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Ginsenoside Rb1 Alleviates DSS-Induced Ulcerative Colitis by Protecting the Intestinal Barrier Through the Signal Network of VDR, PPARγ and NF-κB

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Purpose: Ginseng (*Panax ginseng* Meyer) is an herbal medicine used in traditional Chinese medicine (TCM), has the effects of treating colitis and other diseases. Ginsenoside Rb1 (GRb1), a major component of ginseng, modulates autoimmunity and metabolism. However, the mechanism underlying GRb1 treatment of ulcerative colitis (UC) has not yet been elucidated. UC is a refractory inflammatory bowel disease (IBD) with a high recurrence rate, and researches on new drugs for UC have been in the spotlight for a long time.

Methods: Mice with DSS-induced UC were treated with GRb1 or 0.9% saline for 10 days. Colon tissue of UC mice was collected to detect the levels of intestinal inflammatory cytokines and integrity of the intestinal barrier. RNA-seq and network pharmacology were used to predict the therapeutic targets of GRb1 during UC treatment.

Results: GRb1 treatment alleviated intestinal inflammation and improved intestinal barrier dysfunction in UC mice. Specifically, GRb1 downregulated the levels of pro-inflammatory cytokines such as TNF-α and IL-6, while upregulating the level of the anti-inflammatory cytokine IL-10. Additionally, GRb1 treatment increased the levels of tight junction proteins including ZO-1, Occludin, and E-cadherin, which are crucial for maintaining intestinal barrier integrity. Further analyses using RNA-seq and network pharmacology suggested that these effects might involve the regulation of GRb1 in the signal transduction network of VDR, PPARγ, and NF-κB.

Conclusion: The study demonstrated that GRb1 effectively alleviated UC by modulating intestinal inflammation and protecting the integrity of the intestinal barrier through the signal transduction network of VDR, PPARγ, and NF-κB.

Keywords: ginsenoside Rb1, ulcerative colitis, intestinal barrier, vitamin D receptor, nuclear factor-kappa B

Introduction

Ulcerative colitis (UC), a type of inflammatory bowel disease, is characterized by symptoms such as abdominal pain, diarrhea, and mucopurulent bloody stool.[1] Pathologically, UC mainly affect the mucosa and submucosa, leading to mucosal congestion, edema, erosions, and small superficial ulcers. Meanwhile, a large number of infiltrative neutrophils mixed with mucus and bacteria can be observed in the intestinal crypts, resulting in the formation of trap abscesses and small submucosal abscesses.[2] However, the etiology of UC is not yet clear, and it is currently believed that UC is caused by multi-factorial interactions, including environmental, genetic, intestinal microecological, and immune factors. Inflammation and impaired intestinal barrier play significant roles in the development of UC.[3,4]

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Clinically, UC patients are treated mainly with mesalazine, corticosteroids, immunosuppressants, and monoclonal antibodies.[5] However, these medications may lead to side effects.[6,7] And the high cost of long-term treatment is difficult to afford for UC patients.[8] Therefore, there is a growing focus on natural remedies and alternative therapies for UC.

Ginseng (*Panax ginseng* Meyer), an herbal medicine in traditional Chinese medicine (TCM), powerfully supplements *Qi*, restoring pulse to rescue from collapse syndrome, invigorating the spleen, benefiting the lung, calming the mind, and benefiting intelligence (TCM concept). Ginseng is used in classic TCM formulas combined with other medicines in treating UC for a long time.[9] Recent researches have also demonstrated that ginseng has anti-inflammatory and immunoregulatory effects.[10,11] Recent study reported that GRb1 alleviated endoplasmic reticulum stress and Fas-related apoptosis through the E3 ubiquitin ligase HMG-CoA Reductase Degradation protein (Hrd1) signaling pathway to ameliorate colitis.[12] However, the effect of GRb1 on cytokines and intestinal epithelial cells (IECs) has not been elucidated. In this study, we investigated the therapeutic effect and underlying mechanism of GRb1 in UC from the perspective of intestinal inflammation and barrier function.

Materials and Methods

Drug and Reagents

Ginsenoside Rb1 (CAS: 41753–43-9, HPLC≥98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). GRb1 was dissolved in 0.9% saline and prepared before use. Dextran sulfate sodium (DSS) was purchased from Yeasen (Shanghai, China). Primary antibodies against zonula occludens-1 (ZO-1), occludin, nuclear factor-kappa B (NF-κB), and GAPDH were purchased from Proteintech Group (Wuhan, China); vitamin D receptor (VDR) from Abcam (Cambridge, UK); tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and interleukin 10 (IL-10) from ABclonal Technology Co., Ltd. (Wuhan, China); and peroxisome proliferator-activated receptor γ (PPARγ), phospho-NF-κB p65, and E-cadherin were purchased from Cell Signaling Technology (MA, USA). All secondary antibodies were obtained from Cell Signaling Technology (MA, USA). The bicinchoninic acid (BCA) protein assay kit, immunohistochemical kit, and immunofluorescence kit were obtained from ServiceBio (Wuhan, China).

Animal and Treatment

30 Male C57BL/6 mice (6-week-old) were purchased from Jiangsu GemPharmatech Co., Ltd. All animals were maintained in the specific-pathogen-free (SPF) animal facility with temperature at 22 ± 2 °C, 55 ± 5% humidity and a 12/12 circadian rhythm of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST). All the animal studies were performed according to Laboratory animal—Guideline for ethical review of animal welfare (GBT35892-2018). All animal studies were approved and monitored by the Tongji Hospital Animal Care, Tongji Medical College, HUST (TJH-202205003). After a week of acclimatization, the mice were randomly divided into control, DSS, and GRb1 groups with 3 doses (6 mice each). Mice in the treatment and DSS groups were administered 3% DSS dissolved in drinking water to induce colitis model. DSS was replaced with normal drinking water after one week. Mice in the GRb1 groups were gavaged with 20, 40, and 80 mg/kg GRb1 solution. These three groups were named as G20, G40, G80 respectively. And mice in the control and DSS group were treated with 0.9% saline via oral gavage for 10 days (Figure 1A). At the end of 10 days experiment, all mice were anesthetized with 1% pentobarbital sodium, colon and liver tissues were collected respectively. Then, these tissues were stored in −80 °C refrigerator and 4% paraformaldehyde for subsequent experiments.

Disease Activity Index

Animals were monitored and weighed daily. The disease activity index (DAI) was calculated by weight loss, stool viscosity, and rectal bleeding, as detailed in Table 1, according to a previous study.[9] The sum of the scores was expressed as the DAI.

