Curcumin ameliorates ulcerative colitis via inhibiting STAT3-mediated angiogenesis

Su Xu[1], Zixuan Zhou[2], Aoxun Zhu[1], Yurong Guo[1], Shengnan Jin[2], Maozhen Zhang[2], Yanping Hao[3]*

[1]Yancheng TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Jiangsu, China
[2]Clinical Medical College of Nanjing University of Chinese Medicine, Jiangsu, China
[3]Yancheng Clinical College of Xuzhou Medical University, Jiangsu, China

*Corresponding author:
Yanping Hao
Yancheng Clinical
College of Xuzhou
Medical University
Jiangsu, China
E-mail:
haoyanping1983@163.com

Abstract

Introduction: Ulcerative colitis (UC) is a chronic and persistent inflammatory bowel disease with limited clinical treatment options and significant therapeutic challenges. This study aimed to evaluate whether the therapeutic effect of curcumin against ulcerative colitis is positively correlated with its inhibition of angiogenesis and to elucidate the underlying angiogenesis-related molecular mechanism.

Material and methods: A multi-database analysis was performed to predict the possible targets involved in curcumin inhibition of UC. Tube formation and aortic ring assays were used to evaluate angiogenesis *in vitro*. A dextran sulfate sodium (DSS)-induced ulcerative colitis mouse model was used to evaluate curcumin's effect on UC.

Results: We first employed a comprehensive multi-database analysis to identify overlapping targets connecting curcumin, ulcerative colitis, and angiogenesis, leading to the identification of signal transducer and activator of transcription 3 (STAT3) as a potential mediator of this process. *In vitro* experimental results demonstrated that curcumin significantly inhibited tube formation in human umbilical vein endothelial cells (HUVEC) and suppressed endothelial sprouting in rat aortic rings. Furthermore, curcumin downregulated the expression of vascular endothelial growth factor (VEGF) in HUVEC cells and concurrently inhibited the expression of phosphorylated JAK2 and phosphorylated STAT3 (Y705). Notably, the addition of VEGF partially reversed curcumin's inhibitory effects on p-JAK2 and p-STAT3. *In vivo* studies using a DSS-induced mouse model of ulcerative colitis revealed that curcumin ameliorated DSS-induced colon shortening and various disease symptoms. It also suppressed serum levels of TNF-α and IL-6 and inhibited the expression of STAT3 and VEGF in colonic tissues.

Conclusions: Curcumin ameliorates ulcerative colitis through inhibition of the VEGF-mediated JAK2/STAT3 signaling pathway. These findings position curcumin as a potential clinical candidate drug for the treatment of ulcerative colitis.

Key words: curcumin, ulcerative colitis, vascular endothelial growth factor, angiogenesis, STAT3 pathway.

Introduction

Ulcerative colitis (UC) has become a global disease with approximately 5 million patients worldwide [1]. UC is a complex chronic inflammatory disorder of the colon with an immune-mediated etiology. Several risk

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factors have been implicated, including dietary habits, pharmaceutical exposures, and lifestyle elements that can modulate the host microbiome or antigenic immune responses [1, 2].

UC features complex pathologies including epithelial barrier disruption, dysregulated immune responses, and microbiome alterations [1]. Histologically, active UC shows neutrophil infiltration, crypt abscesses, goblet cell depletion, and mucosal ulceration, while the quiescent phase exhibits glandular distortion, submucosal fibrosis, and lymphatic dilation [3]. Among these multifaceted pathologies, aberrant angiogenesis – the formation of new blood vessels – has emerged as a critical driver of inflammation and tissue damage [4]. Dysfunctional vasculature perpetuates leukocyte infiltration, disrupts mucosal oxygenation, and contributes to the vicious cycle of inflammation [4, 5]. At present, conventional therapies for UC include biologic therapy, herbal therapy, and fecal microbiota transplantation [6]. Furthermore, diet and exercise, which promote recovery from hyperlipidemia, diabetes, and hypertension in older patients, are considered essential adjunctive therapies for UC [7, 8]. Among them, natural compounds from herbs are attracting increasing attention for development of new therapeutic agents for UC [2].

