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Network pharmacology-based identification of the protective mechanisms of taraxasterol in experimental colitis☆

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ABSTRACT

Background and aim: Taraxasterol, a pentacyclic-triterpene, has been reported to exert potent anti-inflammatory activity. However, the molecular mechanisms by which taraxasterol attenuates acute experimental colitis (AEC) remain undocumented.

Methods: A network pharmacology approach was used to identify the candidate and collective targets of taraxasterol and acute colitis, and an AEC model was established by oral administration of dextran sulfate sodium (DSS) in mice. Body weight and colon lengths were then examined, the pathological scoring was assessed by using hematoxylin and eosin staining, and the expression levels of target genes were further confirmed by qRT-PCR and immunohistochemistry (IHC) analysis in taraxasterol treated AEC models.

Results: 14 collective targets of taraxasterol and acute colitis were identified by a network pharmacology analysis, including PPARG, JAK2, MMP3, NR1I2 and PTPN11. Further investigations in an AEC model showed that, taraxasterol alleviated the unfavorable clinical symptoms and attenuated the intestinal inflammation response by reducing the cytokines TNF-α, IL-1β and IL-6 levels. qRT-PCR and IHC analysis evidenced that, taraxasterol decreased MMP3 expression levels, but increased PPARG expression levels in AEC models as compared with the DSS group.

Conclusions: Our findings demonstrated that taraxasterol improved DSS-induced AEC through regulating MMP3 and PPARG expression, providing a new insight into the potential therapeutic strategies for acute colitis.

1. Introduction

Acute colitis is an acute and relapsing inflammatory ailment resulted from dysregulation of mucosal immune response in gastrointestinal tract. It is characterized by infiltration of inflammatory cells into the mucosa, leading to submucosal congestion and edema [1]. The symptoms of acute colitis include acute pain, vomiting, weight loss, diarrhea, and bloody stool [2] and the inflammation cells may involve the whole colon or be limited to colonic segments [1]. Due to its high homogeneity and reproducibility, sodium dextran sulfate (DSS)-induced colitis has been used to elucidate the pathogenesis of acute colitis [3,4]. DSS-induced colitis has been considered to be driven by macrophages which produce inflammatory cytokines and chemokines, cause tissue damage, and induce migration of neutrophils, dendritic cells and natural killer cells in the colon [5]. Quinolones are commonly used to treat acute colitis, but have some side effects. Therefore, discovery of cost effective and efficacious agents for acute colitis is urgently needed.

Taraxasterol is a pentacyclic-triterpene isolated from *Taraxacum officinale* [6]. Accumulating evidence shows that, taraxasterol has anti-inflammatory effects but minimal side effects [7]. Previous studies showed that, taraxasterol represses the release of NO, PGE2, TNF-α, IL-1β and IL-6 in LPS-induced macrophages [8], reduces IL-1β-induced inflammatory response in osteoarthritic chondrocytes [9] and displays

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Table 1
List of primer sequences.

Gene	Forward primer	Reverse primer
GAPDH	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT
MMP3	GGCCTGGAACAGTCTTGGC	TGTCCATCGTTCATCATCGTCA
PTPN11	CCCGAGTCTATGAGAACGTG	GCTGTTCAAATGGTTCCAA
JAK2	TTAAGATGCTTTCTGGGTTGG	CTTGAATGGGATGAACAGCA
NR1I2	GACCTGCCTATTGAGGACCA	GGAAGCCACCATTAGGGTCT
PPARG	GCCAGTTTCGATCCGTAGAA	AATCCTTGGCCCTCTGAGAT
TNF-α	CCCTCACACTCACAAACCACC	CTTTGAGATCCATGCCGTTG
IL-6	GAAATGATGGATGCTACCAAACTG	GACTCTGGCTTTGTCTTTCTTGTT
IL-1β	CACTACAGGCTCCGAGATGAAC	TCCATCTTCTTCTTTGGGTATTGC