Hematoxylin-Eosin Staining

Colon tissues were fixed with 4% paraformaldehyde to prepare paraffin-embedded sections. Colon tissues embedded in paraffin were sectioned into slices of 4–5 μm thickness. Paraffin-embedded colonic sections were dewaxed with

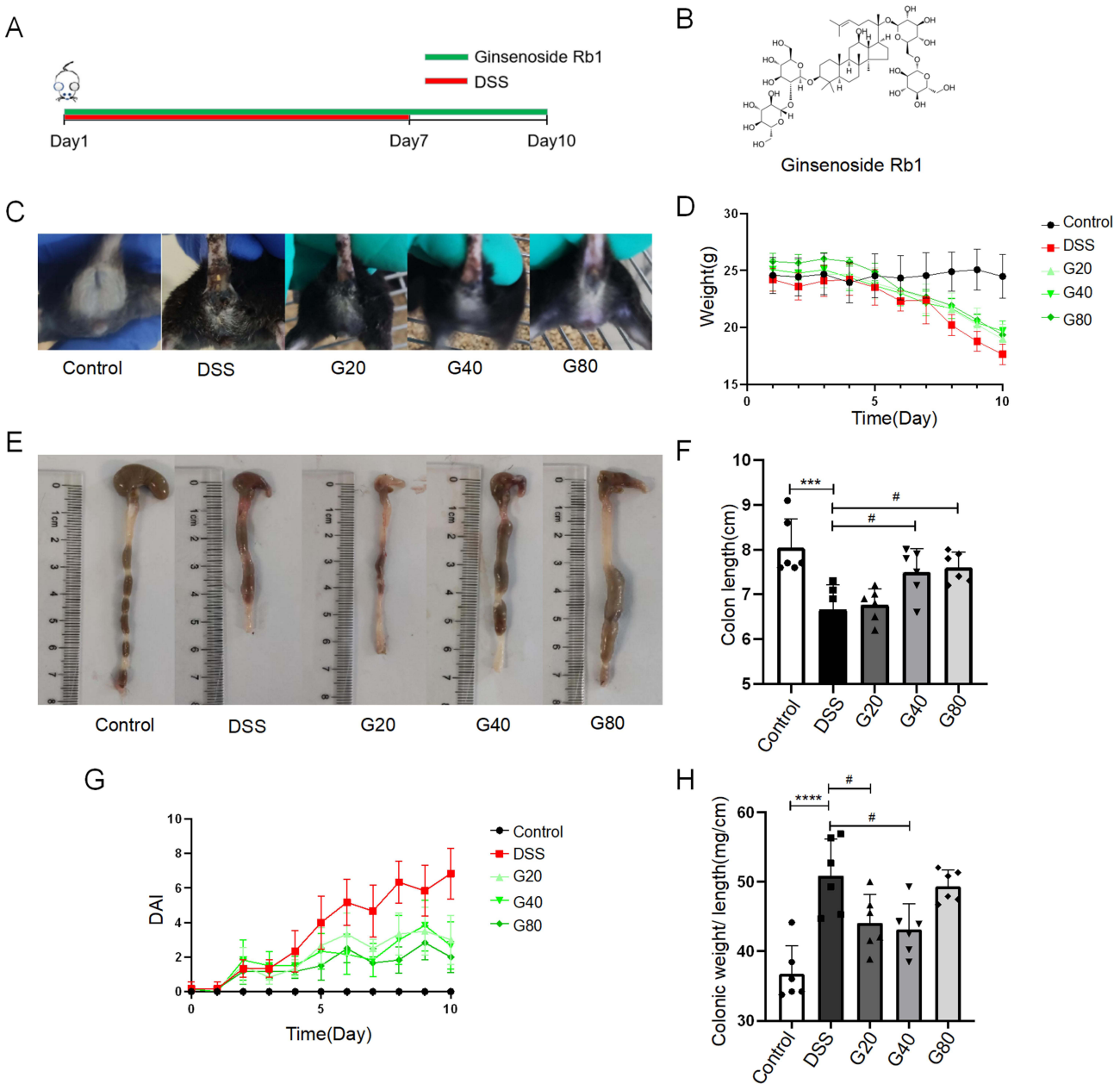

Figure 1 GRb1 alleviates DSS-induced colitis in mice. (**A**) Animal experimental protocol. (**B**) Chemical structure of GRb1. (**C**) Bloody stools of mice. (**D**) The body weight changes of colitis mice treated with 0.9% saline or GRb1 every day. (**E**) Representative colon pictures from the mice in different groups. (**F**) The colon length of mice in different groups. (**G**) Disease activity index (DAI) in different groups. (**H**) The colonic weight/ length in different groups. ***$p < 0.005$, ****$p < 0.001$ vs Control group; $^{\#}p < 0.05$ vs DSS group, (n=6).

dimethylbenzene, sequentially rehydrated with 100%, 95%, and 75% alcohol for 5 min, and then rinsed with water. The sections were stained with hematoxylin for 4 min, rinsed with tap water for 10 min, differentiated with hydrochloric acid alcohol for 3 s, rinsed with tap water for another 10 min, and stained with eosin for 1 min. Hematoxylin-eosin (H&E) staining was used to observe the structure of the colonic sections using microscopy (BX51, Olympus). Histological scoring was performed by H&E staining of colon tissue sections, and the criteria are detailed in Table 2, according to a previous study.[7]

Table 1 Disease Activity Index (DAI)

	Weight Loss (%)	Stool Consistency	Rectal Bleeding
0	None	Well-formed pellets	Negative hemoccult
1	1–5	Soft but formed	Weakly positive hemoccult
2	6–10	Very soft and wet	Positive hemoccult
3	11–20	Pasty and semiformed	Visible blood stool
4	>20	Watery	Gross bleeding

Table 2 Histological Scoring

Index	Extent	Inflammation	Crypt damage	Spread (%)
0	None	None	None	None
1	Mucosa	Slight	Basal one-third lost	0–25
2	Submucosa	Moderate	Basal two-thirds lost	25–50
3	Transmural	Severe	Only surface epithelium intact	50–75
4	–	–	Entire crypt and epithelium lost	75–100

Immunohistochemistry Staining

After dewaxing and rehydration, sections were immersed in sodium citrate antigen repair solution and incubated in aqueous hydrogen peroxide solution for 30 min at room temperature. After blocking with 20% goat serum, sections were incubated with the primary antibody overnight at 4 °C. The next day, the sections were washed and incubated with secondary antibodies for 1 h at room temperature, followed by staining with 3.3'-diaminobenzidine (DAB), re-staining with hematoxylin, dehydration, and sealing with coverslips after dropping neutral gum. Images were obtained using a microscope.

Immunofluorescence Staining

Immunofluorescence staining was performed using immunohistochemistry before incubation with secondary antibodies, except that the sections were washed with 0.1% TritonX-100 for permeabilization before blocking. Secondary antibodies were incubated, and nucleus with 4',6-diamidino-2-phenylindole (DAPI) in the dark. Immunofluorescent images were obtained using a microscope.

Network Pharmacology Analysis

The targets of GRb1 were downloaded from Traditional Chinese Medicine Systems Pharmacy (https://tcmsp-e.com/tcmsp.php) and symmap (http://www.symmap.org/). A protein–protein interaction (PPI) network was constructed using STRING (https://string-db.org/). The target network was visualized using the Cytoscape software.

Molecular Docking

The 3D structures of VDR, PPARγ, and NF-κB were downloaded from Protein Data Bank (https://www.rcsb.org/). The GRb1 structure was downloaded from PubMed (https://pubchem.ncbi.nlm.nih.gov/). AutoDock Vina software was used for docking analysis. PyMOL software was used to visualize the molecular docking.

RNA-Seq Analysis

The colon tissues of mice were immediately cryopreserved and transcriptomics were performed using RNA Sequencing (RNA-seq). Data were analyzed using the Dr. Tom platform from the Beijing Genomic Institute (BGI).

