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ORIGINAL RESEARCH

Investigating the Role and Underlying Mechanisms of 18β-Glycyrrhetinic Acid in the Therapy of Ulcerative Colitis Through Modulation of the PPAR-γ/NF-κB Signaling Pathway

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Background: 18β-glycyrrhetinic acid (18β-GA), a triterpenoid saponin naturally occurring in *Glycyrrhizae uralensis*, has potent anti-inflammatory and antioxidant properties, but the therapeutic efficacy and precise mechanism of 18β-GA in ulcerative colitis (UC) remain unclear.

Methods: To determine the therapeutic potential of 18β-GA, we constructed a dextran sodium sulfate (DSS)-induced UC model on a cohort of thirty-two female BALB/c mice and used mouse peritoneal macrophages to establish a co-culture system for in vitro experiments. We measured body weight, fecal characteristics, colon length, disease activity index (DAI) of mice, and the degree of colonic histological lesions. Changes in the composition of intestinal flora were monitored using high-throughput 16S rDNA sequencing. Combining network pharmacology and molecular docking to predict pharmacological mechanisms and using Western blot for validation.

Results: 18β-GA significantly alleviated DSS-induced weight loss, colon length reduction, an increase in the DAI score, and pathological colon damage. Additionally, 18β-GA promotes a favorable environment that hindered the proliferation of pathogenic bacteria, thereby promoting gut health. Co-culture and scratch assays confirmed that 18β-GA promotes mucosal repair. Network pharmacology and molecular docking predicted potential drug targets, while Western blot analysis revealed that 18β-GA down-regulated phosphorylated nuclear factor kappa-B (p-NF-κB) and activated the peroxisome proliferator-activated receptor γ (PPAR-γ).

Conclusion: The therapeutic application of 18β-GA in UC demonstrates a multifaceted pharmacological process. It fosters harmonious intestinal microbiota, reinstates the integrity of the intestinal barrier, and exerts its beneficial effects through modulating the PPAR-γ/NF-κB signaling pathway, underscoring its potential as a therapeutic agent for UC.

Keywords: 18β-GA, ulcerative colitis, 16S rDNA sequencing, PPAR-γ/NF-κB signaling

Introduction

Ulcerative colitis (UC) is a chronic and nonspecific inflammatory disease of the gastrointestinal tract characterized by persistent diarrhea, discharge of mucopurulent and bloody stools, and abdominal discomfort that imposes a significant burden on those living with it. Its pathogenesis is driven by hyperactive inflammatory pathways, leading to excessive pro-inflammatory cytokine production and an imbalance between pro- and anti-inflammatory mediators in colonic tissues.[1] The pathophysiology of ulcerative colitis usually involves immunological dysregulation, malfunction of the epithelial barrier, dysbiosis of

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the gut microbiota, and so forth.[2,3] Given its unknown cause, substantial risk of recurrence, and often poor prognosis, UC has long posed a formidable challenge in clinical practice. Current pharmacological strategies for UC primarily include 5-aminosalicylic acid (5-ASA), glucocorticoids, thiopurines, biological medications, and anti-cytokine therapies.[4] However, the substantial cost and potential adverse effects of these treatments hinder their long-term sustainability. Consequently, there is an urgent need to identify novel therapeutic options that are more efficacious and safer.

Peroxisome proliferator-activated receptor γ (PPAR-γ), a nuclear receptor and ligand-activated transcription factor, is a key regulator of inflammation and plays a critical role in maintaining gut homeostasis and attenuating inflammatory damage.[5,6] PPAR-γ is essential for maintaining gut homeostasis by regulating inflammatory responses and preventing tissue damage. Pharmacological modulation of PPAR-γ activity, exemplified by 5-ASA, significantly attenuates intestinal inflammation.[7] Through the inhibition of the NF-κB signaling cascade, PPAR-γ activation reduces the generation of inflammatory cytokines. A crucial transcription factor that controls inflammatory and immunological processes, NF-κB is intimately associated with the pathophysiology of ulcerative colitis.[8–10] Consequently, the PPAR-γ/NF-κB pathway holds promise as a potentially effective therapeutic strategy for UC treatment.

In the realm of traditional Chinese medicine, glycyrrhiza uralensis (GU) stands as one of the most revered and frequently employed herbs. 18β-glycyrrhetinic acid (18β-GA), a triterpenoid saponin metabolite of glycyrrhizin, is a key bioactive compound in GU, exhibiting significant anti-inflammatory, antiviral, antitumor, antioxidant, and immunomodulatory properties.[11–14] The chemical structure is illustrated in Figure 1a. This underscores the profound therapeutic potential of 18β-GA, and highlights its significance in modern medicinal research and practice.

To further investigate the anti-colitis efficacy and underlying mechanisms of 18β-GA, we embarked on a comprehensive investigation both in vitro and in vivo. 5-ASA ameliorates UC in mice through dual mechanisms of preserving intestinal epithelial barrier integrity and modulating gut microbiota composition, while simultaneously suppressing JNK and p38 MAPK phosphorylation in colonic macrophages.[15,16] Based on the aforementioned effects and mechanisms of 5-ASA, we utilized 5-ASA as the positive control drug in our in vivo experiments. This study aimed to elucidate the intricate interplay between 18β-GA and mitigating colitis by fostering a more profound understanding of its therapeutic potential.

Materials and Methods

Ethics Statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Jining Medical University (JNMC-2022-DW-162) and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Chemicals and Reagents

18β-GA (purity>99%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Dextran sodium sulfate (DSS) (molecular weight: 36–50 kDa) was purchased from MP Biomedicals (Irvine, CA, USA). A qualitative fecal occult blood detection kit was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Hematoxylin and eosin (H&E) stain was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Antibodies against PPAR-γ and p-NF-κB were purchased from Cell Signaling Technology Co. Ltd. (Danvers, MA, USA). Anti-mouse and anti-rabbit secondary antibodies were obtained from eBioscience (San Diego, CA, USA). Enhanced chemiluminescent substrate was purchased from Beijing Labgic Technology Co., Ltd. (Beijing, China). Transwell inserts (pore size, 0.4 μm) were purchased from Corning Inc. (Kennebunk, ME, USA).

Cell Culture

Mouse colonic epithelial cells (MCECs) were cultivated in a humidified incubator maintained at a constant temperature of 37°C and a CO_2 concentration of 5%. They were nourished with high-sugar Dulbecco's Modified Eagle's Medium (DMEM) and further enriched with 10% fetal bovine serum to support their growth and proliferation. The culture medium was fortified with antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin).