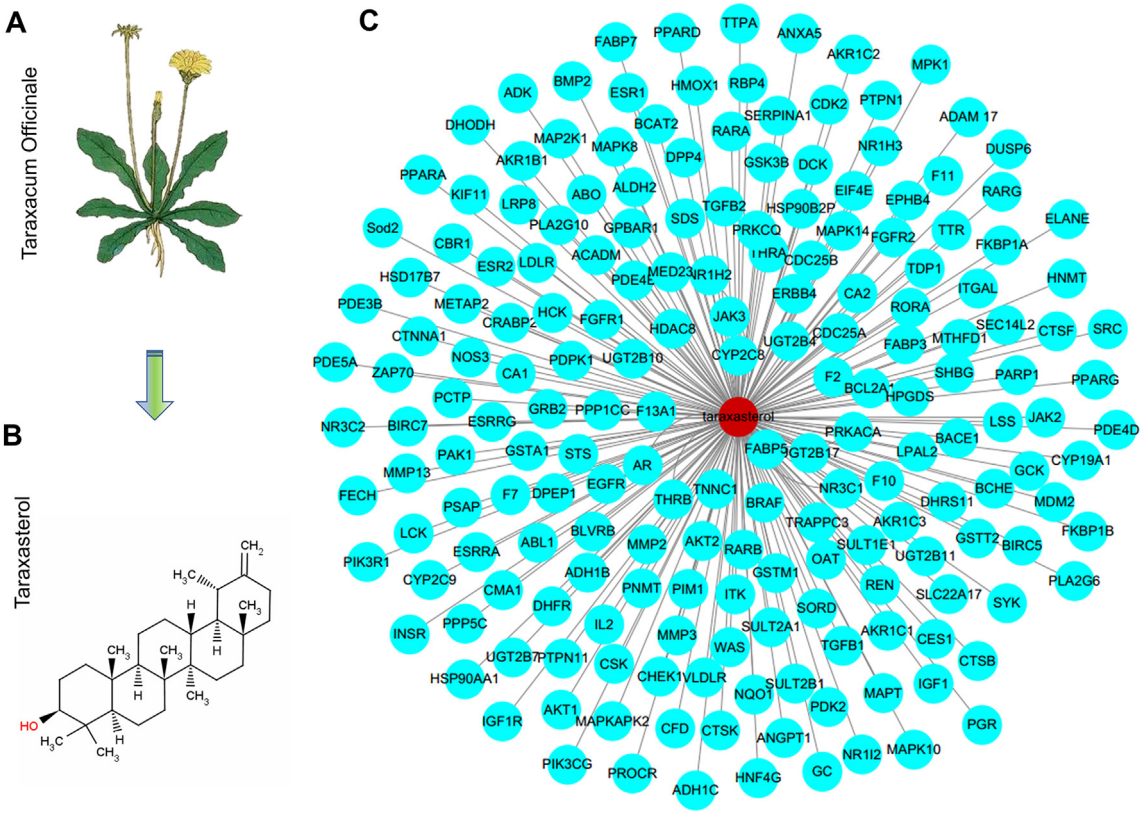

Fig. 1. The targets of taraxasterol were used to establish the protein-protein regulation network. (A) The image of *Taraxacum officinale*. (B) 2-D structure of taraxasterol. (C) 190 candidate targets of taraxasterol were identified to establish the protein-protein regulation network. The green nodes represent the protein targets of taraxasterol, and the red node stands for the taraxasterol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a protective effect against arthritis and cigarette smoke-induced lung inflammation by decreasing TNF-α, IL-1β, IL-6, PGE2 and RANKL levels [10]. However, the molecular mechanisms of taraxasterol in DSS-induced AEC remain unclear.

Network pharmacology emphasizes the prediction of multi-targets of drugs with minimum side effects [11]. In this study, we developed a comprehensive network pharmacology to identify 14 collective targets of taraxasterol and acute colitis, and found that, taraxasterol improved DSS-induced colitis through regulating MMP3 and PPARG expression, providing a new insight into potential therapeutic strategies for acute colitis.

2. Materials and methods

2.1. Materials

Taraxasterol was obtained from Reifensis Biotechnology Co., Ltd. (Chengdu, PR, China). DSS was purchase from MP Bio, USA. Male 6–8 weeks C57BL/6 male mice were supplied by West Pui Kai experimental animal Co., Ltd. (Shanghai, PR, China). The primers used were obtained from Seville Biotechnology Co., Ltd. (Wuhan, PR, China).

2.2. Identification of candidate targets of taraxasterol

The Canonical SMILES of taraxasterol was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). About 190 targets of taraxasterol were screened by Swiss Target Prediction Database (http://www.swisstargetprediction.ch/), STICH Database (http://stitch.embl.de/) and PharmMapper Database (https://lilab.ecust.edu.cn/pharmmapper/index.php).

2.3. Identification of candidate targets of acute colitis

About 297 targets of acute colitis were acquired from OMIM Database (http://omim.org/), Therapeutic Targets Database (http://bidd.nus.edu.sg/group/cjttd/) and PharmGKB Database (https://www.