Western Blot Analysis

Protein samples were quantified using a bicinchoninic acid (BCA) protein assay kit. The samples were electrophoresed in 8–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to 0.45μm polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with 5% skim milk for 1 h at room temperature to block non-specific binding. The membranes were then incubated with primary antibodies overnight at 4 °C. After completing the above steps, the membranes were incubated with secondary antibodies for 1 h at room temperature and washed three times (10 min each) with TBST. Finally, the results were visualized using the Odyssey system and quantified using the ImageJ software.

Statistical Analysis

GraphPad Prism 9.0 Software (San Diego, USA) was used to analyze data and draw graphics. All data were expressed as means ± standard error of the mean (SEM). Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by post hoc Tukey's test for comparisons among groups. P value < 0.05 was considered significant.

Results

GRb1 Alleviates DSS-Induced Colitis in Mice

GRb1 is a tetracyclic triterpenoid isolated from ginseng (Figure 1B). Following GRb1 treatment, DSS-induced colitis mice showed less bleeding and higher stool viscosity in the anal region (Figure 1C). During the experiment, the weight of the mice was measured daily. Weight loss was severe in the DSS group, whereas GRb1 relieved the DSS-induced weight loss in mice (Figure 1D). Moreover, GRb1 treatment reduced colon shortening (Figure 1E and F). Notably, GRb1 slowed the increase of DAI in DSS-induced colitis mice (Figure 1G). In addition, after measuring the weight-to-length ratio of the colon, we found that the GRb1-treated mice exhibited a lower degree of inflammation in the colon (Figure 1H). The therapeutic effects of GRb1 increased as the GRb1 concentration increased. In summary, our results suggest that GRb1 significantly alleviates DSS-induced colitis in mice.

GRb1 Regulates the Levels of Inflammatory Cytokines in the Colon

In H&E-stained colon sections, the GRb1 groups showed reduced colonic ulceration, crypt and epithelial damage, and decreased inflammatory cell infiltration compared to the DSS group (Figure 2A). In addition, mice in the GRb1 group had lower histological scores than those in the DSS group (Figure 2B). Inflammatory cytokines, including pro-inflammatory and anti-inflammatory cytokines, play an important role in the development of colitis. Immunohistochemistry results showed that GRb1 reduced the levels of pro-inflammatory cytokines, including TNF-α and IL-6. In addition, GRb1 elevated the levels of IL-10, an anti-inflammatory cytokine (Figure 2C). In liver sections, no significant differences were observed in any of the samples (Figure 2D). These results suggest that GRb1 can inhibit inflammation in mice with colitis and is not harmful to the liver.

GRb1 Reduces Damage of the Intestinal Barrier in Colitis Mice

ZO-1 and Occludins are tight junction proteins in colon epithelial cells, whereas E-cadherin is an epithelial calcium adhesion molecule-binding protein that is involved in the formation of intercellular adhesive junctions. Immunofluorescence and Western blot were performed to measure the levels of these three proteins. Immunofluorescence results showed a significant increase in ZO-1 levels following GRb1 treatment (Figure 3A). The levels of Occludin and E-cadherin were severely reduced in the colon tissue of colitis mice but significantly increased after treatment with GRb1 (Figure 3B–D). Therefore, GRb1 may improve intestinal barrier function by reducing inflammation in mice with colitis.

RNA-Seq, Network Pharmacology and Molecular Docking Reveal the Potential Targets of GRb1 Regulating DSS-Induced Colitis in Mice

To further investigate the molecular mechanism by which GRb1 alleviates DSS-induced colitis in mice, RNA-seq was used to analyze the differences in gene expression in the colon tissues of mice from the control, DSS, and GRb1 group. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Set Enrichment Analysis (GSEA) revealed that

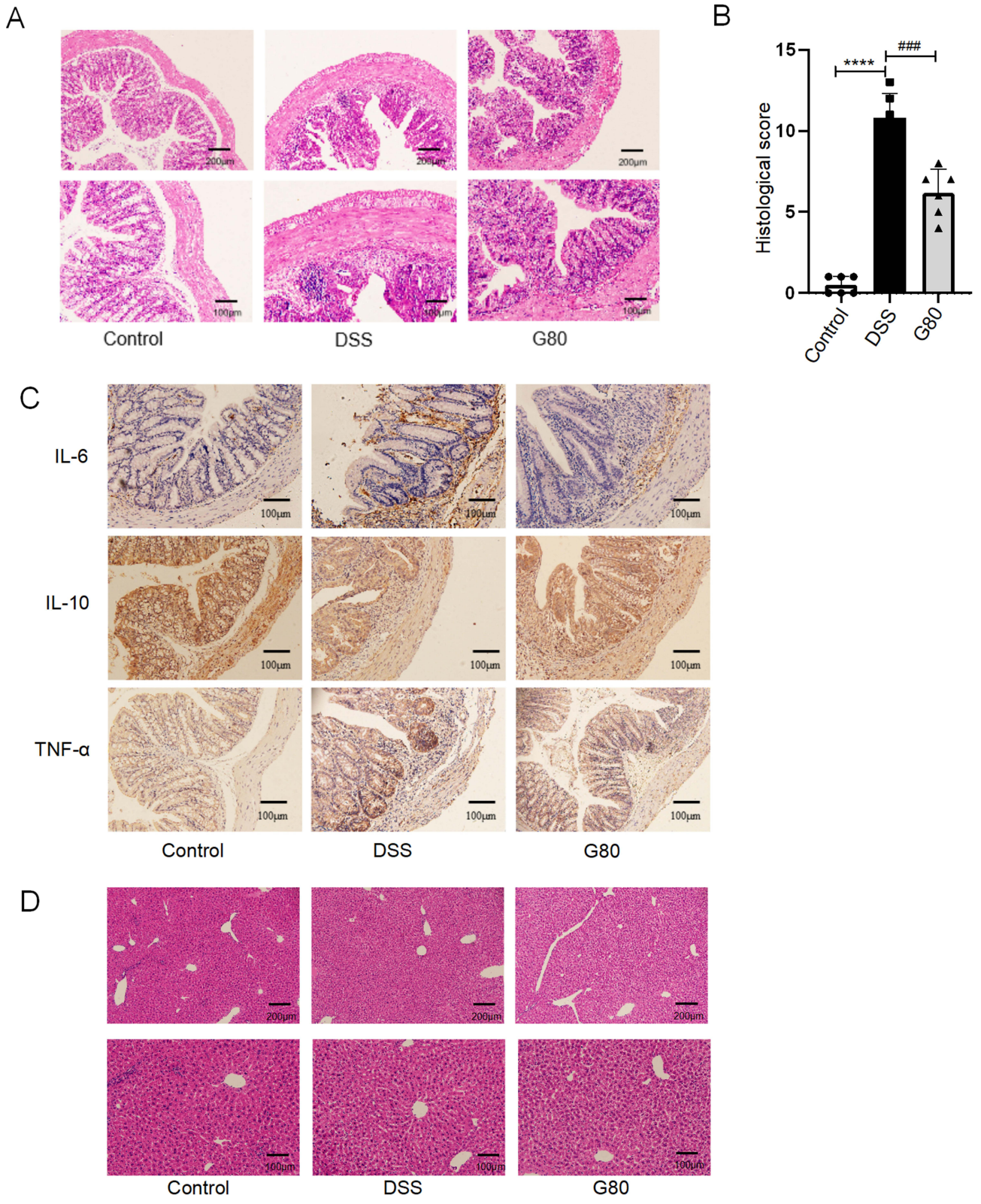

Figure 2 GRb1 regulates the levels of intestinal inflammatory cytokines. (**A**) Representative H&E staining of colon sections from mice in different groups. Scale bar: 200 μm or 100 μm. (**B**) The histological scores for H&E stained colonic sections from the mice in different groups (n=6). (**C**)Representative immunohistochemical staining for IL-6, IL-10 and TNF-α in colon sections. Scale bar: 100 μm. (**D**) Representative H&E staining of liver sections from mice in different groups. Scale bar: 200 μm or 100 μm. ****$p < 0.001$ vs Control group; ###$p < 0.005$ vs DSS group, (n=6).