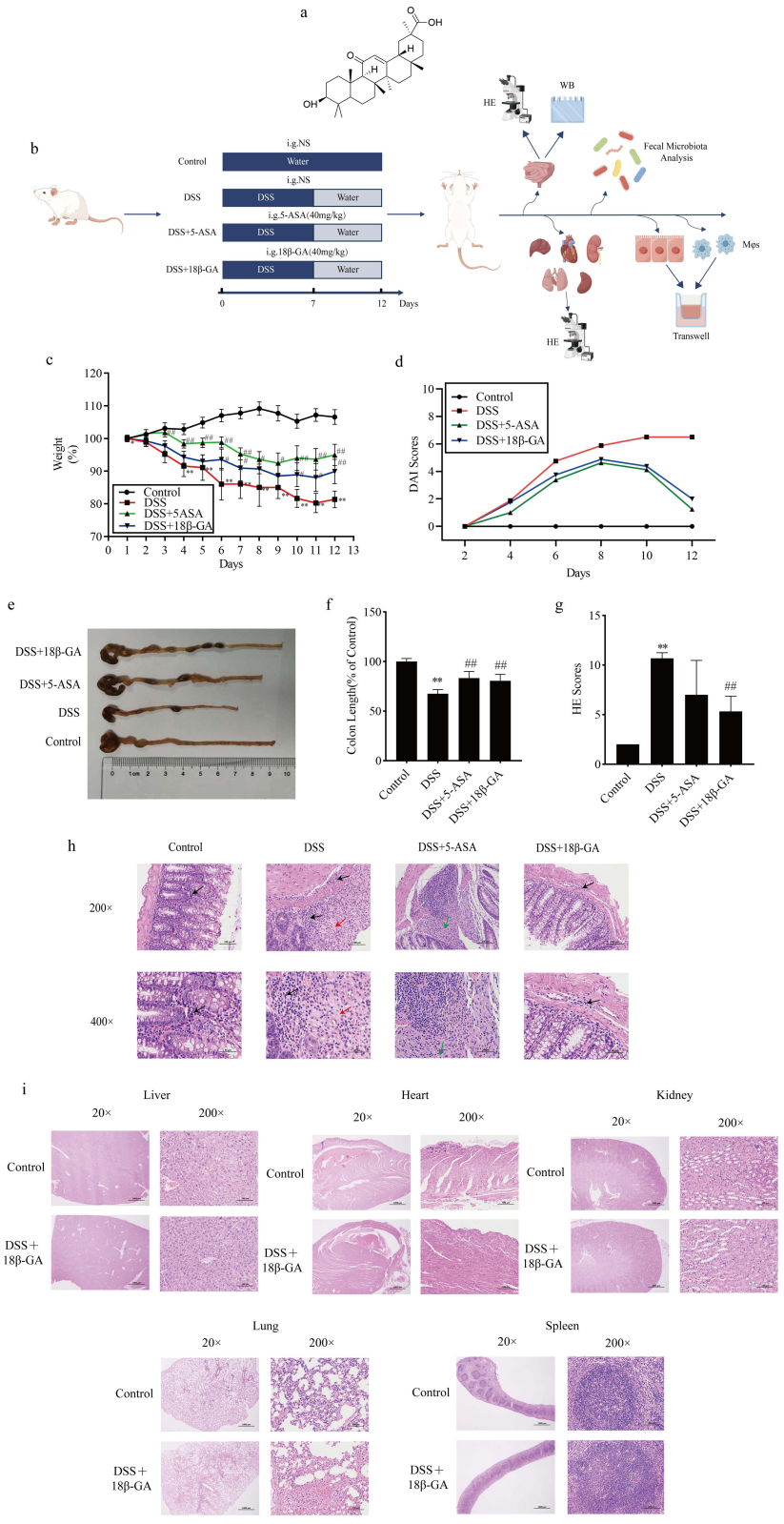

Figure 1 18β-GA ameliorates DSS-induced UC in mice. (**a**) The chemical formula of 18β-GA. (**b**) Experimental process. (**c**) Body weights of mice in all groups. (**d**) DAI scores. (**e** and **f**) Macroscopic appearance and the length of colons from each mouse group. (**g**) Hematoxylin and eosin staining of colonic sections. Black arrows indicated inflammatory cell infiltration, red arrows indicated the loss of intestinal gland necrosis, and green arrows indicated a limited presence of neutrophils. (**h**) Histological scores. *$p < 0.05$, **$p < 0.01$ compared with control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with DSS group. (**i**) H&E staining of the major organs Liver, Heart, Kidney, Lung and Spleen.

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Cell Counting Kit (CCK)-8 Experiment

During the logarithmic growth phase, MCECs were implanted into 96-well plates to ensure a uniform growth density of approximately 30% within each well. Six replicate wells were established using various concentrations of 18β-GA (1000, 500, 250, 125, 62.5, and 31.25 μM). After 24 h of drug treatment, 10μL of the CCK-8 reagent was added to each well. The wells were then incubated for an additional hour. Finally, the absorbance (A) was measured. The experiment described above was repeated thrice. Cell viability was estimated based on these measurements. Cell viability (%) = $(A_{test} - A_{blank})/(A_{control} - A_{blank}) \times 100\%$.

Animals and Experimental Protocols

Female BALB/c mice that were 35–40 days old and 18–22 g in weight) were supplied by Jinan Pengyue Experimental Animal Breeding Co. Ltd. (Jinan, China), which were maintained under optimal conditions, featuring a constant temperature of 21 ± 2 °C and a humidity level maintained at 45 ± 10%. A typical circadian rhythm was established to preserve the mice's regular physiological functions. Mice were randomly assigned to four groups (n=8): untreated control, DSS model, DSS+5-ASA, and DSS+18β-GA. Mice were administered a 4% (w/v) DSS solution for 7 days, followed by normal water for 5 days, with the exception of the control group.[17] Mice in the two treatment groups received daily oral gavage of either 5-ASA (40 mg/kg) or 18β-GA (40 mg/kg) from days 1 to 12, whereas mice in the control and DSS groups received saline during this period. All mice were euthanized on day 13.

Disease Activity Index (DAI) Score

The DAI scale was calculated from the combined scores of 0–4 for body weight loss, stool consistency, and rectal bleeding (Table 1).[18]

Collection of Main Organs and Intestinal Tissues

The main organs, including the heart, liver, spleen, lungs, and kidneys, were collected and weighed. The colon and rectum were separated from the small intestine at the proximal ileocecal end and from the anus at the distal end. Consistent with previous reports, this section of the colon was extracted and straightened without stretching and its length.[19] Following a longitudinal dissection of the colon and subsequent thorough rinsing with saline solution, samples of the colon as well as main organs were preserved in a 10% formalin solution, fixed in paraffin and stained with hematoxylin and eosin (H&E), while the remaining colon tissue was stored at −80°C for further analysis.

H&E Staining

Paraffin-embedded colonic tissue blocks were cut into 4-μm-thick sections using a microtome and stained with H&E, as previously described.[20] The histological change scoring criteria are presented in Table 2.