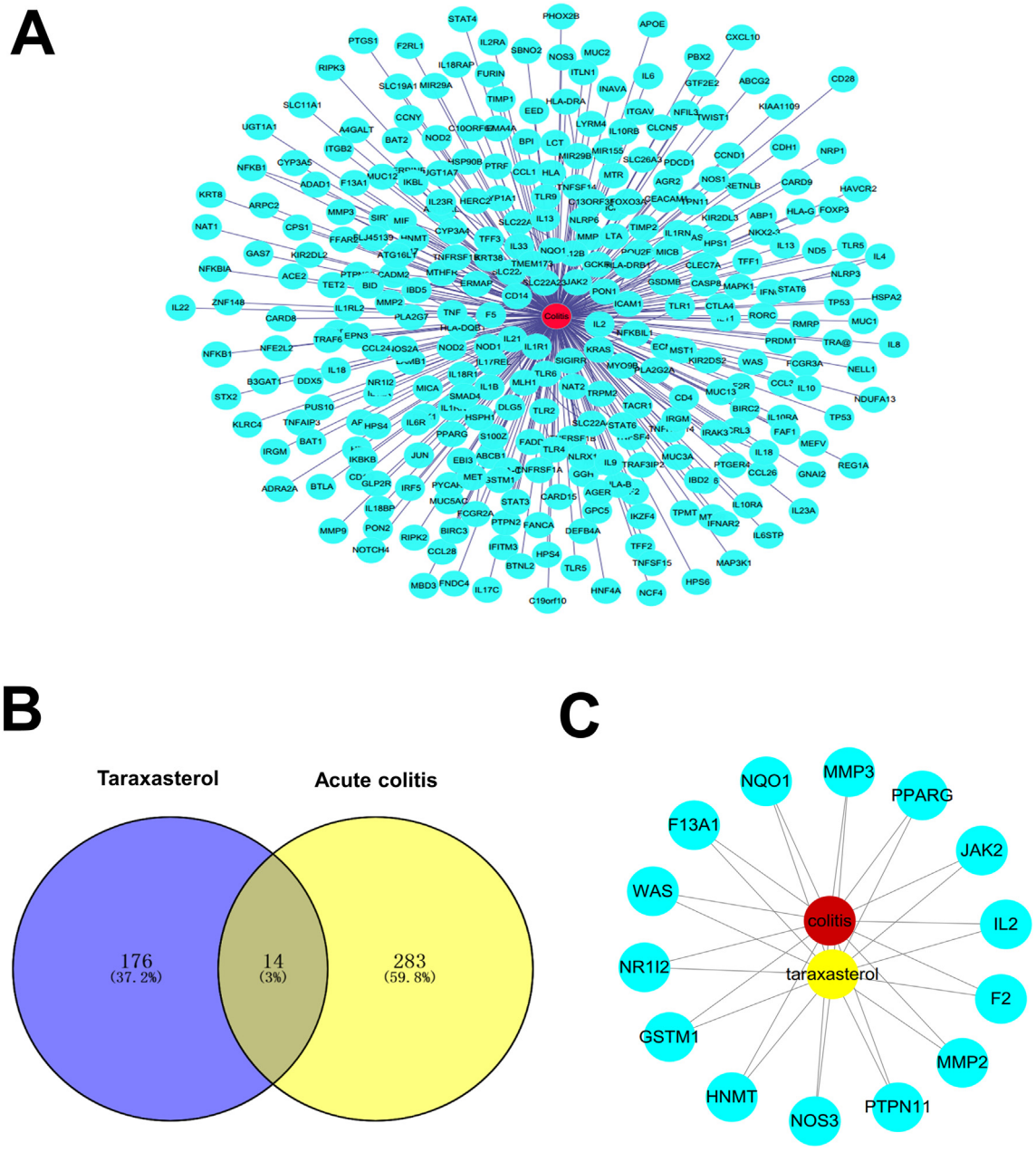

Fig. 2. The collective targets of taraxasterol and colitis were identified. (A) 297 targets of acute colitis were identified to establish the protein-protein regulation network. The green nodes represent the protein targets, and the red node stands for the acute colitis. (B) 14 collective targets of taraxasterol and colitis were identified. (C) These collective targets were used to establish the protein-protein regulation network. The green nodes represent the protein targets, red node stands for acute colitis, and the yellow nod represents taraxasterol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pharmgkb.org/).

2.4. Protein-protein interaction network

The collective targets of taraxasterol and acute colitis were used to establish the protein-protein interaction regulation network by cytoscape database (http://www.cytoscape. org/).

2.5. DSS-induced AEC model

6–8 weeks C57BL/6 male mice were housed in the SPF facility at the Animal Laboratory Center of our hospital. They were freely fed for 5 days and randomly divided into control group (n = 8), DSS group (n = 8) and DSS + taraxasterol (DSS + T) group (n = 8). DSS groups underwent DSS-induced acute colitis by oral administration of 3% DSS, DSS + T groups were administrated orally with 10 mg/kg taraxasterol (T) and 3% DSS, and control groups were orally given with water. After investigations for a week, the mice in these three groups were executed and the colon tissues were used for further analysis. Our animal study was approved by the Ethics Committee of Shanghai Sixth People's Hospital.

