, PBS-treated IBD mice, and OP9-treated IBD mice (n = 3). (F) Representative H&E staining images of control mice, PBS-treated IBD mice, and OP9-treated IBD mice. (G) The histological scores analysis of control mice, PBS-treated IBD mice, and OP9-treated IBD mice (n = 3). The control mice were fed with water without DSS and intravenously injected with PBS as a negative control; PBS-treated IBD mice were fed with 3% DSS water and intravenously injected with PBS as positive control; OP9-treated IBD mice were fed with 3% DSS water and intravenously injected with OP9 cells as treatment group. All data are presented as mean ± SD. Student's t-test for comparison between two groups, one-way ANOVA for comparison more than two groups, ns: not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

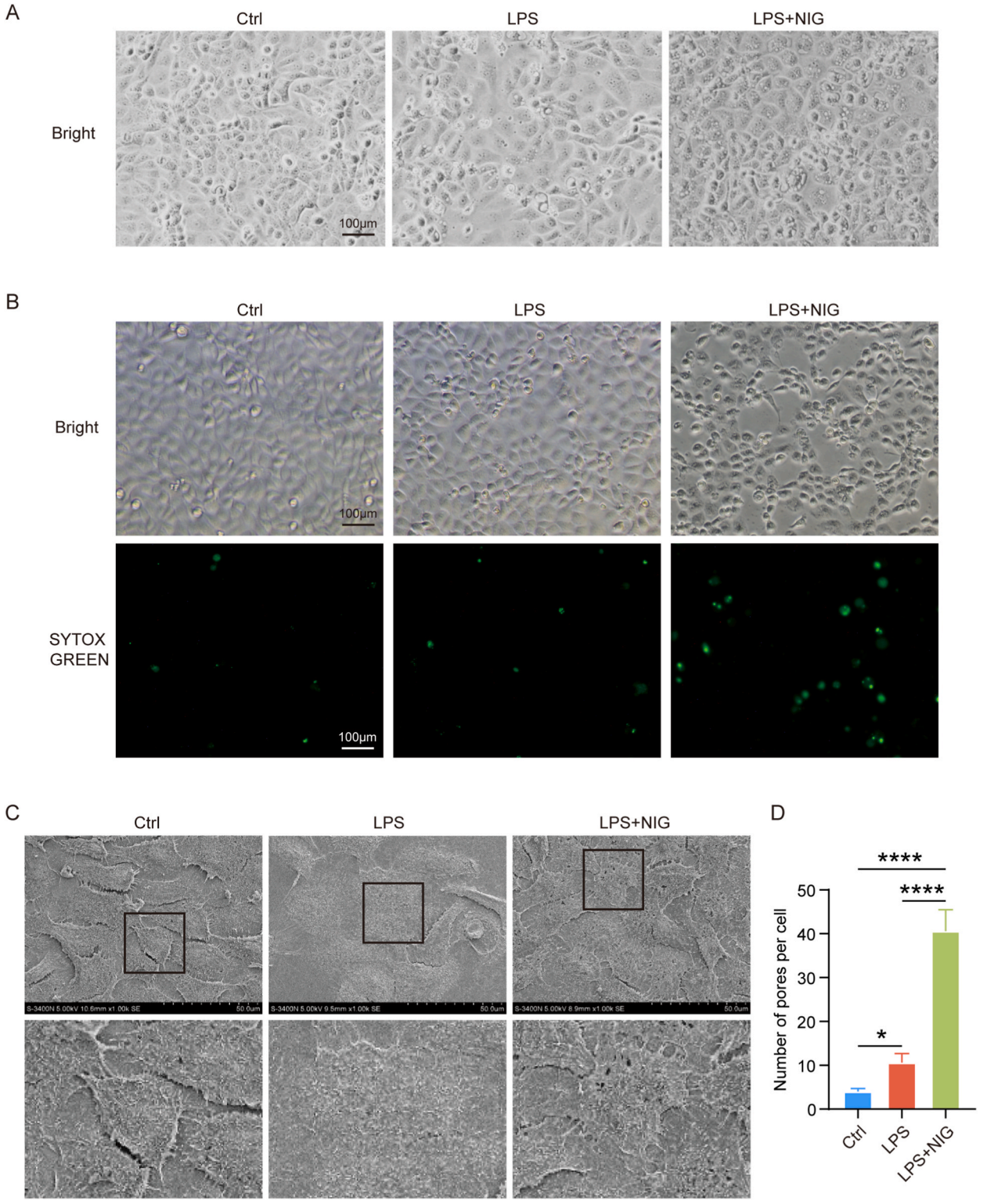


Fig. 2. OP9 cells were treated with LPS and nigericin. (A) Microscopic images of OP9 cells stimulated by LPS and nigericin. (B) After OP9 cells were stimulated by LPS and nigericin and stained with SYTOX Green, bright field and fluorescence microscope images were taken. (C) Representative scanning electron microscopy images of OP9 cells stimulated by LPS and nigericin. The below photos were amplification of the above ones. (D) Statistical analysis of pore number counts of OP9 cells in result (C) (n = 5). All data are presented as mean ± SD. One way ANOVA test, $*p < 0.05$, $****p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