16S rDNA Sequencing and Microbiota Analysis

Using the forward (5'-AGRGTTTGATYNTGGCTCAG-3') and reverse (5'-TASGGHTACCTTGTTASGACTT-3') primer pairs, sequencing of 16S rDNA was performed. Third-generation microbial diversity was based on the PacBio sequencing platform, and single-molecule real-time cell sequencing was used to sequence the marker genes. The circular consensus sequence was filtered, clustered, or denoised, and species annotation and abundance analyses were performed as previously described[21] to reveal the species composition of each sample. The following analyses were performed: species taxonomy and annotation,

Table 1 The Calculation Method of DAI

Score	Weight Loss (%)	Stool Consistency	Fecal Occult Blood Condition
0	0	Normal	Negative
1	1–5	—	Light blue
2	5–10	Loose stool	Blue
3	10–15	—	Dark blue
4	>15	Diarrhea	Gross blood

Table 2 Scoring Criteria for Histological Changes

Score	Number of Ulcers	Epithelial Cell Changes	Lesion Depth
0	0	Normal	Normal
1	1	Goblet cell	Mucous membrane
2	2	Massive goblet cell depletion	Submucosa
3	3	Crypt absence	Muscle
4	>3	Crypt large area absence/polypoid regeneration	Serosa

significant differences, and diversity (alpha and beta diversities). The repository(s) names and accession numbers(s) are available at https://www.ncbi.nlm.nih.gov/ (accessed on August 25, 2024), PRJNA1152111.

Network Pharmacology

Search for 18β-GA-related targets in TCMSP[22] (https://old.tcmsp-e.com/tcmsp.php, accessed on June 3, 2023), The Encyclopedia of Traditional Chinese Medicine[23] (ETCM, http://www.tcmip.cn/ETCM/index.php/Home/Index/, accessed on June 3, 2023), Swiss Target Prediction[24] (http://www.swisstargetprediction.ch/, accessed on June 3, 2023), and TargetNet[25] (http://targetnet.scbdd.com/home/index/, accessed on June 3, 2023) databases. Duplicates were removed, and the remainder were imported into UniProt Protein[26] (UniProt, https://www.uniprot.org/, accessed on June 3, 2023) to standardize target names and ultimately obtain drug-related targets. Using "ulcerative colitis" as the keyword, Gene cards[27] (https://www.genecards.org/, accessed on June 3, 2023), OMIM[28] (http://www.omim.org, accessed on June 3, 2023), Therapeutic Target Database[29] (TTD, https://db.idrblab.net/ttd/, accessed on June 3, 2023), Pharmacogenetics and Pharmacogenomics Knowledge Base[30] (PharmGKB, https://ngdc.cncb.ac.cn/databasecommons/database/id/1436, accessed on June 3, 2023), and DrugBank[31](https://go.drugbank.com/, accessed on June 3, 2023) databases were searched, and UC targets were overloverlated and de-duplicated, and imported into UniProt database to standardize the target names and obtain the final UC disease targets. 18β-GA-related and UC disease targets were imported into the Venny2.1[32] online mapping tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html, accessed on June 3, 2023) to create a unified "Drug-Disease" target. Using STRING (http://cn.string-db.org, accessed on June 3, 2023) to select the "Homo sapiens" species and setting the highest confidence to >0.4, import the results into Cytoscape 3.9.1 software[33,34] (http://cytoscape.org/ver.3.9.1, accessed on June 3, 2023) to construct a protein-protein interaction (PPI) network diagram for common target proteins. Using Metascape (https://metascape.org/gp/ index.html, accessed on 3 June 2023) for gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of common targets between drugs and diseases, the results include GO analysis for biological processes (BPs), cellular components (CCs), and molecular functions (MFs), as well as KEGG enrichment results.[35]

Molecular Docking

The files for the protein and 18β-GA were obtained from the Protein Data Bank (PDB)[36] database (https://www.rcsb.org/, accessed on June 3, 2023) and PubChem[37] database (https://pubchem.ncbi.nlm.nih.gov, accessed on June 3, 2023). AutoDockTools were used to export proteins and 18β-GA in the PDBQT format. Next, use AutoGrid 4 to set up the docking area, export the grid point parameter file, and utilize PyMOL 2.4.0 software for visualization analysis. Finally, the data was uploaded to the DockThor[38] website to determine the matching score.

Co-Culture and Scratch Assay

Mice were induced for 5 days with a 4% DSS solution and and sacrificed on the 6th day. Peritoneal macrophages (Mφs) were collected and cultured in Dulbecco's modified DMEM medium. MCECs were plated in 6-well culture plates and incubated at 37 °C in an incubator with 5% CO_2. And peritoneal macrophages (Mφs) were added to the upper chamber of the Transwell insert (pore size: 0.4 μm) to co-cultured. The co-culture system was treated with 18β-GA (62.5 μM). After the above treatment, monolayers of MCECs were scratched and observed at 0 and 24 h, and the percentage coverage was calculated.

Western Blotting

Protein expression of PPAR-γ and p-NF-κB was examined in colon tissues using a previously described Western blotting method.[20,39]

Statistical Analysis

GraphPad Prism software (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. All information was displayed as mean ± SD. The significant difference between groups were analyzed by Student's t-test (for normal distribution), p values of less than 0.05 were considered significant for all data.

Ethical Statement

This study utilized exclusively publicly available data from databases including TCMSP, ETCM, Swiss Target Prediction, TargetNet, UniProt, GeneCards, OMIM, TTD, PharmGKB, DrugBank, PDB, PubChem, and NCBI (Project PRJNA1152111, accessed on August 25, 2024). All data were anonymized, legally acquired, and devoid of personal identifiers. According to Items 1 and 2 of Article 32 of China's Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (effective February 18, 2023), this research is exempt from ethics approval as it involves secondary analysis of pre-existing public data without privacy risks or commercial interests. The longitudinal design (2023–2024) required phased data validation, consistent with ethical exemption criteria for non-interventional bioinformatics studies.

Results

18β-GA Ameliorates DSS-Induced UC in Mice

All the animal procedures and assays are shown in Figure 1b. The DSS-induced weight loss was reduced by 18β-GA treatment (Figure 1c). The total DAI of DSS-induced mice was decreased by 18β-GA treatment, as evaluated by weight loss and loose and bloody stools (Figure 1d). The colon lengths of mice in the DSS group were significantly shorter than those of mice in the control group, whereas the colon lengths of the 18β-GA-treatment group mice were significantly longer than those of the model mice (Figure 1e and f). Using H&E staining, a thorough examination of colonic injury and inflammatory cell infiltration was conducted, and the results showed that the administration of DSS elicited profound colonic necrosis, characterized by the disappearance of mucosal integrity and glandular architecture, coupled with notable hyperplasia of connective tissue (Figure 1g and h). Furthermore, mice in the DSS group showed a large amount of inflammatory cell infiltration in colon tissues, indicative of a robust inflammatory response, and subsequent treatment with 18β-GA led to a marked improvement in all the aforementioned pathological features, suggesting potent therapeutic efficacy in mitigating DSS-induced colonic damage and inflammatory sequelae (Figure 1g and h). Meanwhile, H&E staining showed that the hearts, livers, spleens, lungs, and kidneys of mice in the administration group had no obvious lesions (Figure 1i–m). These results indicated that 18β-GA has certain safety on the major organs of mice.