Curcumin, a polyphenolic compound derived from the rhizome of *Curcuma longa*, exhibits a broad spectrum of pharmacological activities, making it a subject of extensive research in both preclinical and clinical studies [9]. Its most notable bioactivities include antitumor [10], anti-inflammatory [11], and antifibrotic properties [12], underpinned by its ability to modulate diverse molecular pathways. Many articles have witnessed the improved effect of curcumin on inflammatory diseases including colitis. Curcumin blocks nuclear translocation of NF-κB and suppresses downstream cytokines (e.g., TNF-α, IL-1β, IL-6), resulting in improvement of UC [13]. By binding to Toll-like receptor 4 (TLR4), curcumin disrupts TLR4-mediated NF-κB activation and reduces intestinal permeability [14]. Curcumin downregulates JAK2/STAT3 phosphorylation, curtailing Th1/Th17-driven inflammation and promoting Treg cell activity [15, 16]. Curcumin attenuates ulcerative colitis via regulating the sphingosine kinase 1/NF-κB signaling pathway [17]. Furthermore, curcumin attenuates visceral hyperalgesia through inhibiting phosphorylation of TRPV1 in a rat model of ulcerative colitis [18]. Curcumin arrests dendritic cells of maturation to promote Treg function, shifting the balance toward anti-inflammatory responses [19].

Angiogenesis, the formation of new blood vessels from pre-existing ones, is a tightly regulated process critical for both physiological development and pathological conditions such as cancer and chronic inflammation [20]. It involves complex interactions between endothelial cells, signaling molecules, and extracellular matrix components, enabling tissue growth, repair, and nutrient supply [21]. Pathological angiogenesis, however, often exhibits abnormal vessel structures and hyperpermeability, contributing to tumor progression and metastasis [22]. However, mounting evidence indicates a close relationship between inflammatory bowel disease and angiogenesis [23].

Considering the overall evidence, we hypothesized that curcumin may ameliorate ulcerative colitis via inhibiting angiogenesis. Concurrently, we aimed to elucidate its molecular mechanism.

Material and methods

Chemicals and reagents

Curcumin was obtained from Nanjing Jin Yibai Biological Technology Co. Ltd. (Nanjing, China). TNF-α (KE10002) and IL-6 (KE10007) ELISA kits were purchased from Proteintech. The following antibodies were used: VEGF (Proteintech, 66828-1-lg), VEGFR (Proteintech, 83049-4-RR), JAK2 (Abcam, 245303), p-JAK2 (Abcam, 32101), STAT3 (Proteintech, 51076-1-AP) and p-STAT3 (Y705) (Abcam, 278669), GAPDH (Proteintech, 10494-1-AP).

Animals

The male C57 mice (20–25 g) were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd. Every 5 mice were housed as a group in a cage, and provided with free access to food and water at controlled room temperature (22 ±2°C) and humidity (60–80%) under a 12 h/12 h light/dark cycle. The animal experiment was approved by the Yancheng TCM Hospital affiliated with Nanjing University of Chinese Medicine Institutional Animal Care and Use Committee (SLDW231201).

Cell culture

HUVEC cells were obtained from the American Type Culture Collection (ATCC) and subjected to regular testing for mycoplasma contamination. HUVECs were cultured in DMEM/F12 medium supplemented with 10% FBS (Cellmax, Lanzhou, China) and 1% penicillin-streptomycin (Corning, NY, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and routinely screened for mycoplasma contamination.

Tube formation assay

Tube formation assay was performed as described previously [24]. Briefly, HUVECs, pretreated with or without curcumin for 24 h, in the presence or absence of VEGF-1 (10 ng/ml) or 2% FBS,

were trypsinized and then seeded into a 96-well plate (2×10^4/well) precoated with 40 µl (10 mg/ml) growth factor-reduced Matrigel (BD Biosciences, Billerica, MA). After 2 to 3 h of incubation at 37°C, the capillary tube structures were observed, and representative images were captured with an inverted phase-contrast microscope (Zeiss, Germany) (200×) equipped with the Quick Imaging system. The formed tubes were quantified by ImageJ software. Briefly, three randomly selected fields of view were photographed in each treatment. Tube formation was assessed by counting the circular structures representing tube-like networks. The average of three fields was taken as the value for each treatment.