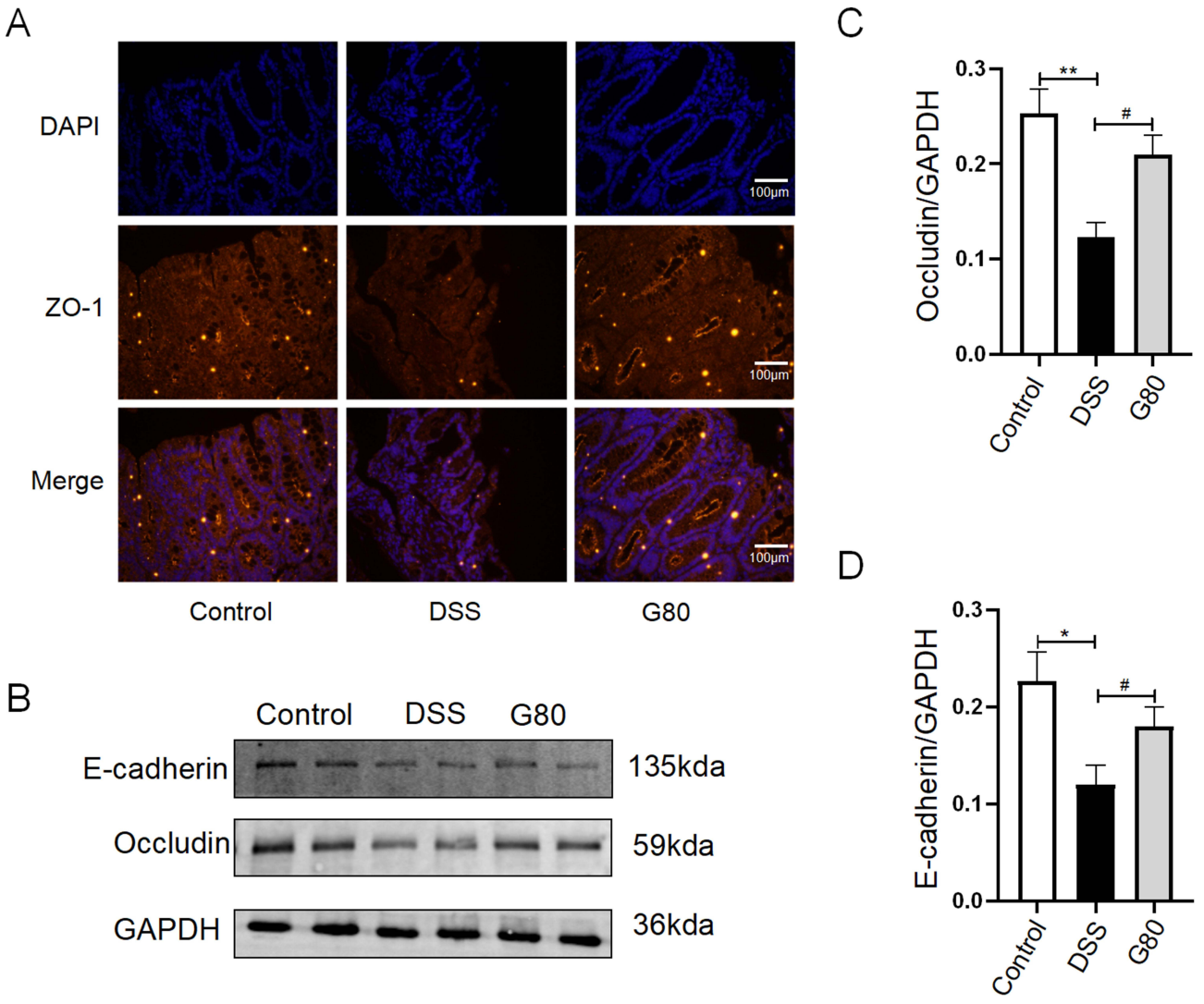

Figure 3 GRb1 reduces damage of the intestinal barrier in colitis mice. (**A**) Representative immunofluorescent staining for ZO-1 in colon sections. Scale bar: 100 μm. (**B**) Representative Western blots for E-cadherin and Occludin. (**C** and **D**) Quantitative analysis of the proteins for (**B**). *p < 0.05, **p < 0.01 vs Control group; #p < 0.05 vs DSS group, (n=6).

Mineral Absorption, NF-κB signaling pathway, and the PPAR signaling pathway were the major differentially enriched pathways (Figure 4A–C). The differential genes were mainly located upstream and downstream of VDR, PPARγ, and NF-κB. Interestingly, network pharmacological analysis revealed that VDR, PPARγ, and NF-κB play important roles in the treatment of colitis by GRb1 (Figure 4D). Molecular docking analysis was used to dock GRb1 with VDR, PPARγ, and NF-κB. Molecular docking was used to predict the interactions between molecules and proteins. The magnitude of the docking energy can be used to evaluate the stability of binding between molecules and proteins, with lower energies indicating more stable binding. The results showed that the molecular docking energies of GRb1 binding to VDR, PPARγ, and NF-κB were below −5 kcal/mol. The three ligand-receptor pairs were docked successfully, indicating that there was some interaction between GRb1 and the three proteins (Figure 4E). These results indicated that GRb1 alleviates UC through the signal transduction network of VDR, PPARγ, and NF-κB.

GRb1 Alleviates Colitis by Regulating the Signal Transduction Network of VDR, PPARγ and NF-κB

To validate the role of signal transduction network of VDR, PPARγ and NF-κB in DSS-induced UC mice treated with GRb1, Western blot was used to measure the protein levels of VDR, PPARγ, and NF-κB in colon tissues. The protein