Fecal Microbiota Analysis

To investigate the effects of 18β-GA on gut microbiota composition, 16S rDNA sequencing was performed. Alpha diversity was evaluated using richness indices (Chao1 and ACE) and diversity indices (Shannon and Simpson). Sparse rarefaction and Shannon curves validated the sufficiency of sample size and sequencing depth (Figure 2a and b). The 18β-GA group displayed lower ACE and Chao1 indices compared to the model group, indicating reduced microbial richness post-treatment. Similarly, the decreased Shannon and Simpson indices in the 18β-GA group suggested diminished species diversity following administration (Figure 2c). Beta diversity, reflecting intergroup variability, was assessed using unweighted UNIFRAC. Principal Coordinates Analysis (PCoA) revealed distinct clustering patterns among groups, highlighting significant compositional differences in intestinal microbiota. 18β-GA administration enhanced microbiota diversity, underscoring its potential to restore microbial homeostasis (Figure 2d). Nonparametric Similarity Analysis confirmed that intergroup differences in community composition and abundance were more pronounced than intragroup variations (Figure 2e). Linear discriminant analysis coupled with effect size measurements

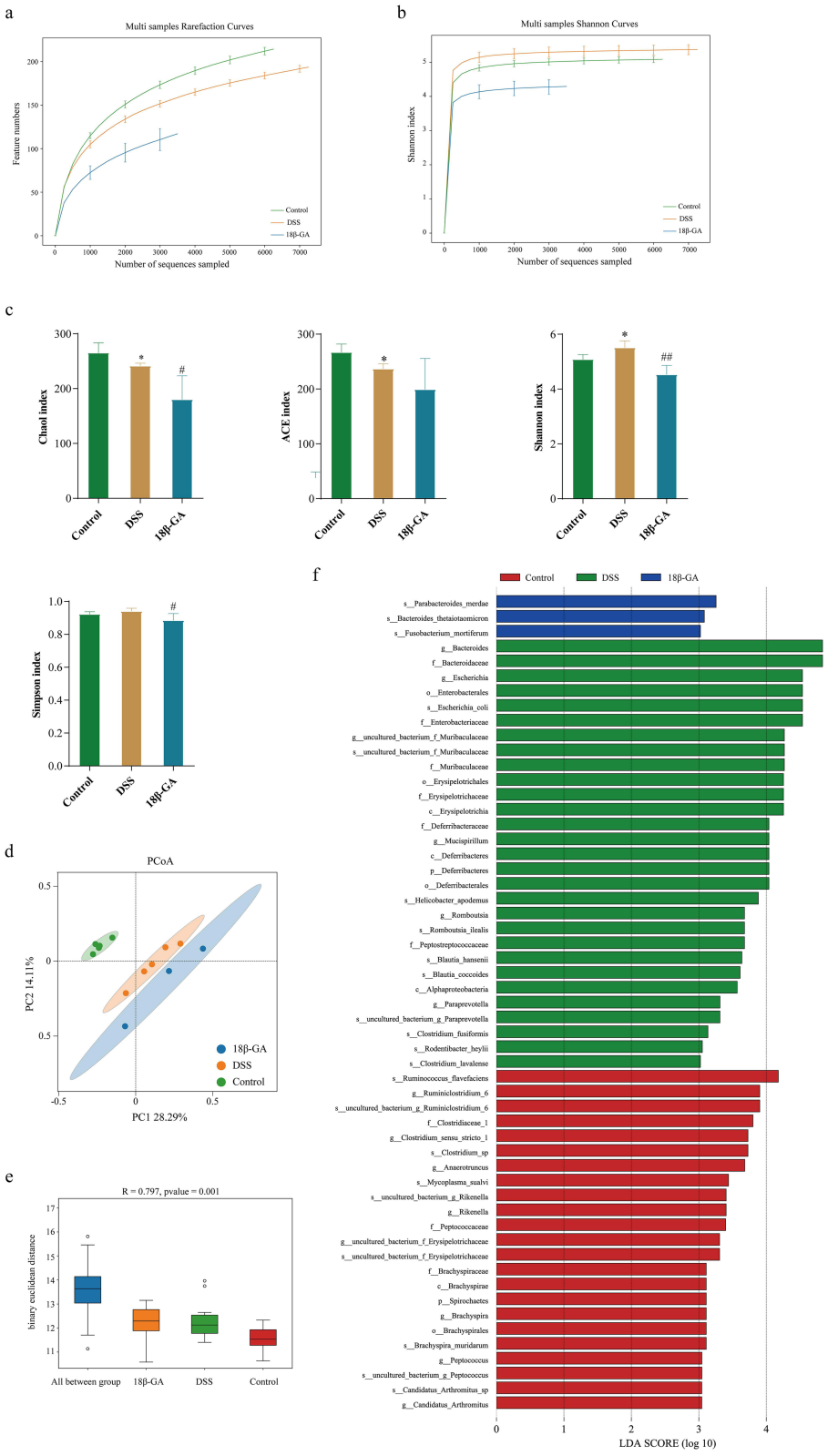

Figure 2 Changes in microbiota in different groups. (**a**) Rarefaction analysis. (**b**) Shannon index curve. (**c**) Microbial community α diversity index of mice in. *$p < 0.05$ compared with the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group. (**d**) PCoA plot of unweighted unifrac distance. (**e**) Analysis of variance of intestinal bacterial communities in mice. (**f**) LEfSe analysis.

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(LEFSe) identified bacterial taxa with significant differential abundance between groups. We found that mice in the control group had a higher abundance of bacteria, including *Ruminococcus_flavefaciens, Ruminiclostridium*, and *Clostridiaceae*, than those in the other groups, and the number of bacteria in the DSS group increased significantly, including *g_Escherichia, o_Enterobacterales, and f_Deferribacteres*, which was *s_Parabacteroides_merdae* in the 18β-GA treatment group (Figure 2f).