Rat aortic ring assay

The rat aortic ring assay was performed following the modified methods as described [25]. Thoracic aortas were excised from Sprague-Dawley male rats and cut into cross sectional, 1-mm-wide rings and flushed with DMEM/F12 medium (Hyclone, USA). Rings were immediately placed into the wells of a 48-well plate containing 400 µl fibrinogen/thrombin solution and then incubated at 37°C until the fibrinogen/thrombin solution polymerized. The wells were then overlaid with 600 µl of DMEM/F12 medium containing 10 ng/ml VEGF and various concentrations of curcumin. On the seventh day, vascular vessels sprouting from each ring were examined using a Leica inverted phase-contrast microscope (Leica, Germany) equipped with the Quick Imaging system to take photos and to facilitate counting.

Western blotting analysis

Proteins were extracted from cells using RIPA lysis buffer. Following separation by SDS-PAGE, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. These membranes were then blocked with 5% skim milk and probed with specific antibodies targeting anti-JAK2 (1 : 1000), anti-p-JAK2 (1 : 1000), anti-STAT3 (1 : 1000), anti-p-STAT3 (1 : 1000), and anti-actin (1 : 1000). After washing with Tris-buffered saline with Tween 20 (TBST), the relative protein expression was determined using an imaging system (Bio-Rad, USA).

Dextran sulfate sodium (DSS)-induced ulcerative colitis in mice

A total of 40 male Balb/c mice were acclimated for one week, and then randomly divided into 4 groups ($n = 10$): the blank group, control group, and two curcumin groups (25 mg/kg and 50 mg/kg). All groups except the blank group were treated with water containing 4% DSS for 7 days. Si-multaneously, the mice of treated groups were administered curcumin (25 mg/kg and 50 mg/kg), whereas the mice of blank and control group were treated with sterile water. During the experiment, all the mice were treated with a standard diet, and the gavage volume was 0.1 ml/10 g. The weight of mice was recorded every day, and the disease activity index score (DAI score) was assessed according to the procedure described by Hao *et al.* [26]. At the endpoint of the experiment, the colon was dissected and the length of the colon was measured after the blood was prepared. The colon tissues were fixed in 4% neutral formalin for H&E staining and immunohistochemical analysis.

RNA isolation and quantitative real-time PCR

Total RNA was isolated from cells using Trizol, and cDNA was synthesized using the HiScript III-RT SuperMix kit (Vazyme, R323-01). The cDNA samples were subjected to a real-time quantitative polymerase chain reaction (RT-qPCR) using the ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711-02) performed on an iQ6 instrument (Bio-Rad, USA). The primer sequences were as follows: VEGF: Forward: 5'-TTGCCTTGCTGCTCTACCTCCA -3'. Reverse: 3'-GATGGCAGTAGCTGCGCTGATA -5'

VEGFR: Forward: 5'-GGAACCTCACTATCCGCAGAGT -3'. Reverse: 3'-CCAAGTTCGTCTTTTCCTGGGC -5'

H&E staining

The harvested lung and liver tissues were first rinsed with PBS and subsequently fixed in 4% paraformaldehyde for over 24 h. After fixation, the tissues underwent dehydration with a gradient of ethanol and xylene before being embedded in paraffin. Sections of 4 µm thickness were then prepared and stained with hematoxylin and eosin (H&E) following standard protocols. Finally, the sections were imaged using a Pathology Workstation (PerkinElmer, USA). Results were quantified using Image J software (Bethesda, NIH, USA).

Immunohistochemical staining

Lung tissue sections collected from mice were first rehydrated and then subjected to antigen extraction in 10 mM sodium citrate and 0.05% Tween solution at 95°C for 3 min. Sections were then blocked with 5% bovine serum albumin (BSA) solution for 30 min, followed by incubation with the indicated primary antibodies overnight at 4°C and then with the corresponding secondary antibodies for 2 h at room temperature. Finally, the sections were stained with 3,3'-diaminobenzidine (DAB) (ZSGB-BIO, ZLI-9018), sealed with neutral gum, and observed under a Pathology Workstation

(PerkinElmer, USA). The immunohistochemical signals were photographed and the images were analyzed using ImageJ software (Bethesda, NIH, USA).

Enzyme-linked immunosorbent assay (ELISA)

The serum was obtained by centrifugation at 2000 RPM for 5 min. The content of TNF-α and IL-6 was determined using ELISA kits according to the manufacturer's instructions, with absorbance values measured at 450 nm.

Statistical analysis

All the experimental data in this paper were processed with GraphPad Prism 8.0 software, presented as mean ± SD. One-way ANOVA and unpaired Student's t-test were applied to the analysis between groups. $P < 0.05$ was considered statistically significant.