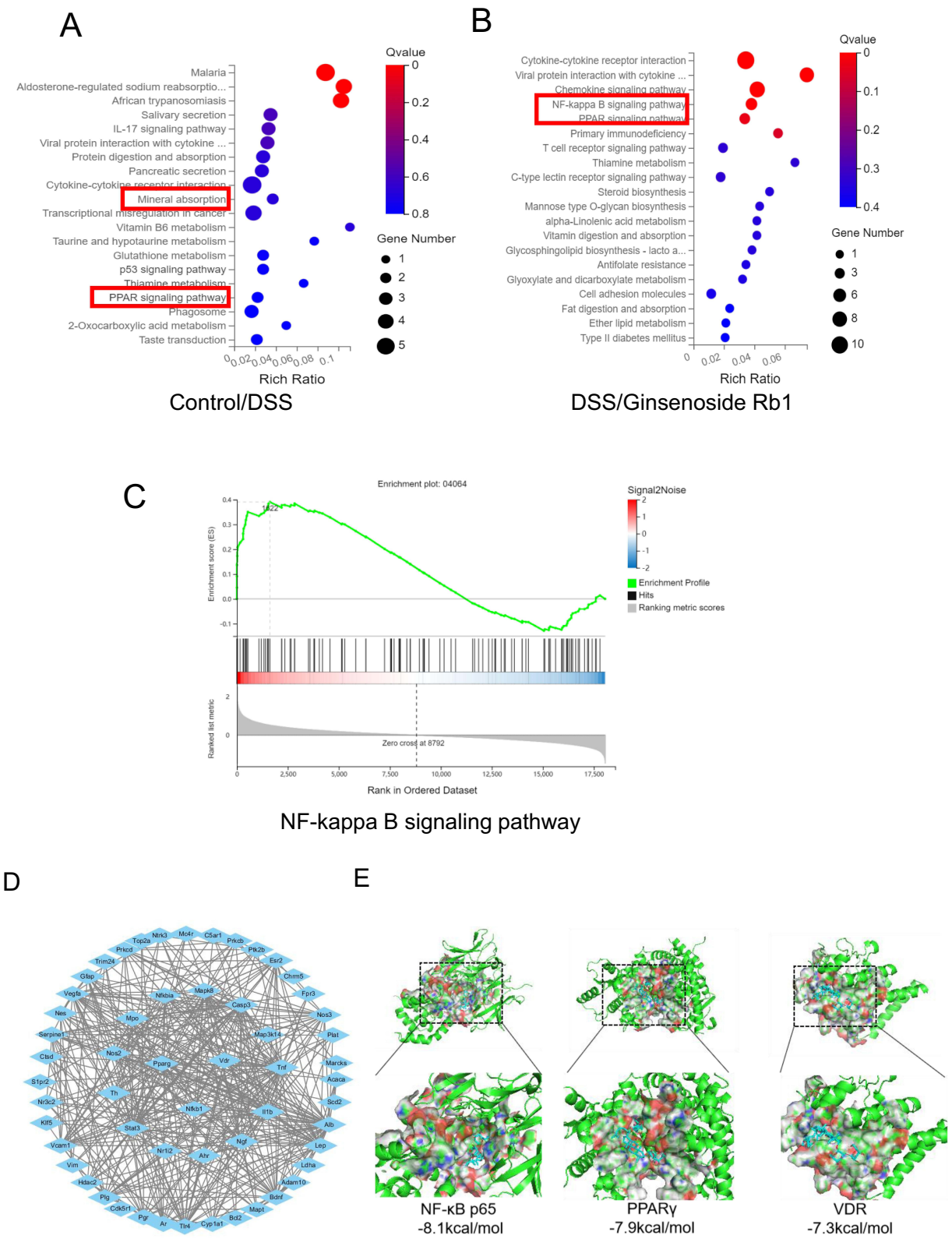

Figure 4 RNA-seq and network pharmacological analysis of GRb1 regulating DSS-induced colitis in mice. (**A** and **B**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of RNA-seq analysis. (**C**) Gene Set Enrichment Analysis (GSEA) of RNA-seq analysis. (**D**) Network pharmacological analysis of the targets of GRb1 treating colitis. (**E**) Molecular docking of GRb1 to VDR, PPARγ and NF-κB p65 protein.

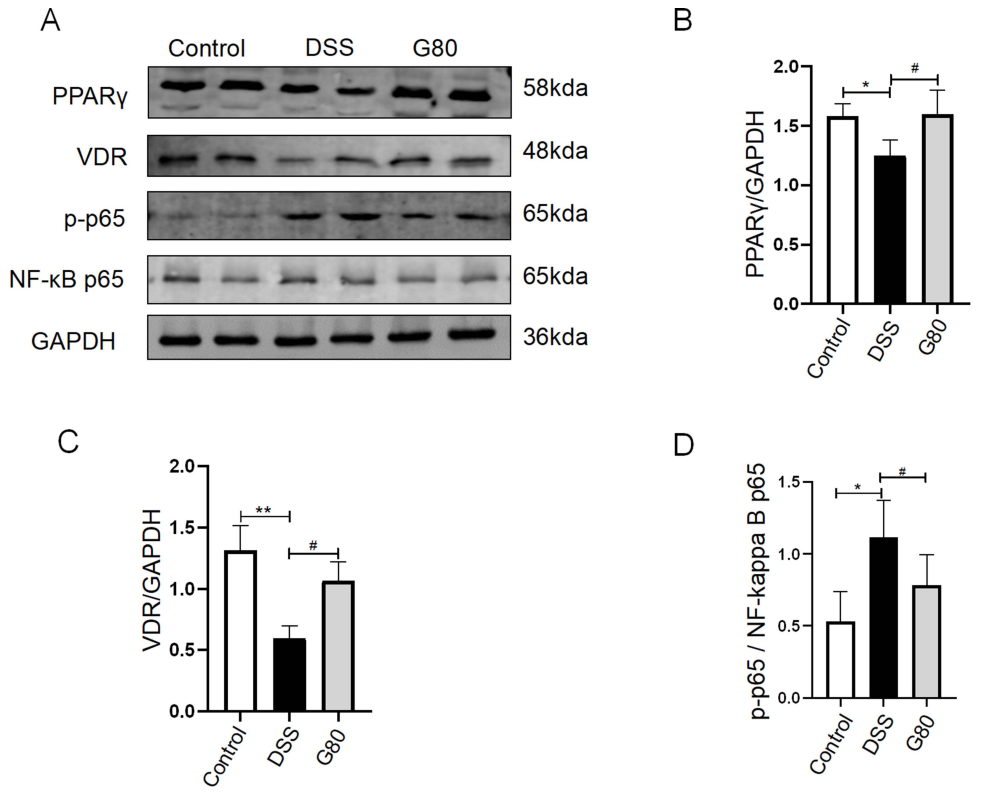

Figure 5 GRb1 treats colitis by regulating the signal transduction network of PPARγ, VDR and NF-κB. (**A**) Representative Western blots for PPARγ, VDR, NF-κB p65 and p-p65. (**B**) Quantitative analysis of the proteins for PPARγ/GAPDH. (**C**) Quantitative analysis of the proteins for VDR/GAPDH. (**D**) Quantitative analysis of the proteins for p-p65 / NF-κB p65. *p < 0.05, **p < 0.01 vs Control group; #p < 0.05 vs DSS group, (n=6).

levels of VDR and PPARγ were elevated after GRb1 treatment compared with those in the DSS group. NF-κB was activated in the DSS group, and the increased phosphorylation of NF-κB p65 was inhibited by GRb1 treatment (Figure 5A–D). In summary, GRb1 protected the intestinal barrier and alleviated DSS-induced ulcerative colitis through the signal transduction network of VDR, PPARγ, and NF-κB.

Discussion

UC has a long course and is prone to recurrence in patients with poor quality of life. Current clinical treatment options for UC have a number of deficiencies, such as primary non-response, secondary non-response, and potential side effects. Many patients with UC eventually have to opt for surgical treatment.[13] Therefore, we investigated the mechanism of GRb1 in the treatment of UC. In this study, we found that GRb1 inhibited intestinal inflammation, protected the intestinal barrier, and alleviated UC by regulating the signaling pathways related to VDR, PPARγ, and NF-κB.