2.6. Hematoxylin and erosion (H&E) staining and histological scoring

The colon tissues were isolated and fixed on a 4% paraformaldehyde solution for 48 h and embedded in paraffin. Histological examinations

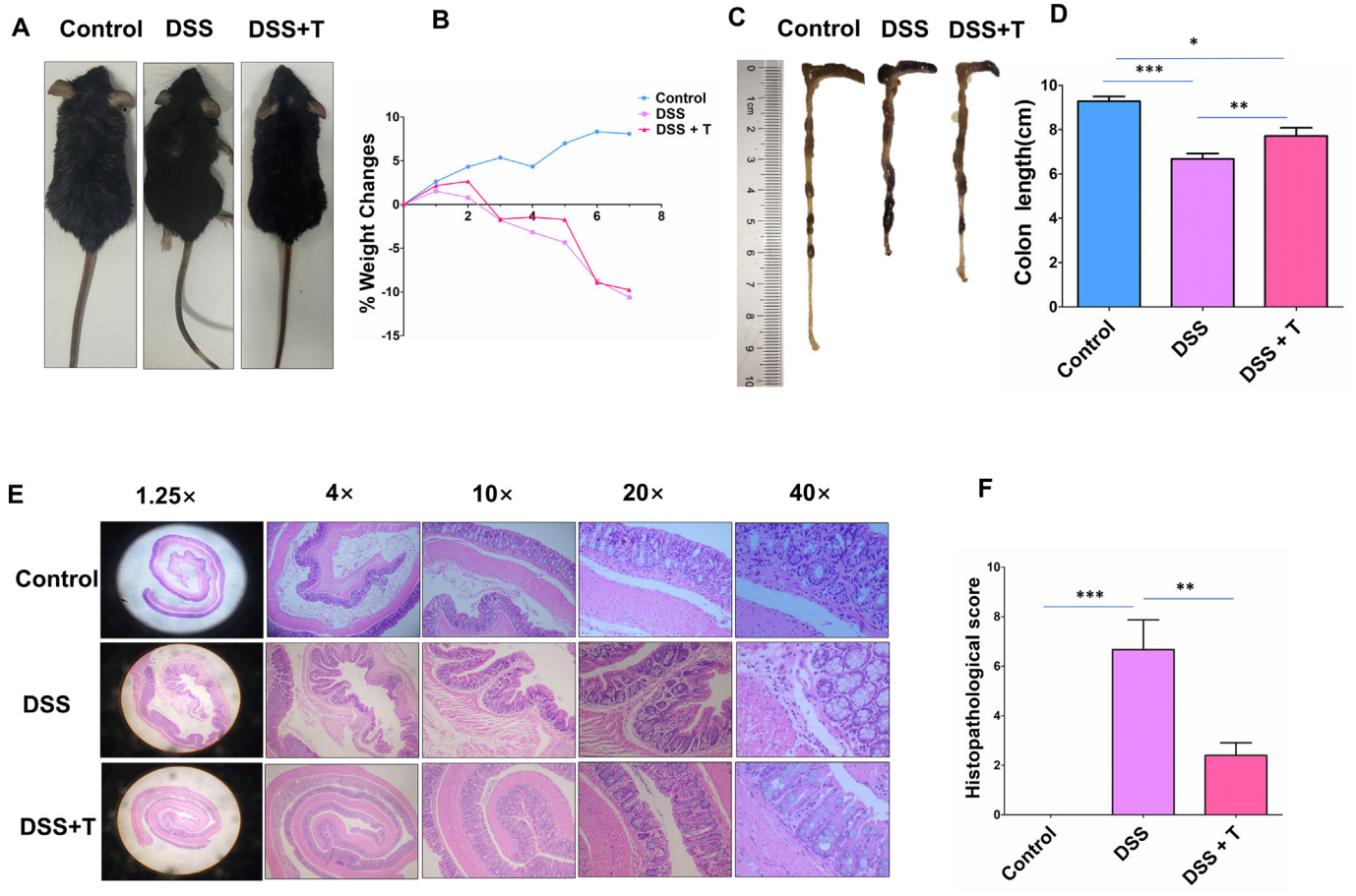

Fig. 3. Taraxasterol ameliorated DSS-induced colitis in mice. (A) Representative schematic of the mice in different groups and comparison of the mean body weight between the three groups. (B) Macroscopic appearance of the colon with the mean colon length and typical injury findings, and comparison of the colon lengths in each group. (C) The histopathological changes in colon tissues were examined by H&E staining (1.25×, 4×, 10×, 20×, 40×), and the pathological scores were further assessed between the three groups. Data are the means ± SEM of three experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

were performed by H&E staining after paraffin sections of these colon tissues. The histological scoring was conducted as previously reported [12,13].