Results

Comprehensive target analysis of curcumin, ulcerative colitis, and angiogenesis using multiple databases

Curcumin, ulcerative colitis, and angiogenesis-related targets were collected to investigate potential mechanisms of action. Using the Swiss Target Prediction website, the SMILES sequence of curcumin (PubChem CID: 969516) was input, selecting *Homo sapiens* as the species. This yielded 65 targets. Using the PharmMapper website, the structural file of curcumin was uploaded, selecting Human Protein Targets Only. Targets were filtered for Norm Fit ≥ 0.5. IDs were then mapped to Gene Names using the UniProt database, resulting in 106 targets. Using the STITCH website, the SMILES sequence of curcumin was input, selecting *Homo sapiens* and filtering for low confidence (0.150). Exported data yielded 10 targets. The curcumin targets obtained from the above sources were combined and duplicate targets were removed, resulting in a total of 170 targets.

Using the GeneCards website, a search was performed with the keyword of Ulcerative Colitis. The downloaded data contained 5,843 genes. Targets with a score greater than the median score of all genes were screened, resulting in 2,921 targets. Using the GeneCards, OMIM, and TTD websites, the same search was performed with the keyword Angiogenesis. Genes were copied into a table. The angiogenesis-related targets obtained from the above sources were combined and duplicate targets were removed, resulting in 1,723 targets.

Finally, a Venn diagram was constructed using the obtained target datasets, revealing 63 common intersection targets (Figure 1 A). A protein-protein interaction (PPI) network was constructed using the STRING website. "Multiple proteins" was selected, and the common intersection targets were input. The resulting image and a TSV data file were exported (Figure 1 B). The TSV data were imported into Cytoscape software. Targets were ranked based on their degree value, and the PPI network diagram was visualized (Figure 1 C). The top ten ranked targets were: AKT1, EGFR, PPARG, ALB, STAT3, CASP3, BCL2, IGF1, PLAU, SRC. Using the DAVID database, the common overlapping targets were imported for GO/KEGG enrichment analysis. The GO analysis results were primarily enriched in processes such as signal transduction, transcriptional regulation by RNA polymerase, and cell proliferation (Figure 1 D). KEGG pathway analysis showed significant enrichment in pathways including prostate cancer, EGFR tyrosine kinase inhibitor resistance, pancreatic cancer, epithelial signaling transduction, and acute myeloid leukemia (Figure 1 E). Among the identified targets, STAT3 was chosen for further investigation.

Curcumin inhibits tube formation and vascular sprouting *in vitro*

To confirm curcumin's effect on angiogenesis, tube formation assay and rat aortic ring assay were used. As shown in Figure 2, VEGF clearly induced tube formation, while curcumin significantly inhibited VEGF-induced tube formation in HUVECs in a concentration-dependent manner. Figure 3 shows that the number of sprouting endothelial cells in the curcumin treatment group was significantly lower than that of the control group. Both results suggested that curcumin inhibited angiogenesis *in vitro*.

Curcumin inhibits VEGF expression and the JAK2/STAT3 pathway in HUVEC cells

We next observed how curcumin affects HUVEC cells in vitro. RT-PCR results showed that curcumin could inhibit transcriptional expression of VEGF and VEGFR (Figures 4 A, B). Western blotting data demonstrated curcumin's inhibitory effect on phosphorylation of JAK2 and phosphorylation of STAT3 but not on total JAK2 and STAT3 protein levels (Figures 4 C–E). These results indicated that the VEGF-mediated JAK2/STAT3 pathway was involved in curcumin's inhibition of angiogenesis.