Network Pharmacological Analysis of 18β-GA

In our comprehensive analysis, we initially identified 6, 48, 92, and 27 potential targets of 18β-GA by leveraging the complementary resources of four distinct databases: TCMSP, ETCM, Swiss Target Prediction, and Target Net. To ascertain the relevant targets of UC, we employed the keyword "ulcerative colitis" and retrieved 3769, 7, 38, 15, and 180 potential targets from GeneCards, OMIM, TTD, PharmGKB, and DrugBank, respectively. To streamline our findings, we removed duplicates and normalized the remaining targets to their respective gene names as recorded in UniProt. This process yielded a refined list of 143 potential targets of 18β-GA and an extensive list of 3821 potential targets of UC. To visually illustrate the overlap between these two sets of targets, we utilized the Venny 2.1 tool to generate a Venn diagram. This analysis revealed a significant intersection, pinpointing 80 common targets that serve as crucial "drug-disease" intersections, underscoring their potential significance in the therapeutic landscape of 18β-GA for UC (Figure 3a). The drug-disease targets were uploaded to the STRING database, 80 nodes and 433 edges were obtained, and the PPI network was constructed using the Cytoscape software (Figure 3b). The average degree of the network was found to be about 10.825, the average number of betweenness was about 97.575, and the average proximity was about 0.0058306, and 16 targets were found to be important targets of 18β-GA in UC. Drug-disease intersection targets were also introduced into the Medscape platform for GO biological function analysis and KEGG pathway enrichment analysis. Using $p < 0.05$ as the main screening criteria, a total of 922 GO biological function items were retrieved, including 808 BPs, 36 CCs, and 78 MFs (Figure 3c). A total of 108 signaling pathways were obtained by KEGG pathway enrichment analysis, which may be related to the same signaling pathway (Figure 3d). Moreover, the results showed that 18β-GA had antimicrobial effects, which was consistent with the results of 16S rDNA sequencing.

18β-GA Molecular Docking with the Top 10 Core Target Proteins in the PPI Network

In the process of conducting molecular docking simulations with 18β-GA against the top ten pivotal target proteins associated with ulcerative colitis (as depicted in Figure 4a–j), it is well established that a lower binding energy signifies a more robust interaction between the ligand (18β-GA) and its acceptor proteins. As shown in Table 3, the affinity score in the molecular docking results reflects the level of binding between 18β-GA and the top ten core target proteins, including IL6 (PDB ID: 1ALU), TNF (1A8M), PTGS2 (5F19), CTNNB1 (7 AFW), ESR1 (7UJO), PPARG (2ZK2), MAPK3 (4QTB), PPARA (4CI4), NR3C1 (1NHZ), CYP2E1 (3T3Z).

18β-GA Regulated the Expression of PPAR-γ and p-NF-κB in DSS-Induced Colon Tissue

Western blotting results showed that the expression of PPAR-γ was increased and the expression of p-NF-κB was decreased by treatment with 18β-GA in colon tissues (Figure 5).

18β-GA Promoted the Migration of MCECs by Mφs

In scratch experiments in the co-culture system, DSS-treated Mφs showed a significant decrease in the migration ability of MCECs, whereas 18β-GA-treated Mφs promoted the migration of MCECs (Figure 6). These results indicate that 18β-GA promotes cell migration to restore the intestinal barrier function.

Discussion

In this study, we used a DSS-induced animal model of colitis and isolated macrophages to construct a co-culture system, both in vivo and in vitro experiments to highlight the promising therapeutic effects of 18β-GA and the underlying

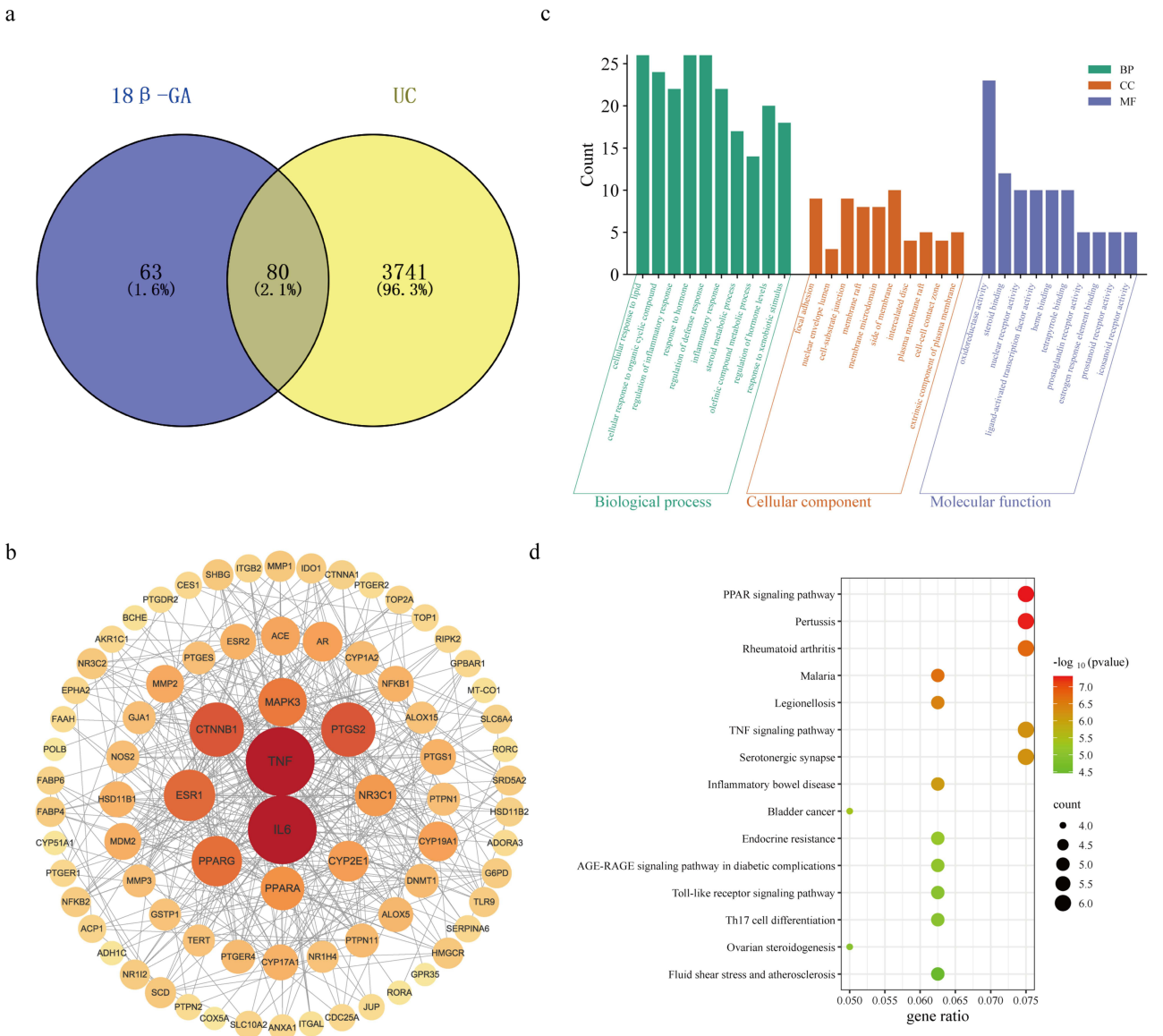

Figure 3 Network pharmacology analysis of 18β-GA. (**a**) Venn diagram intersection between 18β-GA and UC. (**b**) PPI network diagram of intersection targets of 18β-GA and UC. (**c**) Gene ontology analysis. (**d**) KEGG pathway enrichment analysis. The intensity of red deepens signifies a decrease in the p-value, and the expansion of the bubble's size is proportional to the greater number of genes encapsulated within the pathway.