2.7. Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from colon tissues using the Trizol reagent (Invitrogen, USA). Complementary DNA (DNA) is produced by RNA reverse transcription by using the PrimeScript™ Reverse Transcription Kit (TakaRa, Japan). The procedures were performed as follow: Stage 1: Pre-denaturation at 95 °C for 30 s, 1 cycle. Stage 2: PCR reaction, at 95 °C for 5 s and 60 °C for 34 s, 40 cycles. Stage 3: at 95 °C for 30 s and 60 °C for 1 min, 1 cycle. The relative expression levels of TNF-α, IL-6, IL-1β, JAK2, NR1I2, MMP3, PTPN11 and PPARG were calculated using the $2^{-\Delta\Delta Ct}$ method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control. All qPCR reactions were performed in duplicate. The primers used in this study were listed in Table 1.

2.8. Immunohistochemistry (IHC)

The AEC colon tissues were immune-stained for anti-TNF alpha (17590-1-ap, proteintech, PR, China), anti-IL-6 (21865-1-AP, proteintech, China), anti-IL-1β (ab33591, Lianke Biotechnology Co., Ltd., China), anti-MMP3 (ab52915, Abcam, United Kingdom) and anti-PPAR gamma (ab59256, Abcam, United Kingdom) as previously described [14].

2.9. Statistical analysis

SPSS18.0 software was used to analyze the experiment data and graph presentation were achieved by using the GraphPad Prism 5 Software (GraphPad, San Diego, CA, USA). All quantitative data were expressed as Mean ± SD. The Student's *t*-test and Analysis of Variance were used to compare quantitative variables. $P < 0.05$ is considered as a statistical significance.

3. Results

3.1. The targets of taraxasterol were used to establish the protein-protein regulation network

Taraxasterol is isolated from *Taraxacum officinale* (Fig. 1A), and its 2-D structure is shown in Fig. 1B. Swiss Target Prediction, STICH and PharmMapper databases were used to identify 190 target genes of taraxasterol, which were further used for constructing the protein-protein regulation network consisting of about 191 link nodes (Fig. 1C).

3.2. The collective targets of taraxasterol and colitis were used to construct the protein-protein regulation network

OMIM, Therapeutic Targets and PharmGKB databases were then used to identify 297 targets of acute colitis, which were performed for constructing the protein-protein regulation network consisting of about 298 link nodes (Fig. 2A). Thus, 14 collective targets of taraxasterol and