Inflammation is a hallmark of UC, and inflammatory cytokines are central to the disease's clinical manifestations and pathogenic mechanisms. Intestinal inflammation, usually caused by external irritation or infection, can lead to recurrent epithelial cell stimulation and immune cell infiltration. Intestinal inflammation may provide an ideal microenvironment for the development of UC and colitis-associated cancer.[14] The prolonged state of intestinal inflammation not only causes symptoms such as abdominal pain, diarrhea, and constipation, but may also lead to changes in the structure and function of the intestines. The exacerbation of intestinal inflammation may further progress into chronic inflammatory bowel diseases such as ulcerative colitis and Crohn's disease.[15] In this study, GRb1 alleviated the weight loss caused by DSS-induced intestinal inflammation, reduced disease activity, and improved the damage to intestinal structure.

Inflammation of the intestine is inextricably linked to cytokines. TNF-α is a pleiotropic mediator of the systemic inflammatory response and a pro-inflammatory cytokine involved in the immune inflammatory response. TNF-α can synergistically regulate cell proliferation and apoptosis by promoting the release of other chemokines and inflammatory

factors.[16] TNF-α stimulates macrophage fibroblasts, epithelial cells and endothelial cells to secrete arachidonic acid metabolites, cytokines and proteases. And this process could lead to necrosis, apoptosis and edema, resulting in tissue damage of intestinal cells and UC.[17] In addition, TNF-α, in concert with IFN-γ, can alter the morphological structure and barrier properties of intestinal epithelial cells, leading to increased mucosal permeability.[18] IHC results showed that GRb1 decrease the level of TNF-α in colon tissue, thus reduce the level of intestinal inflammation. The interleukin family members play an essential role in inflammatory processes. IL-6 is a pro-inflammatory cytokine that is secreted mainly by activated macrophages, lymphocytes, and epithelial cells and plays a key role in the pathogenesis of the chronic intestinal inflammatory response.[19] IL-6 initiates a cell surface signaling complex in the inflammatory response, plays a role in UC pathogenesis, and is associated with colon cancer development via the STAT3 signaling pathway.[20,21] It was found that IL-6 levels increased in the colon of DSS-induced colitis mice, while IL-6 levels significantly decreased after treatment with GRb1. In contrast, IL-10 is an anti-inflammatory factor that plays a predominantly negative regulatory role in the immune response.[22] IL-10 can combine with transforming growth factor-β (TGF-β) to produce a broad non-specific anti-inflammatory effect, thereby reducing intestinal inflammation.[23,24] The detection of IL-10 revealed that GRb1 significantly increased the level of IL-10 in the colon tissue of mice with DSS-induced colitis. Decreased secretion of anti-inflammatory cytokines and increased secretion of pro-inflammatory cytokines leads to persistent inflammation of the intestinal mucosa and impairment of barrier function. In this study, GRb1 was found to downregulate the protein levels of TNF-α and IL-6, and upregulate the protein levels of IL-10 in colon tissues. Therefore, GRb1 regulates inflammation in colon tissues of mice with colitis.

Inflammatory cytokines cause damage to the intestinal barrier function. The intestinal barrier is an important defense mechanism against external pathogens by invading and maintaining a stable internal environment. Tight junctions (TJs) between the intestinal epithelial cells play an essential role in the intestinal barrier. Intestinal inflammation disrupts tight junction structures and increases intestinal mucosal permeability Then intestinal bacteria and endotoxins enter the intestinal lumen through the intestinal epithelium, further leading to damage to the intestinal barrier.[25,26] Epithelial calcium adhesion molecule binding protein E-cadherin is involved in the formation of intercellular adhesive junctions.[27] A study reported that GRb1 could alleviate apoptosis of intestinal epithelial cells to maintain the integrity of the intestinal barrier and treat UC.[12] In this study, we found that GRb1 could up-regulate protein levels of ZO-1, Occludin and E-cadherin in colon tissues of colitis mice. Therefore, GRb1 may repair intestinal permeability and improve the intestinal barrier, thereby inhibiting the further deterioration of the intestinal environment.

UC pathogenesis is related to many factors and is influenced by several signal transduction pathways. Traditional Chinese medicine monomers do not rely solely on a single pathway or target to treat UC.[28] The results of network pharmacology and molecular docking analysis revealed that GRb1 may treat UC through the signal transduction network of VDR, PPARγ, and NF-κB. RNA-seq analysis of the colon of mice revealed that GRb1 influenced the RNA levels of genes involved in mineral absorption and the NF-κB and PPAR signaling pathways. The mineral absorption pathway involves the uptake and transportation of minerals such as vitamin D, while the PPARγ and NF-κB pathways are closely related to processes such as inflammatory response, cell proliferation, and differentiation. These pathways play an important role in the development of colitis.[29–31] The differentially expressed genes in these three pathways are upstream and downstream of VDR, PPARγ, and NF-κB. Through network pharmacology, molecular docking, and RNA-seq analysis, the signal transduction network of VDR, PPARγ, and NF-κB was considered a possible signal transduction network of GRb1 in UC treatment.

Vitamin D is necessary for maintaining calcium and phosphorus metabolism in the body and regulates cell proliferation and differentiation. Vitamin D exerts its biological effects mainly through the active metabolite 1.25-(OH)2-D bound to VDR.[32] VDR was found to regulate the expression and signaling of target genes related to intestinal inflammation, including ATG16L1 and others.[33] Overexpression of VDR in IECs revealed that the degree of inflammation in colon tissues of colitis mice was reduced and the expression of tight junction protein claudin15 was increased, which was eventually able to protect the intestinal barrier.[34] In addition, the role of VDR in inhibiting NF-κB of IECs has been confirmed.[35] In our study, GRb1 treatment could increase the level of VDR in colonic tissue, which could promote the biological effects of vitamin D, suppress inflammation in the colon and protect the intestinal barrier. Upregulation of VDR further inhibited NF-κB phosphorylation levels in IECs and synergistically produced anti-inflammatory effects.

Studies have shown that PPARγ, which has anti-inflammatory effects, can inhibit neutrophil and monocyte aggregation, lipid peroxidation, and the secretion of pro-inflammatory factors such as IL-6 and TNF-α.[36] In addition, studies have shown that PPARγ can treat UC by inhibiting NF-κB.[37] PPARγ is an important target for the anti-inflammatory drug mesalazine for UC. Mesalazine is considered to be the first choice for the treatment of mild to moderate UC and has the longest history of use for UC.[38] GRb1 can activate PPARγ to enhance the intestinal barrier and reduce the infiltration of harmful bacteria or other pathogenic agents, thereby protecting the intestine from further damage. In the present study, we found that GRb1 increased PPARγ activity, thereby inhibiting the levels of pro-inflammatory cytokines and reducing inflammation. Based on this target, we speculated that GRb1 has a therapeutic effect similar to that of mesalazine.

NF-κB participates in inflammatory and immune responses and regulates cell apoptosis and stress responses. Overactivation of NF-κB has been confirmed to be associated with inflammatory changes in many diseases, such as cancer, rheumatoid arthritis and cardiovascular diseases.[39] The colonic mucosa colitis mouse was damaged severely, and further study proved significantly elevated levels of NF-κB in the colonic mucosa of colitis mice, suggesting that NF-κB was correlated with the severity of UC.[40] Meanwhile, cytokines such as TNF-α and IL-6 are affected by NF-κB, which can regulate immunity and inflammation through different pathways and affect the progression of UC.[41] Study has shown that GRb1 can alleviate inflammation by attenuating NF-κB activity.[42] Our study found that GRb1 can decrease the activity of NF-κB in colon tissues, thus inhibiting the inflammatory response and treating UC. Previous results have indicated that GRb1 significantly alleviates inflammation in mice with colitis by inhibiting the activation of NF-κB.