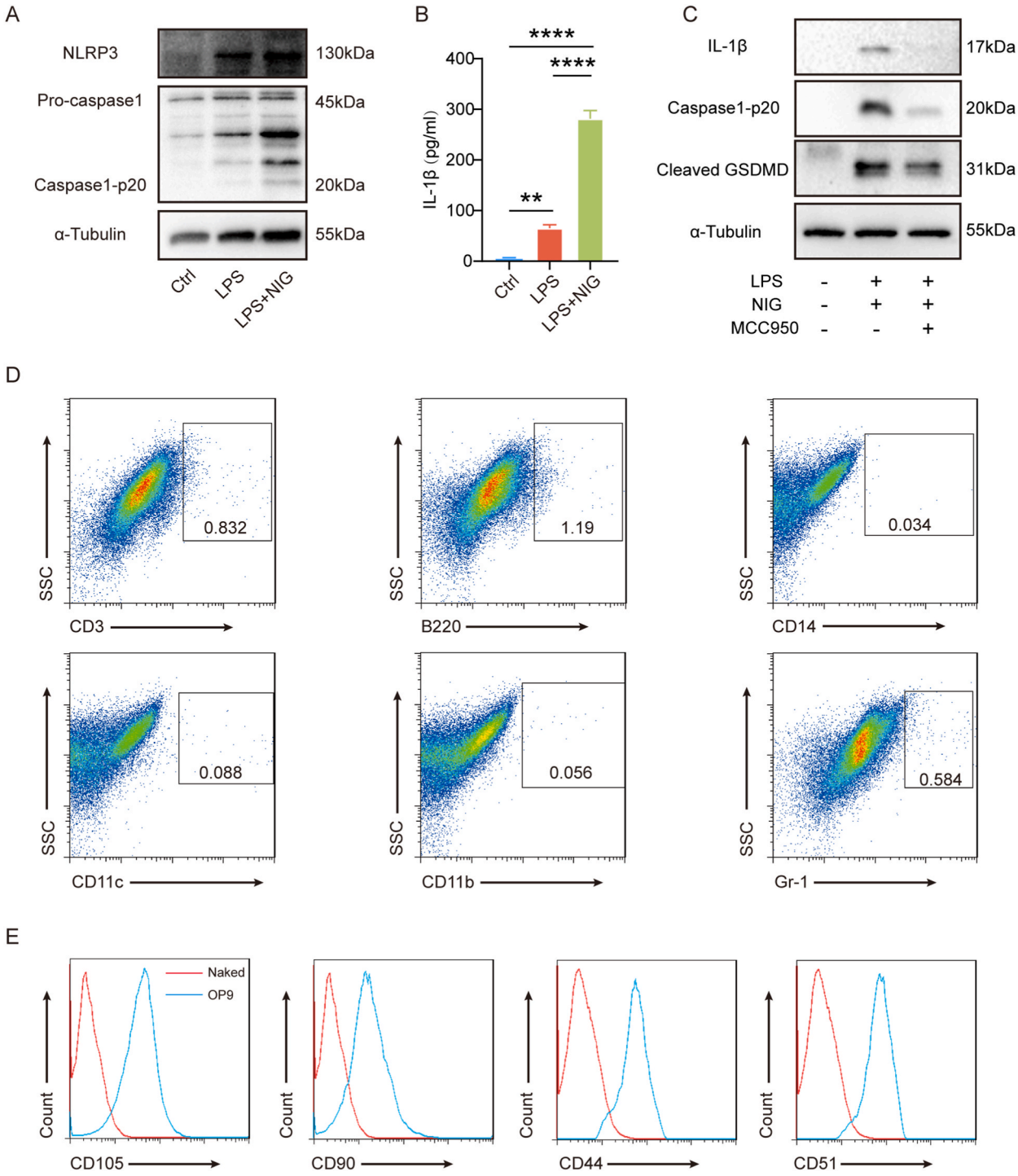
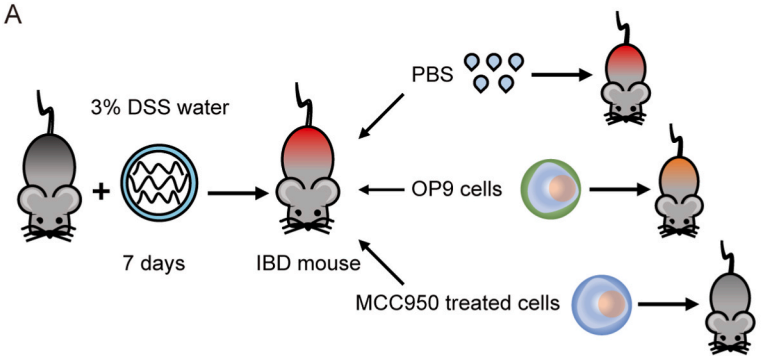
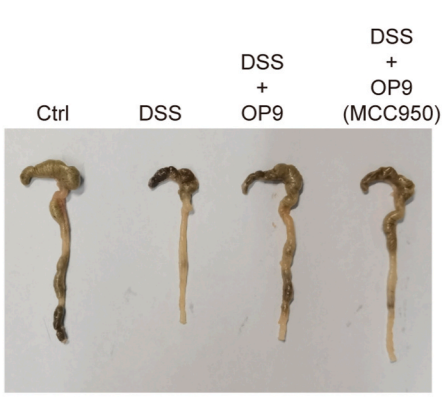
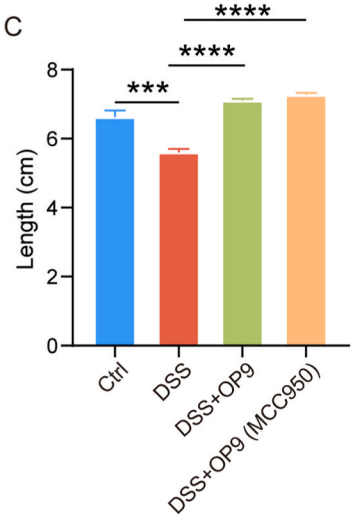
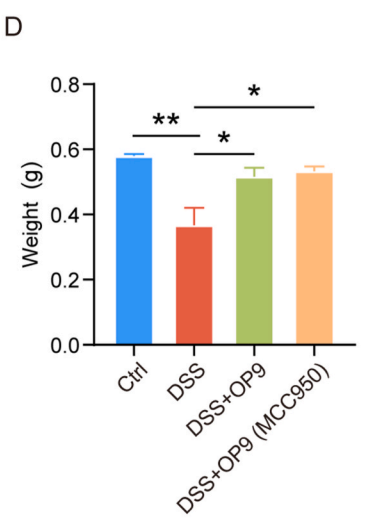