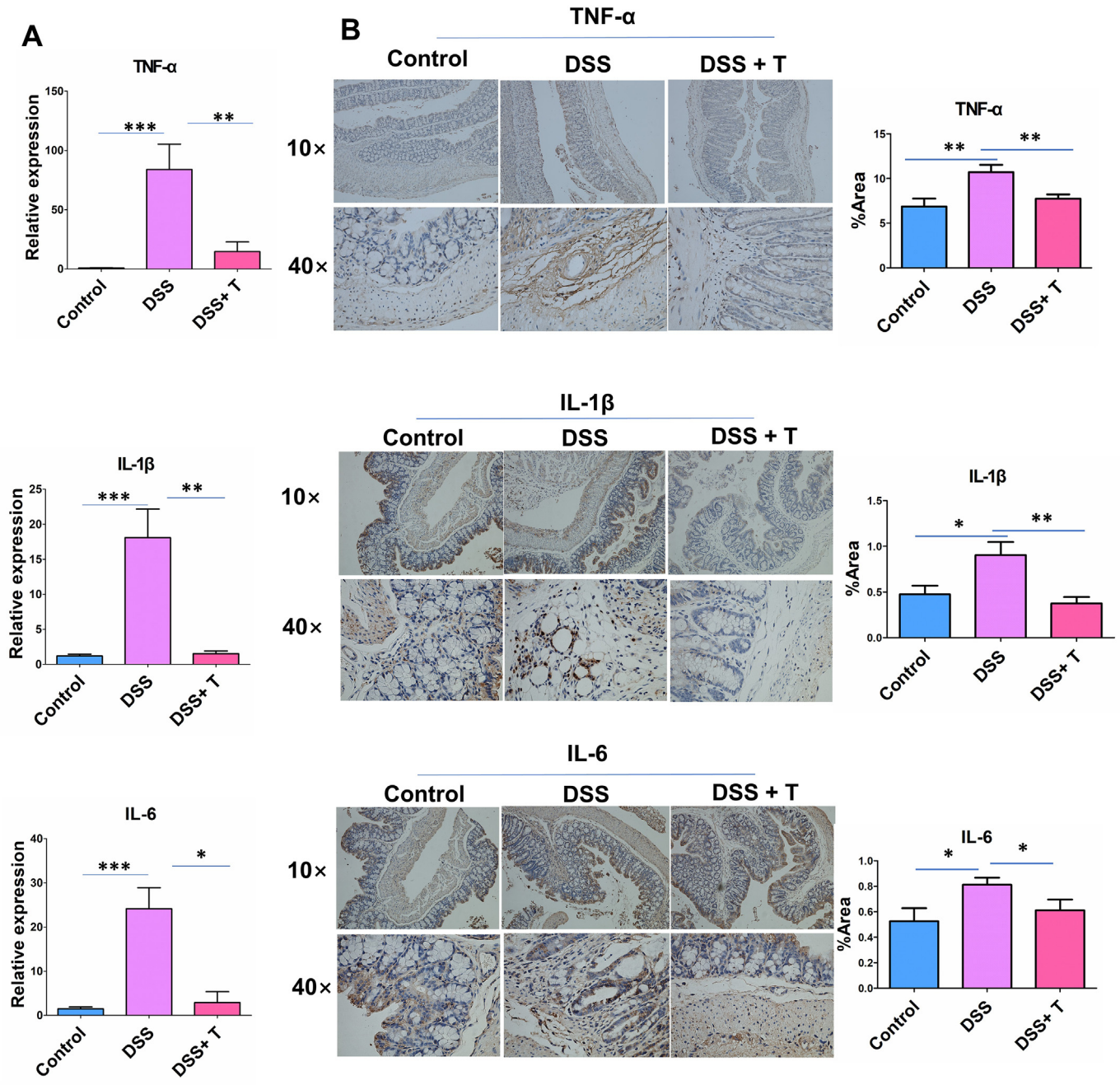

Fig. 4. Taraxasterol suppressed the production of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in AEC models (A) The mRNA expression levels of inflammatory cytokines TNF-α, IL-6, and IL-1β were determined by qRT-PCR analysis in colon tissues in three groups. (B) The protein expression levels of TNF-α, IL-6 and IL-1β were verified by IHC analysis in colon tissues in these three groups. Data are the means ± SEM of three experiments. *$P < 0.05$, **$P < 0.01$.

colitis were acquired (Fig. 2B), and were used to construct the protein-protein regulation network consisting of 16 link nodes (Fig. 2C).

3.3. Taraxasterol ameliorated DSS-induced colitis

After the mice with acute colitis were induced by 3% DSS for a week, they gradually manifested loose stool, occult blood and weight loss in DSS and DSS + T groups as compared with the control group (Fig. 3A). The colon lengths of these three groups were measured, and the mean colon length (cm) for DSS group, DSS + T group and control group were 6.7 ± 0.2, 7.7 ± 0.4, 9.3 ± 0.2, respectively (Fig. 3B). The colon lengths were dramatically decreased by DSS as compared with the control group ($P < 0.0001$), but increased by taraxasterol as compared with DSS group ($P < 0.01$, Fig. 3B).

HE staining was used for assessing the pathological scoring in colon tissues, and showed that, DSS could induce the severe inflammation response, colon ulceration and crypt damage, and the pathological scoring was substantially elevated in DSS group as compared with the control group, but taraxasterol reduced DSS-caused this effect in colon tissues ($P = 0.0085$, Fig. 3C).

3.4. Taraxasterol reduced pro-inflammatory cytokines TNF-α, IL-1β and IL-6 levels in DSS-induced colitis

Inflammatory cytokines such as TNF-α, IL-1β and IL-6 act key roles in regulating the inflammatory response in acute colitis. qRT-PCR analysis showed that, the mRNA expression levels of TNF-α, IL-1β and IL-6 were significantly increased by DSS as compared with the control