mechanisms. Our investigation revealed that 18β-GA is a promising candidate for the prevention and treatment of DSS-induced UC in mice. Notably, the therapeutic efficacy of 18β-GA was evident through the marked amelioration of UC symptoms, including a reduction in DSS-induced weight loss, colon length, pathological colon damage, and a substantial decline in the DAI score. Histopathological assessments further corroborated the therapeutic benefits of 18β-GA, revealing substantial improvements in the 18β-GA-treated group, which exhibited a notable reduction in mucosal ulceration, necrosis, and inflammatory cell infiltration in contrast to the pronounced pathological changes observed in the DSS-treated group. H&E staining showed that 18β-GA had no significant pathological damage to the vital organs of mice. These findings not only reinforce the potential of 18β-GA as a viable therapeutic option for UC but also pave the way for further exploration of its mechanisms of action and clinical translation.

The gut microbiota is pivotal in modulating immune, metabolic, and nervous system interactions and is essential for maintaining critical physiological functions.[40] UC pathogenesis is strongly linked to gut dysbiosis, which initiates a cascade of intestinal inflammatory responses, driving disease development and progression.[41] Therefore, 16S rDNA

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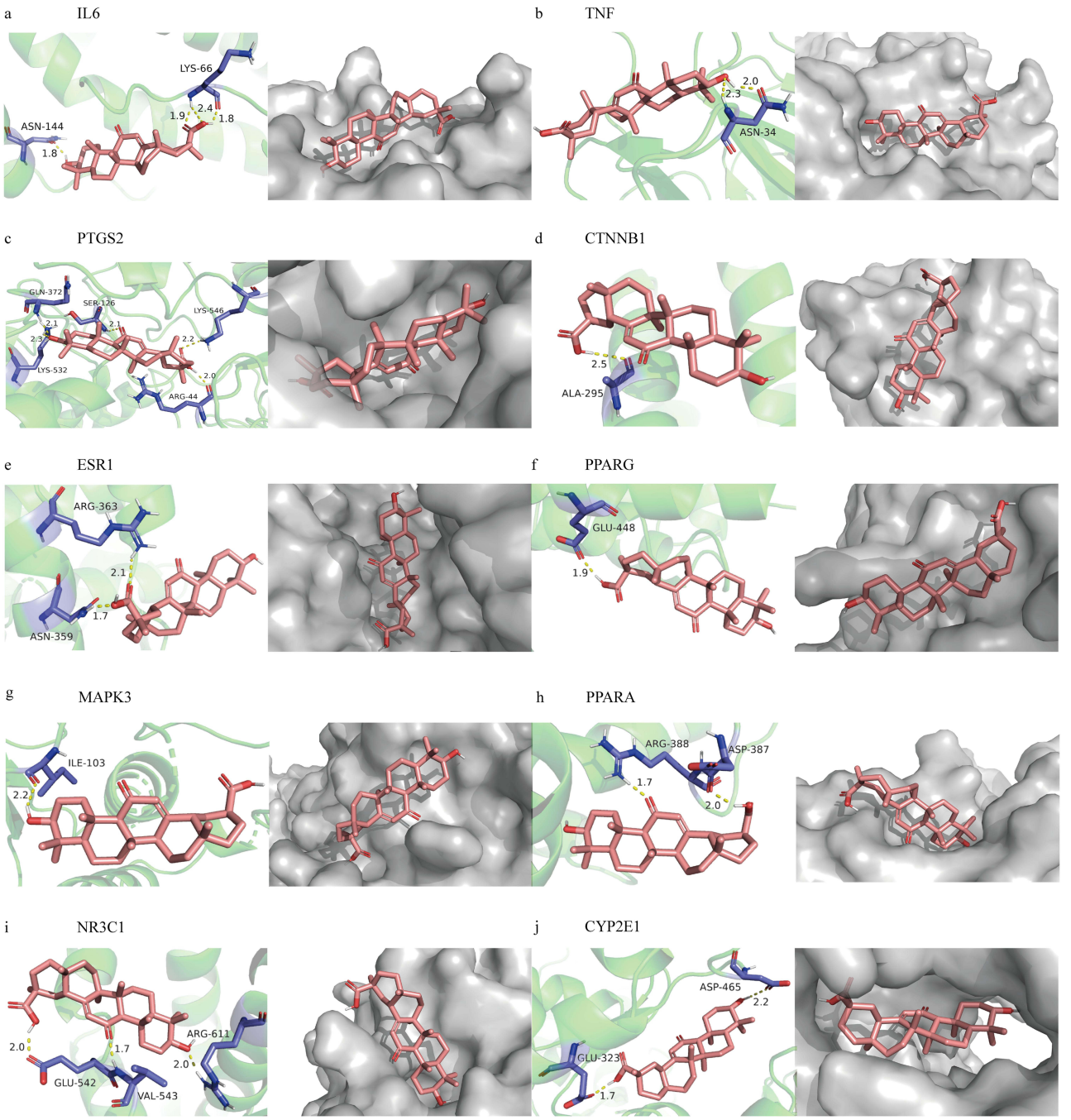

Figure 4 Partial diagram of molecular docking of 18β-GA with the following targets. (**a**) IL6; (**b**) TNF; (**c**) PTGS2; (**d**) CTNNB1; (**e**) ESR1; (**f**) PPARG; (**g**) MAPK3; (**h**) PPARA; (**i**) NR3C1; (**j**) CYP2E1.

sequencing was employed to investigate the impact of 18β-GA on gut microbiota composition. All sample banks used for microbiological analyses had a coverage rate of over 99%, indicating that the sample banks were large enough to contain the majority of microorganisms. The number of operational taxonomic units in each group was saturated, appropriately representing the majority of species. *Parabacteroides* are core members of the human gut flora.[42] *Parabacteroides* may modulate colonic inflammation, improve intestinal flora homeostasis and alleviate UC by generating PPAR-γ agonists and enhancing the integrity of the colonic mucosal epithelial barrier.[43,44] Our results demonstrated that compared to the DSS group, 18β-GA-treated mice had a considerably higher number of *Parabacteroides*. *Deferribacteres* have been found in significantly higher quantities in colitis mice according to previous studies.[45] Additionally, the prevalence of