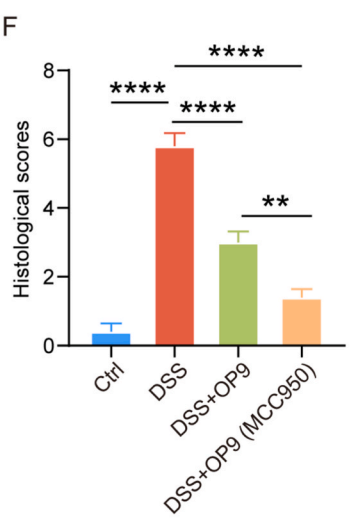
Fig. 3. NLRP3 inflammasomes and surface marker in OP9 cell line. (A) Western blot analysis of the expression of NLRP3, pro-caspase1 and caspase1-p20 in OP9 cells stimulated by LPS and nigericin. (B) ELISA analysis of IL-1β levels in the supernatants of OP9 cells stimulated by LPS and nigericin (n = 5). (C) Western blot analysis of the expression of IL-1β, caspase1-p20, cleaved GSDMD in OP9 cells pretreated with MCC950 and then stimulated by LPS and nigericin. (D) Flow cytometry analysis of immune cells common surface molecular markers (CD3, B220, CD14, CD11c, CD11b and Gr-1). (E) Flow cytometry analysis of MSCs common surface molecular markers (CD105, CD90, CD44 and CD51). The uncropped versions of Fig. 3(A, C) were provided in the Supplementary Material Fig. S2. All data are presented as mean ± SD. One way ANOVA test, $^{**}p < 0.01$, $^{****}p < 0.0001$.