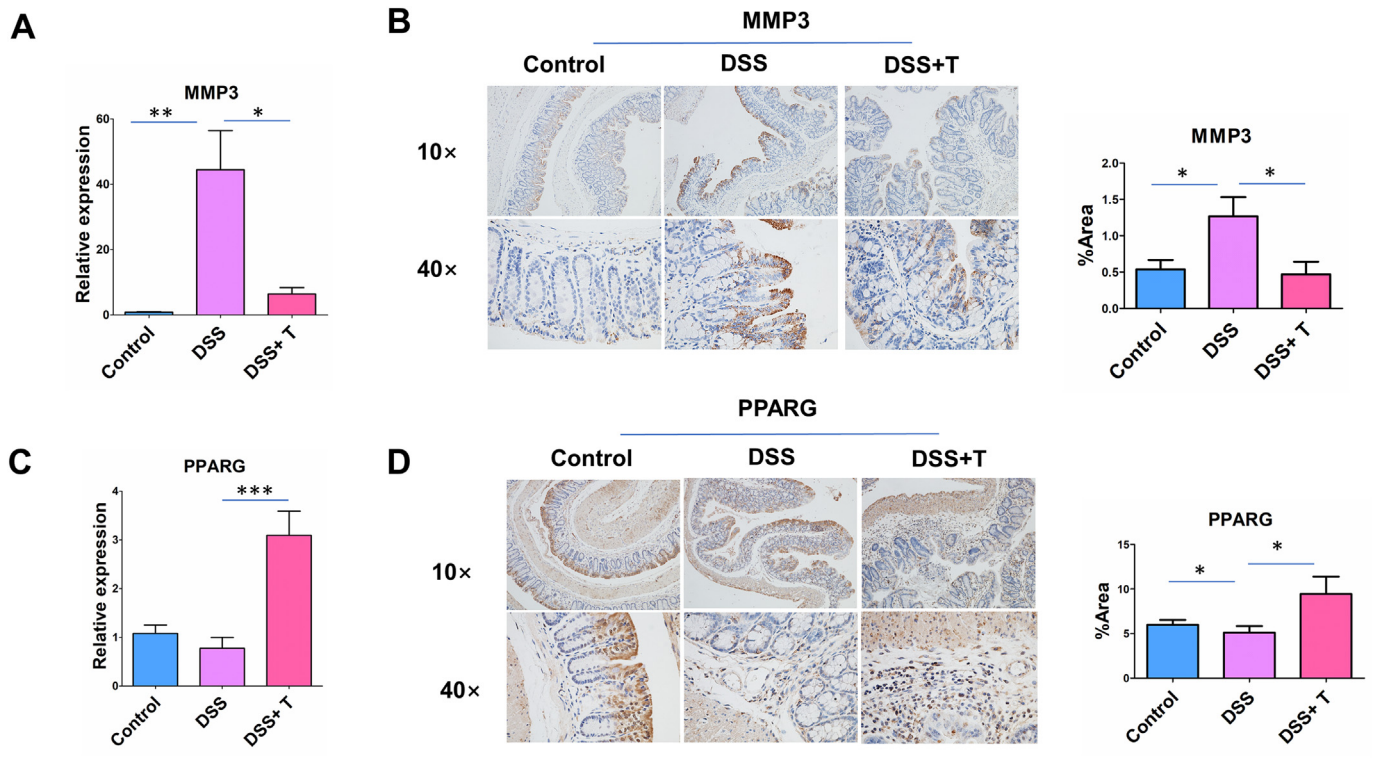

Fig. 5. Taraxasterol regulated the expression of MMP3 and PPARG in DSS-induced colitis in mice. (A, C) The mRNA expression levels of MMP3 and PPARG were determined by qRT-PCR analysis in colon tissues of the three groups. (B) The protein expression levels of MMP3 and PPARG were confirmed by IHC analysis in colon tissues of the three groups. Data are the means ± SEM of three experiments. * $P < 0.05$, ***$P < 0.001$.

group, but taraxasterol reduced DSS-induced their expression levels in colon tissues ($P < 0.05$, Fig. 4A). The similar results were further confirmed by IHC analysis in colon tissues of AEC models ($P < 0.05$, Fig. 4B).

3.5. Taraxasterol regulated MMP3 and PPARG expression in DSS-induced colitis

According to the network pharmacology, 14 collective targets of taraxasterol and colitis were identified (Fig. 2C), among which five targets (JAK2, NR1I2, MMP3, PTPN11 and PPARG) had a close association with acute colitis and were selected for further validation by qRT-PCR and IHC analysis, which indicated that, the expression levels of MMP3 (Fig. 5A, B) were increased, but PPARG expression (Fig. 5C, D) was decreased by DSS, as compared with the control group. However, taraxasterol reversed DSS-induced MMP3 and PPARG expression ($P < 0.05$, Fig. 5), but had no effects on PTPN11, JAK2 and NR1I2 expression as compared with the DSS group in colon tissues (Supplementary Fig. S1).

4. Discussion

Acute colitis mainly occurs in large intestine, and its etiology and pathogenesis are still unclear. Some studies show that, taraxasterol exhibits anti-inflammatory responses by reducing the secretion of TNF-α, IL-1β and IL-6 [8–11]. In this study, an AEC model was established, and DSS-caused shortened colon lengths and higher inflammatory pathological scoring, but taraxasterol reversed these effects in DSS-induced colitis. Moreover, we found that, DSS increased TNF-α, IL-1β and IL-6 expression levels, but taraxasterol reversed DSS-caused their expression in colon tissues. Suggesting that, taraxasterol ameliorated DSS-induced colitis in mice (see also Fig. 6).

A network pharmacology approach was used to identify 14 collective targets of taraxasterol and colitis, among which PPARG and MMP3 had a close association with acute colitis. Peroxisome proliferator-activated receptor gamma (PPARG), a member of the nuclear hormone receptor family, participates in adipocyte differentiation, insulin sensitivity and inflammatory responses [15–17]. Its ligands reduce the disease severity in AEC models [18–20] and are associated with the susceptibility of inflammatory bowel disease (IBD) [21]. PPARG agonists such as thiazolidinediones and 5-ASA reduce the clinical remission of IBD [22–24]. Herein, we found that, taraxasterol increased PPARG expression and reversed DSS-induced PPARG downregulation in AEC colon tissues.