Table 3 Molecular Docking Affinity Scores

Target Protein Name	Protein	Affinity
IL6	1ALU	−7.179
TNF	1A8M	−7.401
PTGS2	5F19	−5.972
CTNNB1	7AFW	−7.44
ESR1	7UJO	−7.435
PPARG	2ZK2	−8.511
MAPK3	4QTB	−7.399
PPARA	4CI4	−8.229
NR3C1	1NHZ	−6.776
CYP2E1	3T3Z	−8.781

Enterobacteriaceae and *escherichia coli* has been observed at elevated levels in UC patients,[46] which is consistent with our current findings that revealed a heightened relative abundance of these three bacterial entities within the DSS-treated group. In summary, upon modifying the composition of the intestinal flora, 18β-GA orchestrated the restoration of the

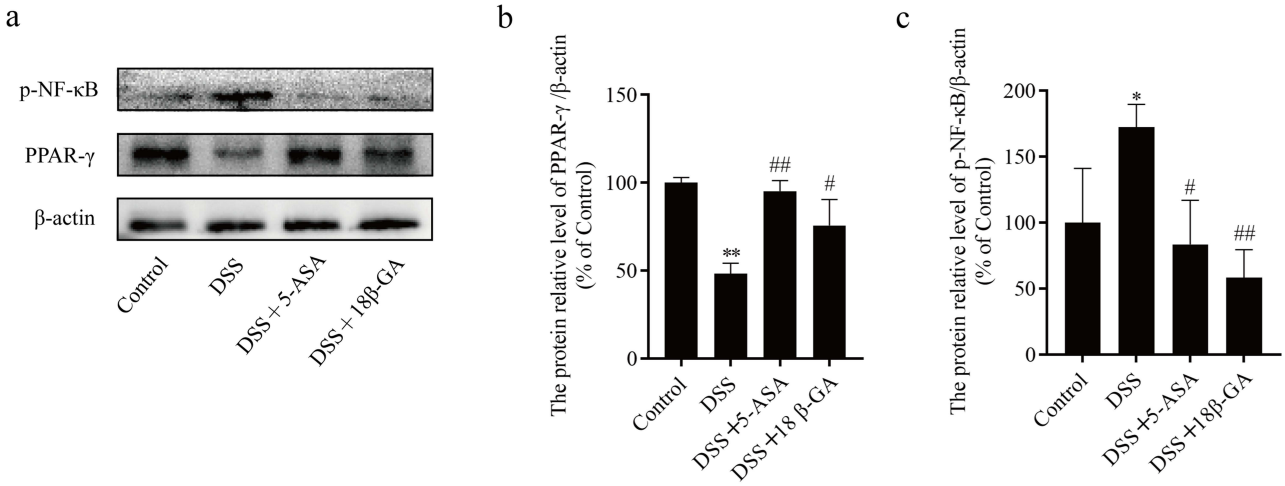

Figure 5 18β-GA regulated the activation of PPAR-γ/NF-κB signaling pathway. (**a–c**) Western blot analysis of PPAR-γ and p-NF-κB expression in the colon tissues. *$p < 0.05$, **$p < 0.01$ compared with control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with DSS group.

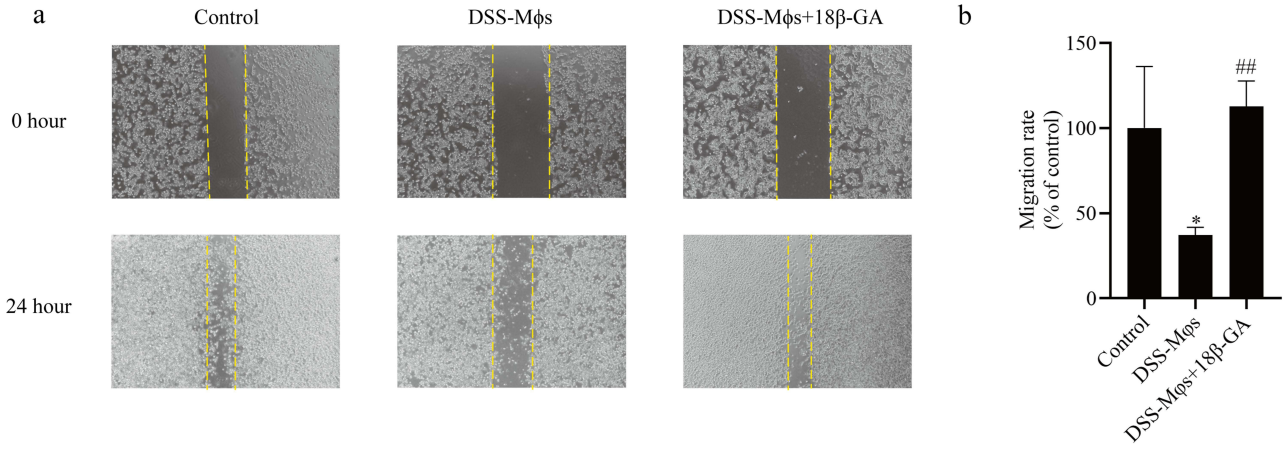

Figure 6 Scratch assay showing the migration capacity of mouse colon epithelial cells (MCECs) co-cultured in vitro with macrophages under 18β-GA treatment. **(a)** Time-dependent changes in epithelial cell migration rate (0 and 24 hours) across experimental groups. **(b)** Comparative migration rates (% of control) among Control, DSS-Mφs, and DSS-Mφs+18β-GA groups. *$p < 0.05$ compared with control group, $^{\#\#}p < 0.01$ compared with DSS group.

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microbial ecosystem towards optimal health, with enhancement of beneficial bacterial populations coupled with a concerted reduction in pathogenic bacteria, fostering an environment conducive to mucosal healing, a cornerstone in the remission of UC. By meticulously manipulating the microbial dynamics, 18β-GA has illuminated a promising avenue for promoting gut health and facilitating the resolution of UC symptoms. Our research findings highlight the association between changes in gut microbiota and UC disease, but they have not yet demonstrated a causal relationship between drug effects and microbiota changes through specific experiments. This will be the focus of our next work.

In the top ten key target proteins associated with 18β-GA and ulcerative colitis, IL6 and TNF function as pro-inflammatory cytokines, promoting inflammation.[47] PTGS2, a crucial polyamine-related gene in UC, can accurately predict the course of patients.[48] The CTNNB1 gene produces the protein β-catenin, which plays a key function in the colon via the Wnt/β-catenin pathway.[49] ESR1 is expressed as the genomic signaling isoform ERα. SERM2, an ERα antagonist, has a moderate but consistent anti-inflammatory effect.[50] PPARγ, an immune-related gene, governs the immune response, which plays a vital role in the progression and development of UC by facilitating interactions between characteristic biomarkers and immune invading cells.[51] MAPK3 encodes a protein from the MAP kinase family, which regulates the start and progression of UC via the MAPK/ERK/JNK signaling pathway.[52] PPAR-alpha antagonism or knockdown dramatically reduces the clinical activity of DSS-induced colitis in mice.[53] NR3C1 has a negative effect in DSS-induced experimental colitis, inhibiting epithelial proliferative responses, resulting in poor wound healing and decreased endogenous corticosterone production.[54] CYP2E1 can be utilized as a biomarker to assess ulcerative colitis.[55] All of the above ten targets are closely related to UC.