The activation of NF-κB leads to increased inflammation, while PPARγ and VDR are involved in maintaining balance by suppressing excessive inflammation. VDR can regulate NF-κB-mediated inflammation. VDR inhibit the nuclear translocation of NF-κB and suppress the levels of pro-inflammatory cytokines.[43] The activation of PPARγ also inhibit NF-κB activation. PPARγ cooperates with E2 UBCH3 to cause the ubiquitination of NF-κB, which leads to proteolytic degradation of the NF-κB subunit.[44] In summary, the activation of NF-κB is a critical in UC, and VDR and PPARγ are modulators of this inflammation process. In this experiment, we found that GRb1 treat UC by regulating the signal transduction network of VDR, PPARγ, and NF-κB (Figure 6).

The limitation of this study may include the following aspects. Firstly, the sample of the study is relatively small. Secondly, experimental model in this study is acute UC model and GRb1 treatment in chronic UC remains unclear. Thirdly, the mechanism of the interaction of VDR, PPARγ, and NF-κB with GRb1 needs further elucidate. Thus, a study

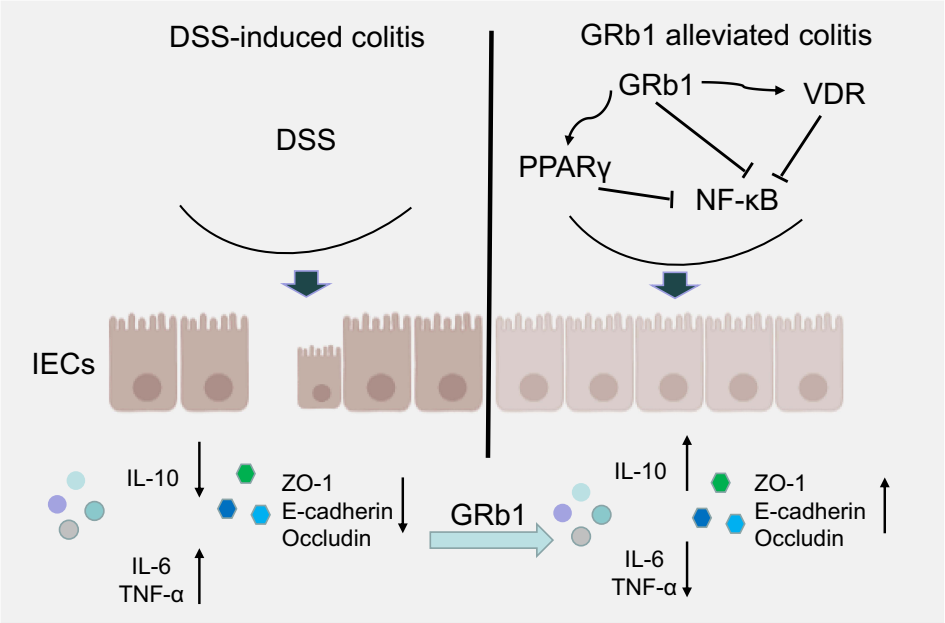

Figure 6 The graphical abstract in this study. GRb1 alleviates colitis by inhibiting inflammation and protecting intestinal barrier through PPARγ, VDR and NF-κB.

with large samples and chronic UC model is warranted in the future to further explore the mechanism. In addition, the combination of GRb1 treatment with other treatments would be investigated.

Conclusion

Taken together, this study investigated the effect of GRb1 in treating UC from the perspective of intestinal barrier. And GRb1 has been demonstrated to modulate the levels of VDR and PPARγ to inhibit NF-κB activation to treat UC. Collectively, our findings explored the mechanism of GRb1 treating UC and could be helpful in the development of new treatment methods in UC.

Abbreviations

BCA, bicinchoninic acid; DAB, 3.3'-diaminobenzidine; DAI, Disease activity index; DAPI, 4',6-diamidino-2-phenylindole; DSS, Dextran sulfate sodium; GRb1, Ginsenoside Rb1; GSEA, Gene Set Enrichment Analysis; Hrd1, HMG-CoA Reductase Degradation protein; IBD, inflammatory bowel diseases; IECs, intestinal epithelial cells; IL-10, interleukin 10; IL-6, interleukin 6; KEGG, Kyoto Encyclopedia of Genes and Genomes; NF-κB, nuclear factor-kappa B; PPARγ, peroxisome proliferator-activated receptor γ; RNA-seq, RNA Sequencing; SPF, specific pathogen-free; TCM, traditional Chinese medicine; TGF-β, transforming growth factor-β; TJs, tight junctions; TNF-α, tumor necrosis factor-α; UC, Ulcerative colitis; VDR, vitamin D receptor; ZO-1, zonula occludens-1.

Data Sharing Statement

Data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

All the animal studies were performed according to Laboratory animal—Guideline for ethical review of animal welfare (GBT35892-2018). The animal study was reviewed and approved by Tongji Hospital Animal Care, Tongji Medical College, HUST (TJH-202205003).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no competing interests that could have influenced the work reported in this study.

References

1. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol*. 2010;28:573–621. doi:10.1146/annurev-immunol-030409-101225
2. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet*. 2017;389(10080):1756–1770. doi:10.1016/S0140-6736(16)32126-2
3. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol*. 2014;14(3):141–153. doi:10.1038/nri3608
4. Zhao J, Lin Z, Ying P, et al. circSMAD4 promotes experimental colitis and impairs intestinal barrier functions by targeting janus kinase 2 through sponging miR-135a-5p. *J Crohns Colitis*. 2023;17(4):593–613. doi:10.1093/ecco-jcc/jjac154
5. Raine T, Bonovas S, Burisch J, et al. ECCO guidelines on therapeutics in ulcerative colitis: medical treatment. *J Crohns Colitis*. 2022;16(1):2–17. doi:10.1093/ecco-jcc/jjab178