Matrix metalloproteinases (MMPs), a kind of protease, regulate the extracellular matrix remodeling and turnover [25], facilitate the leukocyte extravasation and act as potential targets for IBD [26–31]. MMP3 has been reported upregulated in inflammatory colonic mucosa, leading to the tissue damage in IBD and AEC models [27,28,32–35], and controls the colonic pathogenic bacteria and T lymphocyte mobilization in lamina propria [36]. Herein, we found that, DSS induced the MMP3 upregulation, but taraxasterol reversed DSS-induced this effect in AEC colon tissues.

In conclusion, we developed a comprehensive network pharmacology to identify 14 collective targets of taraxasterol and colitis, and found that, taraxasterol improved DSS-induced colitis through regulating MMP3 and PPARG expression, providing a new insight into potential therapeutic strategies for acute colitis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.03.042.

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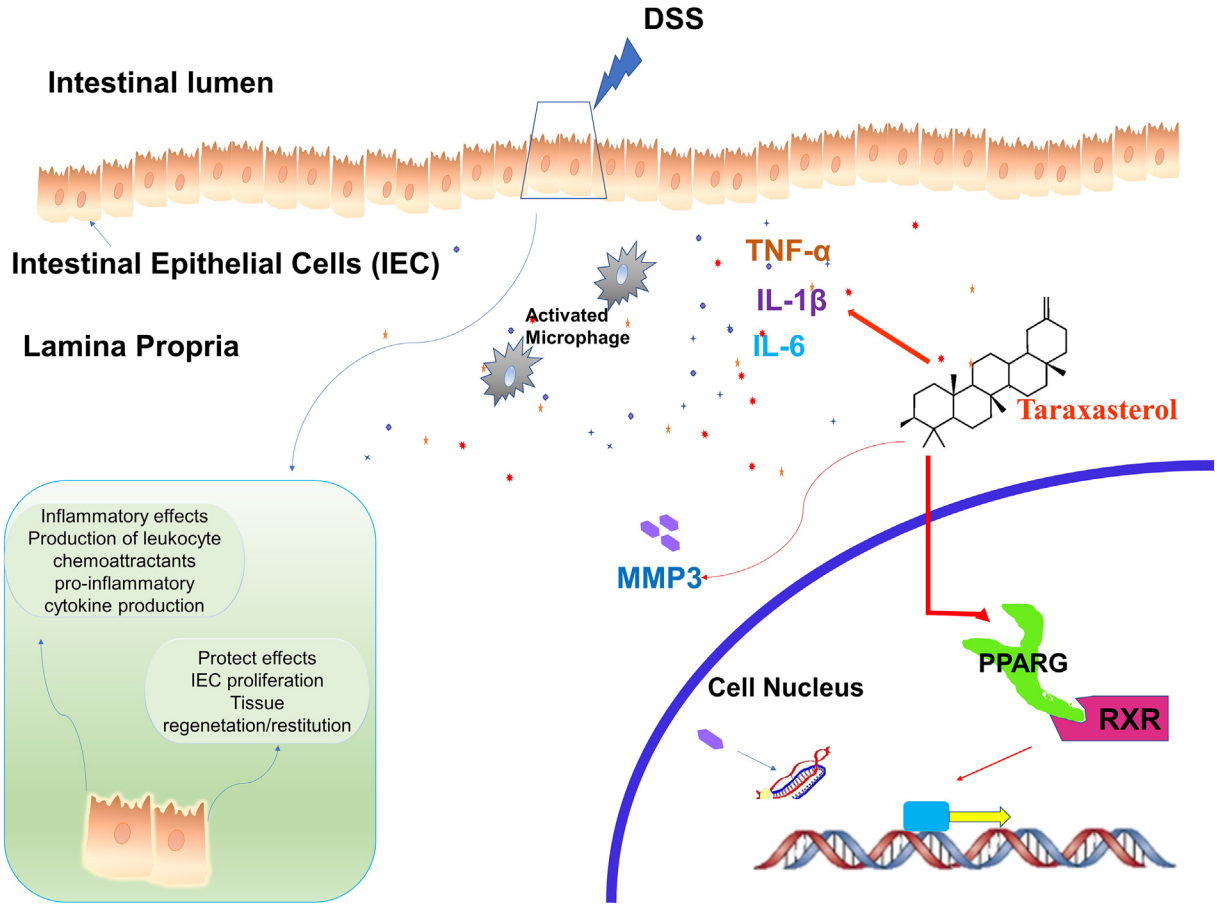

Fig. 6. Schematic representation of the proposed mechanism of taraxasterol in AEC. DSS was used to disrupt the integrity of the mucosal barrier, and induce the inflammatory response in colon tissues. But taraxasterol reduced DSS-induced secretion of inflammatory cytokines TNF-α, IL-1β and IL-6, decreased MMP3 expression levels, but increased PPARG expression levels in DSS-induced colitis.

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