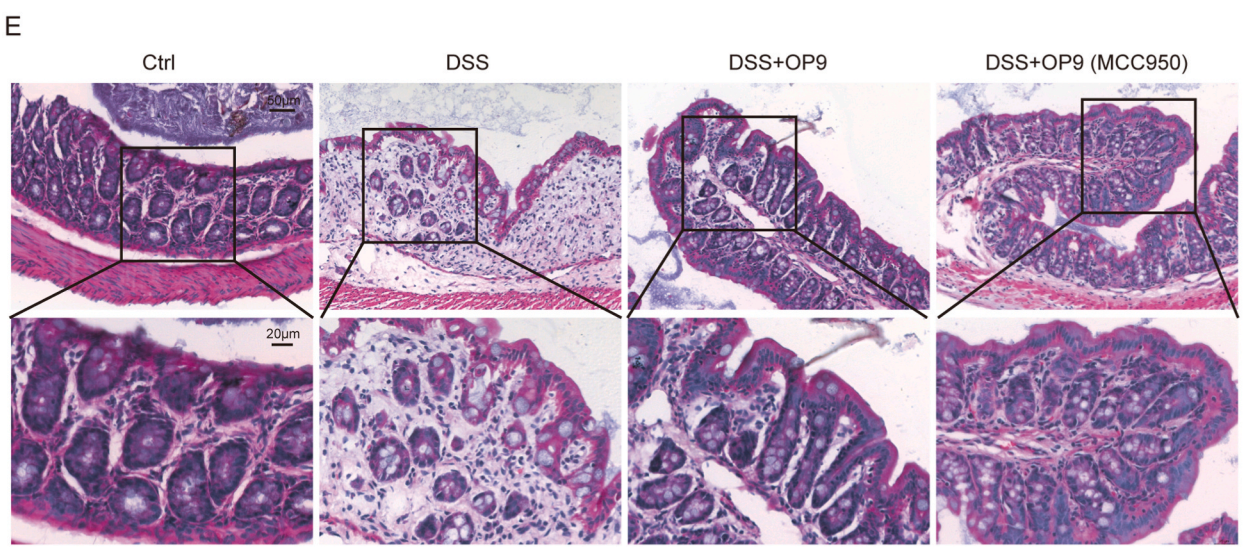


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Fig. 4. Inhibition of NLRP3 inflammasome improves the OP9 cells therapy for IBD. (A) Schematic diagram of IBD mice treated with PBS, OP9 cells and OP9 cells pretreated with MCC950 (OP9 (MCC950)). (B) Colonic morphology of control mice, PBS-treated IBD mice, OP9-treated IBD mice, and OP9 (MCC950)-treated IBD mice. (C) The colon length of control mice, PBS-treated IBD mice, OP9-treated IBD mice, and OP9 (MCC950)-treated IBD mice (n = 3). (D) The weight of control mice, PBS-treated IBD mice, OP9-treated IBD mice, and OP9 (MCC950)-treated IBD mice (n = 3). (E) Representative H&E staining images of control mice, PBS-treated IBD mice, OP9-treated IBD mice, and OP9 (MCC950)-treated IBD mice. (F) The histological scores analysis of control mice, PBS-treated IBD mice, OP9-treated IBD mice, and OP9 (MCC950)-treated IBD mice (n = 3). Detailed explanation of control mice, PBS-treated IBD mice and OP9-treated IBD mice were descripted as before. OP9 (MCC950)-treated IBD mice were fed with 3% DSS water and intravenously injected with OP9 cells pretreated with MCC950 as improved treatment group. All data are presented as mean ± SD. One way ANOVA test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

2.11. ELISA assay

The concentration of IL-1β were measured using a Mouse IL-1β ELISA Kit (#P16807, R&D Systems), and all experimental procedures were carried out according to the manufacturer's instructions. In the ELISA experiments, 10^5 cells/well were inoculated into 24-well plates. OP9 cells were stimulated by LPS for 4 h and nigericin for 1 h, and then supernatants were collected for detection.

2.12. Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 8.0.2 (La Jolla, CA, USA). Results are presented as mean ± standard deviation (SD). Two groups and multiple groups comparisons were analyzed by Student's t-test and one-way analysis of variance (one-way ANOVA), respectively. For the above parameters, a value of $p < 0.05$ was considered statistical significance. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$; ns: no statistical significance.

3. Results

3.1. OP9 cells can effectively relieve IBD syndrome

To explore whether OP9 cells can alleviate on IBD, we established an IBD mouse model (Fig. S1A) and treated IBD mice with OP9 cells (Fig. 1A). Body weight of the IBD mice decreased, and the clinical score increased after 3%DSS water feeding (Figs. S1B and C). When OP9 cells were injected into mice, the symptoms of hematochezia were reduced, and the vitality was restored. In consistent with the observation, clinical scores of IBD mice declined (Fig. 1B), and the colon length and weight recovered (Fig. 1C–E), indicating the colon injury was alleviated. In addition, as shown in the histological analysis of colon H & E staining sections, the normal tissue structure of the colon mucosa was reconstructed by OP9 cells administration (Fig. 1F). Correspondingly, the histological scores were reduced (Fig. 1G). These results demonstrated that OP9 cells were effective to alleviate IBD syndrome.

3.2. NLRP3 inflammasome can be activated in OP9 cell line

The NLRP3 inflammasome exists in MSCs, as proved by our previous studies [20]. But it remains unclear whether NLRP3 inflammasome can be activated in OP9 cells. Thus, we stimulated OP9 cells with LPS and nigericin. The microscopic observations subsequently revealed a marked cell reduction and increased cell damage (Fig. 2A and B). As shown in Fig. 2A, some of the OP9 cells were swollen and deformed. To figure out whether OP9 cells appeared cell death, we used SYTOX Green, a nucleic acid stain, to distinguishing dead from live cells. It was obvious that OP9 cells died significantly after LPS and nigericin stimulation (Fig. 2B). In addition, scanning electron microscope observations showed that OP9 cells formed many membrane pores following LPS and nigericin stimulation (Fig. 2C and D). We hypothesized that the NLRP3 inflammasome might have been activated, and the damage to OP9 cells was probably caused by NLRP3 inflammasome-mediated pyroptosis since LPS and nigericin can induce the assembly of the NLRP3 inflammasome.