To investigate the effects of 18β-GA on enhancing ulcer-healing capabilities in UC mice, peritoneal Mφs were isolated from diseased animals and subsequently co-cultured with MCECs. This intricate setup was devised to mimic the inflammatory microenvironment that characterizes the intestinal epithelium, thereby providing a valuable platform for investigating the potential therapeutic mechanisms of 18β-GA in promoting ulcer repair. The results showed that 18β-GA promoted wound healing in the co-culture system. The M1 phenotype of macrophages plays a pivotal role in amplifying inflammatory cascades by upregulating inflammatory factors and chemokines, and fostering the generation of reactive oxygen species and reactive nitrogen intermediates.[56] Conversely, the M2 phenotype embodies an anti-inflammatory demeanor, curbing inflammatory reactions and fostering an environment conducive to wound healing and tissue repair.[56] Based on the above research, we postulated that 18β-GA may either facilitate the conversion of M1-Mφs to M2-Mφs or hinder the conversion of M2-Mφs to M1-Mφs, thereby reducing the levels of pro-inflammatory ILs in DSS-induced colitis mice and promoting mucosal healing. Additionally, the effect of the drug on macrophage homing in vivo is a crucial aspect we will address nexts.

PPAR-γ is a ligand-produced transcription factor belonging to the nuclear receptor superfamily and is considered a key target for the treatment of inflammation.[57] The classical view of PPAR-γ function is that it forms a heterodimer with the retinoid X receptor[58] and the complex binds to peroxisome proliferator response elements (PPREs) in the promoter region of the target gene to inhibit the expression of inflammatory factors.[59,60] As a nuclear transcription factor, NF-κB regulates both apoptosis and secretion of pro-inflammatory cytokines (such as TNF-α and IL-1β), adhesion molecules, and chemokines.[61] By modulating these fundamental biological processes, NF-κB plays a central role in the complex web of molecular events underlying the development and progression of this inflammatory bowel condition. In summary, the PPAR-γ/NF-κB signaling pathway exerts satisfactory anti-inflammatory effects in colitis. Western blot analysis revealed an elevation in the expression levels of PPAR-γ, accompanied by a decline in the expression of phosphorylated NF-κB (p-NF-κB), in the group subjected to 18β-GA treatment, in contrast to the DSS group. This observation aligns well with the results of our network pharmacology investigations, further corroborating the significance of our findings. Currently, we are only using WB experiments for preliminary detection of the mechanism, and we will use siRNA and plasmid transfection in our next study to delve deeper into the drug mechanism.

As shown in Figure 7, 18β-GA played an important role in UC treatment by regulating the PPAR-γ/NF-κB signaling pathway.

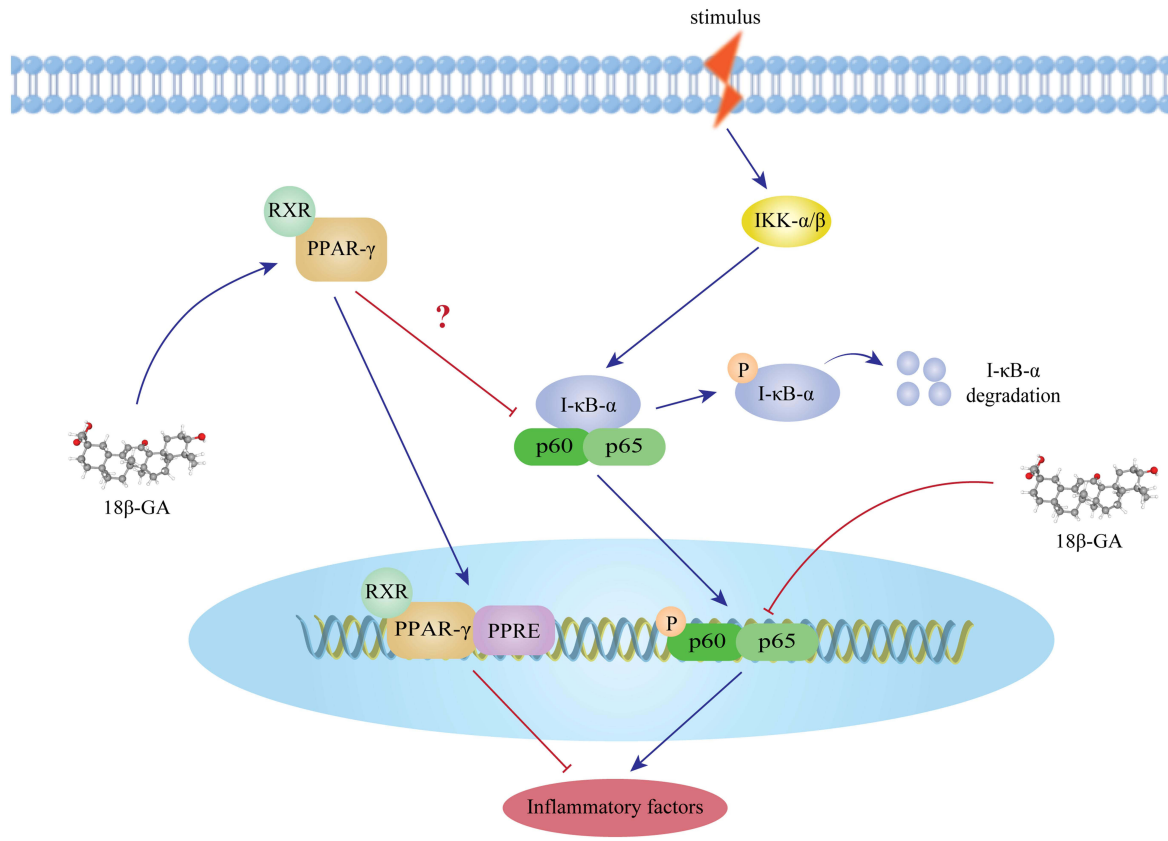

Figure 7 The role of the peroxisome proliferator-activated receptor γ (PPAR-γ)/nuclear factor kappa-B (NF-κB) signaling pathway in 18β-GA's inhibition of DSS-induced UC.

Conclusion

18β-GA exhibited therapeutic efficacy by orchestrating the intricate balance of the intestinal microbiota, restoring the compromised intestinal barrier, and intricately modulating the PPAR-γ/NF-κB signaling pathway without inflicting notable tissue damage or eliciting untoward side effects. Thus, 18β-GA is a promising candidate for UC treatment.

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Disclosure

The authors report no conflicts of interest in this work.

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