6. Chen H, Lei P, Ji H, et al. Escherichia coli Nissle 1917 ghosts alleviate inflammatory bowel disease in zebrafish. *Life Sci*. 2023;329:121956. doi:10.1016/j.lfs.2023.121956
7. Lei P, Yu H, Ma J, et al. Cell membrane nanomaterials composed of phospholipids and glycoproteins for drug delivery in inflammatory bowel disease: a review. *Int J Biol Macromol*. 2023;249:126000. doi:10.1016/j.ijbiomac.2023.126000
8. Feuerstein JD, Cheifetz AS. Ulcerative colitis: epidemiology, diagnosis, and management. *Mayo Clin Proc*. 2014;89(11):1553–1563. doi:10.1016/j.mayocp.2014.07.002
9. Wu F, Shao Q, Hu M, et al. Wu-Mei-Wan ameliorates chronic colitis-associated intestinal fibrosis through inhibiting fibroblast activation. *J Ethnopharmacol*. 2020;252:112580. doi:10.1016/j.jep.2020.112580
10. Mancuso C, Santangelo R. Panax ginseng and Panax quinquefolius: from pharmacology to toxicology. *Food Chem Toxicol*. 2017;107(Pt A):362–372. doi:10.1016/j.fct.2017.07.019
11. Ratan ZA, Youn SH, Kwak YS, et al. Adaptogenic effects of Panax ginseng on modulation of immune functions. *J Ginseng Res*. 2021;45(1):32–40. doi:10.1016/j.jgr.2020.09.004
12. Dong JY, Xia KJ, Liang W, et al. Ginsenoside Rb1 alleviates colitis in mice via activation of endoplasmic reticulum-resident E3 ubiquitin ligase Hrd1 signaling pathway. *Acta Pharmacol Sin*. 2021;42(9):1461–1471. doi:10.1038/s41401-020-00561-9
13. Tripathi K, Feuerstein JD. New developments in ulcerative colitis: latest evidence on management, treatment, and maintenance. *Drugs Context*. 2019;8:212572. doi:10.7573/dic.212572
14. Federici S, Kredo-Russo S, Valdes-Mas R, et al. Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell*. 2022;185(16):2879–2898e2824. doi:10.1016/j.cell.2022.07.003
15. Annese V. Genetics and epigenetics of IBD. *Pharmacol Res*. 2020;159:104892. doi:10.1016/j.phrs.2020.104892
16. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer*. 2009;9(5):361–371. doi:10.1038/nrc2628
17. Zhou L, Zhou W, Joseph AM, et al. Group 3 innate lymphoid cells produce the growth factor HB-EGF to protect the intestine from TNF-mediated inflammation. *Nat Immunol*. 2022;23(2):251–261. doi:10.1038/s41590-021-01110-0
18. Woznicki JA, Saini N, Flood P, et al. TNF-alpha synergises with IFN-gamma to induce caspase-8-JAK1/2-STAT1-dependent death of intestinal epithelial cells. *Cell Death Dis*. 2021;12(10):864. doi:10.1038/s41419-021-04151-3
19. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295. doi:10.1101/cshperspect.a016295
20. Lin H, Feng L, Cui KS, et al. The membrane-associated E3 ubiquitin ligase MARCH3 downregulates the IL-6 receptor and suppresses colitis-associated carcinogenesis. *Cell Mol Immunol*. 2021;18(12):2648–2659. doi:10.1038/s41423-021-00799-1
21. Hailemichael Y, Johnson DH, Abdel-Wahab N, et al. Interleukin-6 blockade abrogates immunotherapy toxicity and promotes tumor immunity. *Cancer Cell*. 2022;40(5):509–523e506. doi:10.1016/j.ccell.2022.04.004
22. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol*. 2010;10(3):170–181. doi:10.1038/nri2711
23. Wang S, Xia P, Chen Y, et al. Regulatory innate lymphoid cells control innate intestinal inflammation. *Cell*. 2017;171(1):201–216e218. doi:10.1016/j.cell.2017.07.027
24. Yang W, Liu H, Xu L, et al. GPR120 inhibits colitis through regulation of CD4(+) T cell interleukin 10 production. *Gastroenterology*. 2022;162(1):150–165. doi:10.1053/j.gastro.2021.09.018
25. Paradis T, Begue H, Basmaciyan L, Dalle F, Bon F. Tight junctions as a key for pathogens invasion in intestinal epithelial cells. *Int J Mol Sci*. 2021;22(5):2506. doi:10.3390/ijms22052506
26. Sayoc-Becerra A, Krishnan M, Fan S, et al. The JAK-inhibitor tofacitinib rescues human intestinal epithelial cells and colonoids from cytokine-induced barrier dysfunction. *Inflamm Bowel Dis*. 2020;26(3):407–422. doi:10.1093/ibd/izz266
27. Jawhari A, Farthing M, Pignatelli M. The importance of the E-cadherin-catenin complex in the maintenance of intestinal epithelial homoeostasis: more than intercellular glue? *Gut*. 1997;41(5):581–584. doi:10.1136/gut.41.5.581
28. Zheng S, Xue T, Wang B, Guo H, Liu Q. Chinese medicine in the treatment of ulcerative colitis: the mechanisms of signaling pathway regulations. *Am J Chin Med*. 2022;50(7):1781–1798. doi:10.1142/S0192415X22500756
29. Ghishan FK, Kiela PR. Vitamins and minerals in inflammatory bowel disease. *Gastroenterol Clin North Am*. 2017;46(4):797–808. doi:10.1016/j.gtc.2017.08.011
30. Laurindo LF, Santos A, Carvalho ACA, et al. Phytochemicals and Regulation of NF-kB in inflammatory bowel diseases: an overview of in vitro and in vivo effects. *Metabolites*. 2023;13(1):96. doi:10.3390/metabo13010096
31. Li D, Feng Y, Tian M, Ji J, Hu X, Chen F. Gut microbiota-derived inosine from dietary barley leaf supplementation attenuates colitis through PPARgamma signaling activation. *Microbiome*. 2021;9(1):83. doi:10.1186/s40168-021-01028-7
32. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *Lancet*. 2014;383(9912):146–155. doi:10.1016/S0140-6736(13)61647-5
33. Triantos C, Aggeletopoulou I, Mantzaris GJ, Mouzaki A. Molecular basis of vitamin D action in inflammatory bowel disease. *Autoimmun Rev*. 2022;21(8):103136. doi:10.1016/j.autrev.2022.103136
34. Chatterjee I, Zhang Y, Zhang J, Lu R, Xia Y, Sun J. Overexpression of vitamin D receptor in intestinal epithelia protects against colitis via upregulating tight junction protein claudin 15. *J Crohns Colitis*. 2021;15(10):1720–1736. doi:10.1093/ecco-jcc/jjab044
35. Lu Y, Guan T, Xu S, et al. Asperuloside inhibited epithelial-mesenchymal transition in colitis associated cancer via activation of vitamin D receptor. *Phytomedicine*. 2022;101:154070. doi:10.1016/j.phymed.2022.154070
36. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem*. 2008;77:289–312. doi:10.1146/annurev.biochem.77.061307.091829
37. Dubuquoy L, Jansson EA, Deeb S, et al. Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology*. 2003;124(5):1265–1276. doi:10.1016/S0016-5085(03)00271-3
38. Prantera C, Rizzi M. 5-ASA in ulcerative colitis: improving treatment compliance. *World J Gastroenterol*. 2009;15(35):4353–4355. doi:10.3748/wjg.15.4353
39. Hoesel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. *Mol Cancer*. 2013;12:86. doi:10.1186/1476-4598-12-86
40. Wang S, Lin Y, Yuan X, Li F, Guo L, Wu B. REV-ERBalpha integrates colon clock with experimental colitis through regulation of NF-kappaB/NLRP3 axis. *Nat Commun*. 2018;9(1):4246. doi:10.1038/s41467-018-06568-5

41. Grivennikov S, Karin E, Terzic J, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell*. 2009;15(2):103–113. doi:10.1016/j.ccr.2009.01.001
42. Shaukat A, Guo YF, Jiang K, et al. Ginsenoside Rb1 ameliorates Staphylococcus aureus-induced Acute Lung Injury through attenuating NF-kappaB and MAPK activation. *Microb Pathog*. 2019;132:302–312. doi:10.1016/j.micpath.2019.05.003
43. Chen Y, Zhang J, Ge X, et al. Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. *J Biol Chem*. 2013;288(27):19450–19458. doi:10.1074/jbc.M113.467670
44. Korbecki J, Bobiński R, Dutka M. Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. *Inflamm Res*. 2019;68(6):443–458. doi:10.1007/s00011-019-01231-1