To testify the speculation, we further tested the changes of protein levels. As shown in Fig. 3A, NLRP3 levels were up-regulated following LPS and nigericin stimulation. The pro-caspase1 was cleaved into caspase1-p20 upon stimulation by LPS and nigericin (Fig. 3A). A successful assembly of NLRP3 inflammasome would initiate the cleavage of pro-caspase1 into caspase1-p20, which would promote the production of mature IL-1β from pro-IL-1β [21,22]. As expected, following the stimulation of OP9 cells with LPS and nigericin, we found that the level of mature IL-1β in the supernatant was significantly increased (Fig. 3B). MCC950 is a highly potent and highly selective NLRP3 inhibitor [23,24]. When OP9 cells were pretreated with MCC950 and then subjected to LPS and nigericin stimulation, caspase1-p20, IL-1β and cleavaged GSDMD was decreased, indicating that MCC950 successfully inhibited the activation of NLRP3 inflammasome in OP9 cells (Fig. 3C). These results indicated that NLRP3 inflammasome was activated by LPS and nigericin in OP9 cells, and can be inhibited by MCC950.

We also assayed OP9 cells for surface markers. The flow cytometry data in Fig. 3D indicated that OP9 cells did not express molecular markers that are typical of immune cells, such as CD3, B220, CD14, CD11c, CD11b and Gr-1. But it is worth noting that OP9 cells expressed molecular markers similar to those expressed by MSCs, specifically CD105, CD90, CD44 and CD51 (Fig. 3E). These data further suggest that OP9 cells were much close to MSCs.

3.3. Inhibition of NLRP3 inflammasome in OP9 cells improved IBD alleviation

Since OP9 cells contain the NLRP3 inflammasome signal, they could respond to inflammation. To investigate whether the activation of NLRP3 inflammasome in OP9 cells would impact the therapy in IBD, we employed the NLRP3 inhibitor MCC950. As it was demonstrated that MCC950 can inhibit NLRP3 inflammasome activation in OP9 cells in *vitro*, animal experiments were subsequently conducted to explore whether MCC950 could enhance the efficacy of OP9 cells in the treatment of IBD. OP9 cells and OP9 cells pretreated with MCC950 for 30 min were intravenously injected into IBD mice alongside PBS-only control (Fig. 4A). After treatment, the morphology (Fig. 4B), length (Fig. 4C), and weight (Fig. 4D) of colon tissue returned to normal. Evidently, both OP9 cells and MCC950 treated OP9 cells relieved IBD symptoms in mice. However, the histological analysis of colonic mucosa found that compared with the OP9 group, the glandular structures of the colonic mucosa tissue in MCC950 treated group were better recovered, and the goblet cells were more orderly arranged (Fig. 4E). Correspondingly, histological scores in the MCC950 treated group were lower than those of the OP9 group (Fig. 4F). These results demonstrated that the inhibition of NLRP3 inflammasome in OP9 cells could achieve better alleviation of IBD symptoms.

4. Discussion

In the current study, we demonstrated that OP9 cells have a therapeutic effect in IBD mice, not only by inhibiting inflammatory responses but also by promoting the repair of colonic mucosal tissues in the mice. At the same time, we also observed that OP9 cells had intracellular NLRP3 inflammasome that could be activated by LPS and nigericin. Additionally, when NLRP3 inflammasome activation of OP9 cells was inhibited by MCC950, the therapy of OP9 cells in the IBD mice was enhanced.

OP9 cells play a similar role to MSCs in the study of hematopoietic cell development and differentiation. Co-culture of OP9 cells with mouse embryonic stem cells can induce the embryonic stem cells to differentiate into red blood cell-derived, bone marrow-derived, and B-cell lineage blood cells [16,25,26]. Previous studies from our group have shown that there are NLRP3 inflammasome in MSCs. In terms of clinical application, MSCs, as an important candidate in stem cell therapy, have shown an obvious therapeutic effect in the treatment of inflammatory bowel disease [18]. However, MSCs are hard to obtain [27]. In addition, the original characteristics of stem cells cannot be maintained long term as the number of cell passages increases. Moreover, the number of passages is not infinite, and the culturing process requires a demanding nutritional environment and a high cost of culture maintenance [10]. In contrast, OP9 cells have a strong proliferation ability and can be massively amplified, as well as having less stringent nutritional requirements in culture. Due to similar molecular marker expression and hematopoietic regulation function, OP9 can be used as a substitute for MSCs to facilitate research [28]. OP9 cells not only express cell surface molecular markers similarly to MSCs, but also possess NLRP3 inflammasome, as in MSCs. These properties of OP9 cells would contribute to a better understanding of MSCs by modeling the functions and characteristics of MSCs.

MSCs have immunomodulatory functions, and experimental animal models of IBD have shown the regulatory effects of MSCs on the immune system. These researches indicate that MSCs are beneficial for intestinal integrity and tissue repair. What's more, a number of clinical trials have registered the application of MSCs in the treatment of IBD patients, and have shown reliable safety, but the research related to its clinical immunity is still lacking [29]. In our study, OP9 cells have also shown a good effect on the treatment of IBD in animal experiments, indicating that OP9 cells, like MSCs, have an immunomodulatory effect. However, the specific mechanism is still unclear. There are few studies on the immunomodulatory mechanism of OP9 cells in inflammation. Exploring the immunomodulatory mechanism of OP9 cells in the treatment of IBD mice will help us to further understand the application potential of OP9 cells in the regulation of inflammation. In this study, the role of OP9 cells in regulating intestinal inflammation in IBD mice was explored from the perspective of NLRP3 inflammasome in OP9 cells. Numerous studies have shown that NLRP3 activation induces inflammatory effects and promotes disease progress *in vivo*, while the inhibition of NLRP3 inflammasome can inhibit the disease deterioration. For example, inhibition of NLRP3 inflammasome with MCC950 can attenuate liver inflammation and fibrosis in mice with experimental non-alcoholic steatohepatitis (NASH) [30], reduce renal injury and apoptosis in contrast-induced acute kidney injury (CI-AKI) [31], and improve vascular function in diabetic mice [32]. Moreover, in several neurological diseases, the NLRP3 inhibitor MCC950 plays a neuroprotective role [33,20]. Notably, in the mouse model of spontaneous chronic colitis, the effect of MCC950 reduced the release of proinflammatory cytokines IL-1α, IL-1β, IL-18, IFN-γ and TNF-α in colonic explants. In addition, IL-1β release and caspase-1 activation in colonic explants were also significantly reduced by MCC950. MCC950 is effective in treating ulcerative colitis in mice [34]. In our study, MCC950 inhibits NLRP3 inflammasome in OP9 cells, which molecular pathway improves the effect of OP9 cells in the treatment of colitis deserves attention. Therefore, MCC950 plays an effective regulatory role in many diseases and has potential for clinical application.

There are also some limitations in this study that need further exploration. First, the mechanism by which NLRP3 inflammasome in OP9 cells affects the establishment of mouse IBD model and the repair of colonic inflammation in IBD mice has not been elucidated. Second, whether MCC950 caused confounding changes in OP9 cells in addition to inhibiting NLRP3 inflammasome remains unclear. Additionally, the mechanism that governs inflammatory response regulation and colonic mucosa structure recovery in OP9 cells is presently unclear. In other words, we have not explored the changes in the levels of proinflammatory cytokines such as IL-1β and IL-6 and the proportion of immune cells such as macrophage of M1 phenotype and M2 phenotype in IBD mice. OP9 cells are similar to MSCs both in phenotype and function, but they are not completely similar in terms of self-renewal and multidirectional differentiation potential. Evidently, future explorations on the mechanisms involved in the treatment of IBD by OP9 cells and on the similarities and differences between OP9 cells and MSCs in treating IBD are highly necessary. It is also vital to study the role of NLRP3 inflammasome in OP9 cells in inflammatory regulation.

In summary, OP9 cells would facilitate research on the functions and characteristics of MSCs in stem cell therapy. Like MSCs, OP9 cells have similar molecular markers on the cell membrane, and NLRP3 inflammasome is present in the cytoplasm. When NLRP3 inflammasome was suppressed by MCC950, OP9 cells showed better therapeutic efficacy on IBD. Our discovery provides a new perspective for exploring the practical application of OP9, a new possibility for the treatment of IBD in a mouse model, and a new scope for the study of regulation in inflammation.

Ethics statement

All experiments on mice were reviewed and approved by the Welfare and Ethics Committee of the Laboratory Animal Center of the Fourth Military Medical University (No. IACUC-20211150).

Author contribution statement

Yutong Chen, Weicheng Meng: Performed the experiments; Wrote the paper.
Guangming Ren, Ning An, Jing Zhang, Xingbin Hu, Zheng Liu: Analyzed and interpreted the data.
Zhixin Liu, Xiaoshuang Wu, Wen Yin: Contributed reagents, materials, analysis tools or data.
Fan Feng, Yaozhen Chen: Conceived and designed the experiments.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] A. Naji, M. Eitoku, B. Favier, F. Deschaseaux, et al., Biological functions of mesenchymal stem cells and clinical implications, Cell. Mol. Life Sci. 76 (17) (2019) 3323–3348, https://doi.org/10.1007/s00018-019-03125-1.
[2] S. Soontararak, L. Chow, V. Johnson, J. Coy, et al., Mesenchymal stem cells (MSC) derived from induced pluripotent stem cells (iPSC) equivalent to adipose-derived MSC in promoting intestinal healing and microbiome normalization in mouse inflammatory bowel disease model, Stem Cells Transl Med 7 (6) (2018) 456–467, https://doi.org/10.1002/sctm.17-0305.
[3] C. Hu, Z. Wu, L. Li, Mesenchymal stromal cells promote liver regeneration through regulation of immune cells, Int. J. Biol. Sci. 16 (5) (2020) 893–903, https://doi.org/10.7150/ijbs.39725.
[4] F.F. Cruz, P.R.M. Rocco, The potential of mesenchymal stem cell therapy for chronic lung disease, Expet Rev. Respir. Med. 14 (1) (2020) 31–39, https://doi.org/10.1080/17476348.2020.1679628.
[5] P.T. Do, C.C. Wu, Y.H. Chiang, C.J. Hu, et al., Mesenchymal stem/stromal cell therapy in blood-brain barrier preservation following ischemia: molecular mechanisms and prospects, Int. J. Mol. Sci. 22 (18) (2021), 10045, https://doi.org/10.3390/ijms221810045.
[6] C.W. Yun, S.H. Lee, Potential and therapeutic efficacy of cell-based therapy using mesenchymal stem cells for acute/chronic kidney disease, Int. J. Mol. Sci. 20 (7) (2019) 1619, https://doi.org/10.3390/ijms20071619.
[7] A. Naji, N. Rouas-Freiss, A. Durrbach, E.D. Carosella, et al., Concise review: combining human leukocyte antigen G and mesenchymal stem cells for immunosuppressant biotherapy, Stem Cell. 31 (11) (2013) 2296–2303, https://doi.org/10.1002/stem.1494.
[8] V. Holan, K. Palacka, B. Hermankova, Mesenchymal stem cell-based therapy for retinal degenerative diseases: experimental models and clinical trials, Cells 10 (3) (2021) 588, https://doi.org/10.3390/cells10030588.
[9] J. Galipeau, L. Sensébé, Mesenchymal stromal cells: clinical challenges and therapeutic opportunities, Cell Stem Cell 22 (6) (2018) 824–833, https://doi.org/10.1016/j.stem.2018.05.004.
[10] Y. Gu, T. Li, Y. Ding, L. Sun, et al., Changes in mesenchymal stem cells following long-term culture in vitro, Mol. Med. Rep. 13 (6) (2016) 5207–5215, https://doi.org/10.3892/mmr.2016.5169.

[11] B. Parekkadan, J.M. Milwid, Mesenchymal stem cells as therapeutics, Annu. Rev. Biomed. Eng. 12 (2010) 87–117, https://doi.org/10.1146/annurev-bioeng-070909-105309.
[12] C.R. Harrell, N. Jovicic, V. Djonov, N. Arsenijevic, et al., Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases, Cells 8 (12) (2019) 1605, https://doi.org/10.3390/cells8121605.
[13] Y. Hu, B. Lou, X. Wu, R. Wu, et al., Comparative study on in vitro culture of mouse bone marrow mesenchymal stem cells, Stem Cell. Int. 2018 (2018), 6704583, https://doi.org/10.1155/2018/6704583.
[14] Y.K. Yang, C.R. Ogando, C. Wang See, T.Y. Chang, et al., Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro, Stem Cell Res. Ther. 9 (1) (2018) 131, https://doi.org/10.1186/s13287-018-0876-3.
[15] H. Kodama, M. Nose, S. Niida, S. Nishikawa, et al., Involvement of the c-kit receptor in the adhesion of hematopoietic stem cells to stromal cells, Exp. Hematol. 22 (10) (1994) 979–984.
[16] T. Nakano, H. Kodama, T. Honjo, Generation of lymphohematopoietic cells from embryonic stem cells in culture, Science 265 (5175) (1994) 1098–1101, https://doi.org/10.1126/science.8066449.
[17] N.E. Wolins, B.K. Quaynor, J.R. Skinner, A. Tzekov, et al., OP9 mouse stromal cells rapidly differentiate into adipocytes: characterization of a useful new model of adipogenesis, J. Lipid Res. 47 (2) (2006) 450–460, https://doi.org/10.1194/jlr.D500037-JLR200.
[18] Y. Chen, X. Qin, Q. An, J. Yi, et al., Mesenchymal stromal cells directly promote inflammation by canonical NLRP3 and non-canonical caspase-11 inflammasomes, EBioMedicine 32 (2018) 31–42, https://doi.org/10.1016/j.ebiom.2018.05.023.
[19] Y.J. Liu, B. Tang, F.C. Wang, L. Tang, et al., Parthenolide ameliorates colon inflammation through regulating Treg/Th17 balance in a gut microbiota-dependent manner, Theranostics 10 (12) (2020) 5225–5241, https://doi.org/10.7150/thno.43716.
[20] B. Hou, Y. Zhang, P. Liang, Y. He, et al., Inhibition of the NLRP3-inflammasome prevents cognitive deficits in experimental autoimmune encephalomyelitis mice via the alteration of astrocyte phenotype, Cell Death Dis. 11 (5) (2020) 377, https://doi.org/10.1038/s41419-020-2565-2.
[21] I. Jorgensen, E.A. Miao, Pyroptotic cell death defends against intracellular pathogens, Immunol. Rev. 265 (1) (2015) 130–142, https://doi.org/10.1111/imr.12287.
[22] W.P. Arend, G. Palmer, C. Gabay, IL-1, IL-18, and IL-33 families of cytokines, Immunol. Rev. 223 (2008) 20–38, https://doi.org/10.1111/j.1600-065X.2008.00624.x.
[23] G.P. van Hout, L. Bosch, G.H. Ellenbroek, J.J. de Haan, et al., The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction, Eur. Heart J. 38 (11) (2017) 828–836, https://doi.org/10.1093/eurheartj/ehw247.
[24] J. Jiao, G. Zhao, Y. Wang, P. Ren, et al., MCC950, a selective inhibitor of NLRP3 inflammasome, reduces the inflammatory response and improves neurological outcomes in mice model of spinal cord injury, Front. Mol. Biosci. 7 (2020) 37, https://doi.org/10.3389/fmolb.2020.00037.
[25] Y. Matsubara, Y. Ono, H. Suzuki, F. Arai, et al., OP9 bone marrow stroma cells differentiate into megakaryocytes and platelets, PLoS One 8 (3) (2013), e58123, https://doi.org/10.1371/journal.pone.0058123.
[26] M. Yoshimoto, T. Heike, H. Chang, M. Kanatsu-Shinohara, et al., Bone marrow engraftment but limited expansion of hematopoietic cells from multipotent germline stem cells derived from neonatal mouse testis, Exp. Hematol. 37 (12) (2009) 1400–1410, https://doi.org/10.1016/j.exphem.2009.09.006.
[27] S. Xu, A. De Becker, B. Van Camp, K. Vanderkerken, et al., An improved harvest and in vitro expansion protocol for murine bone marrow-derived mesenchymal stem cells, J. Biomed. Biotechnol. 2010 (2010), 105940, https://doi.org/10.1155/2010/105940.
[28] J. Gao, X.L. Yan, R. Li, Y. Liu, et al., Characterization of OP9 as authentic mesenchymal stem cell line, J Genet Genomics 37 (7) (2010) 475–482, https://doi.org/10.1016/s1673-8527(09)60067-9.
[29] J. Zhang, Y. Chen, D. Yin, F. Feng, et al., Caspase-3/NLRP3 signaling in the mesenchymal stromal niche regulates myeloid-biased hematopoiesis, Stem Cell Res. Ther. 12 (1) (2021) 579, https://doi.org/10.1186/s13287-021-02640-y.
[30] A.R. Mridha, A. Wree, A.A.B. Robertson, M.M. Yeh, et al., NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice, J. Hepatol. 66 (5) (2017) 1037–1046, https://doi.org/10.1016/j.jhep.2017.01.022.
[31] Q. Lin, S. Li, N. Jiang, H. Jin, et al., Inhibiting NLRP3 inflammasome attenuates apoptosis in contrast-induced acute kidney injury through the upregulation of HIF1A and BNIP3-mediated mitophagy, Autophagy 17 (10) (2021) 2975–2990, https://doi.org/10.1080/15548627.2020.1848971.
[32] A. Sharma, J.S.Y. Choi, N. Stefanovic, A. Al-Sharea, et al., Specific NLRP3 inhibition protects against diabetes-associated atherosclerosis, Diabetes 70 (3) (2021) 772–787, https://doi.org/10.2337/db20-0357.
[33] S. Huang, Z. Chen, B. Fan, Y. Chen, et al., A selective NLRP3 inflammasome inhibitor attenuates behavioral deficits and neuroinflammation in a mouse model of Parkinson's disease, J. Neuroimmunol. 354 (2021), 577543, https://doi.org/10.1016/j.jneuroim.2021.577543.
[34] A.P. Perera, R. Fernando, T. Shinde, R. Gundamaraju, et al., MCC950, a specific small molecule inhibitor of NLRP3 inflammasome attenuates colonic inflammation in spontaneous colitis mice, Sci. Rep. 8 (1) (2018) 8618, https://doi.org/10.1038/s41598-018-26775-w.