VEGF attenuates curcumin's effect on the JAK2/STAT3 pathway

To further confirm whether VEGF mediates curcumin inhibition of the STAT3 pathway, 10 ng/ml VEGF was added to the culture medium of HUVEC cells. Western blotting data showed that VEGF activated p-JAK2 and p-STAT3 in HUVEC cells and
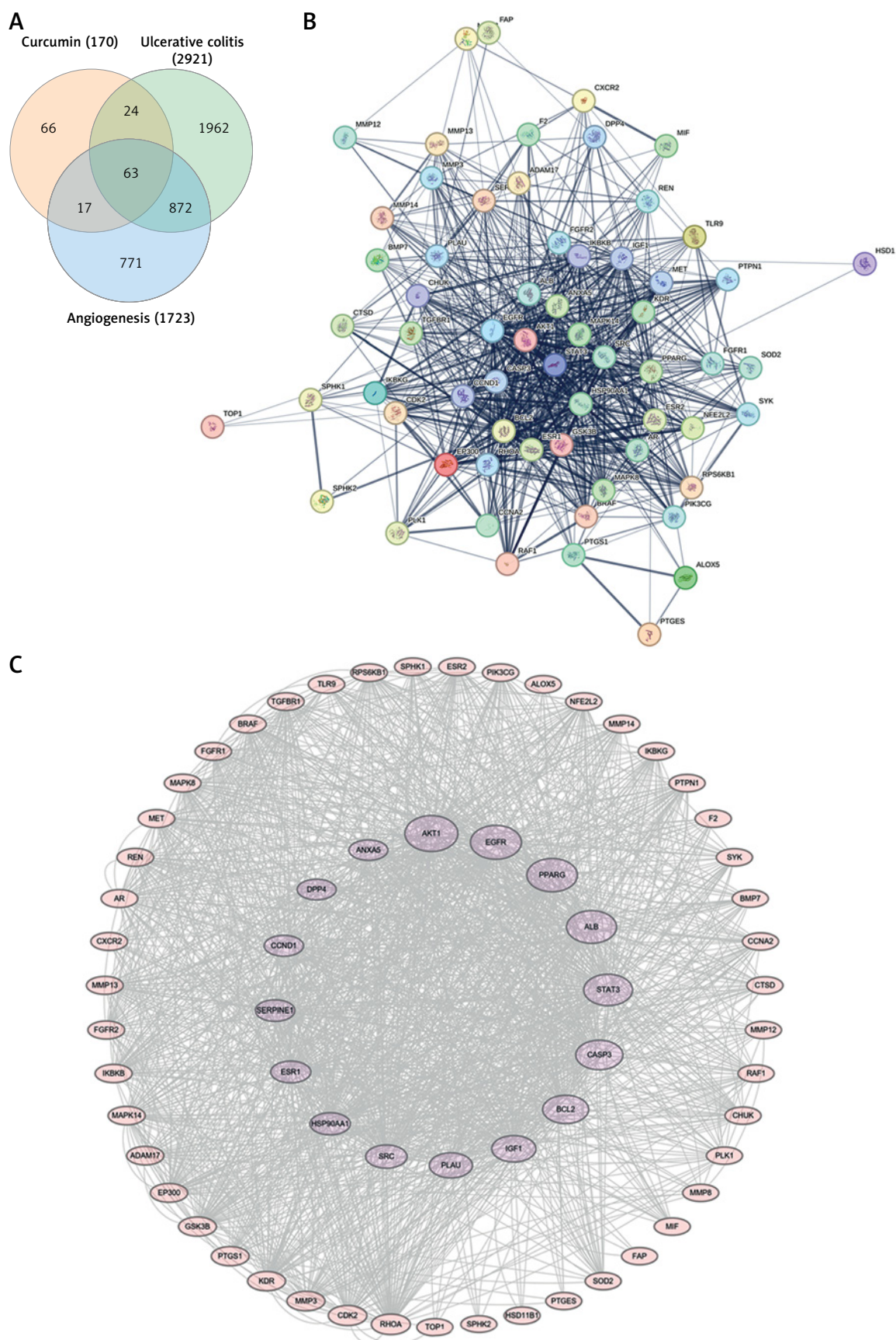
Figure 1. Comprehensive target analysis using multiple databases. Multiple databases were used to comprehensively analyze the possible targets for curcumin, ulcerative colitis, and angiogenesis, then the following diagrams were drawn using the corresponding software in the website: **A** – Venn diagram, **B** – Network diagram of targets, **C** – PPI diagram
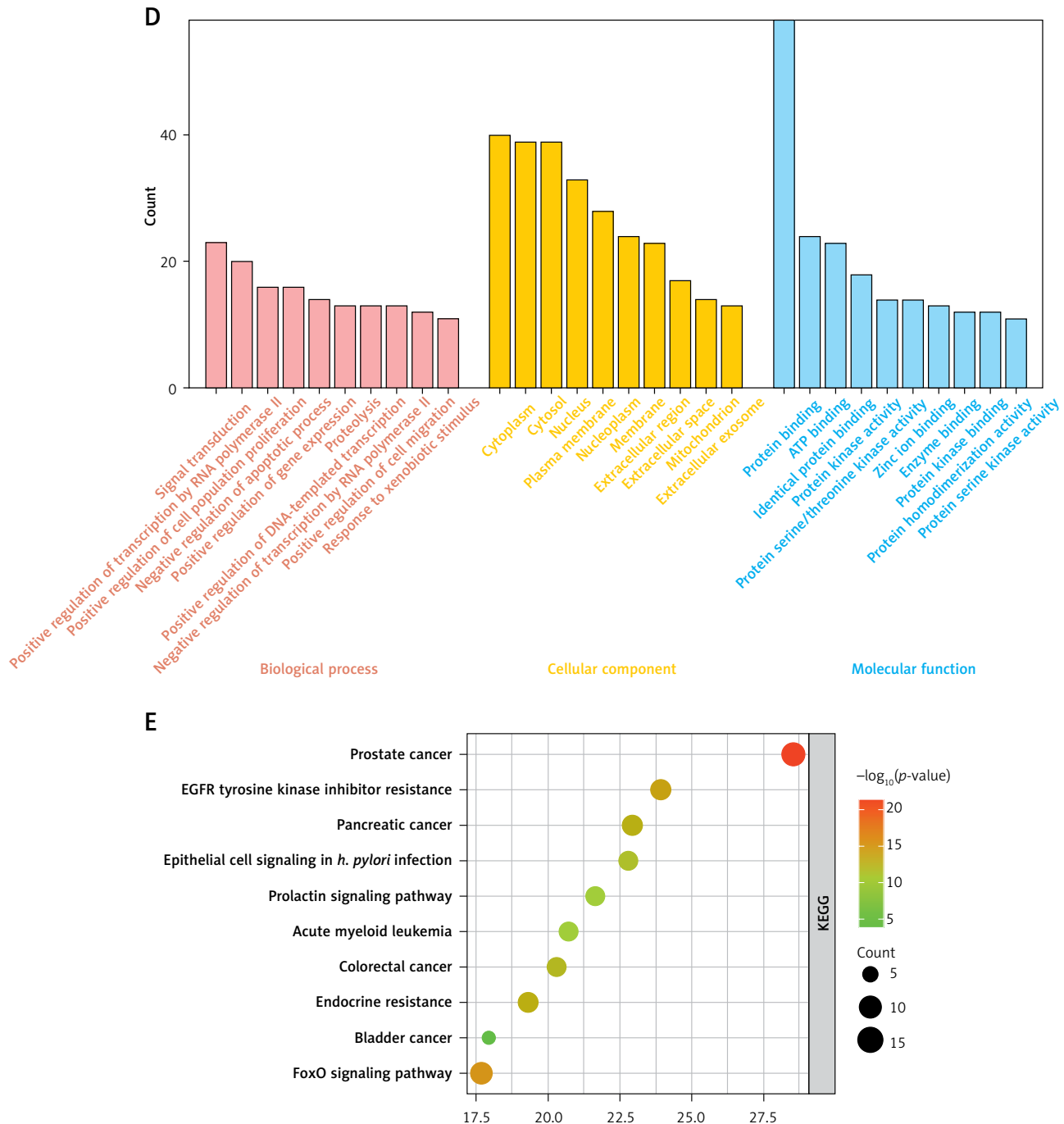
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D

Biological process   Cellular component   Molecular function

E

Figure 1. Cont. **D** – GO analysis, **E** – KEGG analysis

curcumin inhibited p-JAK2 and p-STAT3, whereas the inhibitory effect on p-JAK2 and p-STAT3 was significantly attenuated when VEGF was co-administered (Figure 5). This indicated that VEGF mediated the inhibitory effect of curcumin on the JAK2/STAT3 signaling pathway.

Curcumin inhibits DSS-induced colitis in mice through STAT3-mediated angiogenesis

DSS-induced colitis in mice was established according to the experimental flow diagram (Fig-

ure 6 A). The DSS-treated mice exhibited marked weight loss, diarrhea, bloody stools, and other colitis symptoms. Compared with the blank group, the length of the colon of the model group was shorter (Figure 6 B), and the DAI scores of weight loss, diarrhea, and bloody stool were significantly higher (Figure 6 C). Compared with the control group, curcumin alleviated colon shortening in DSS-induced colitis mice (Figure 6 B). A similar result was achieved in terms of DAI score; curcumin notably reduced the DAI score by improv-
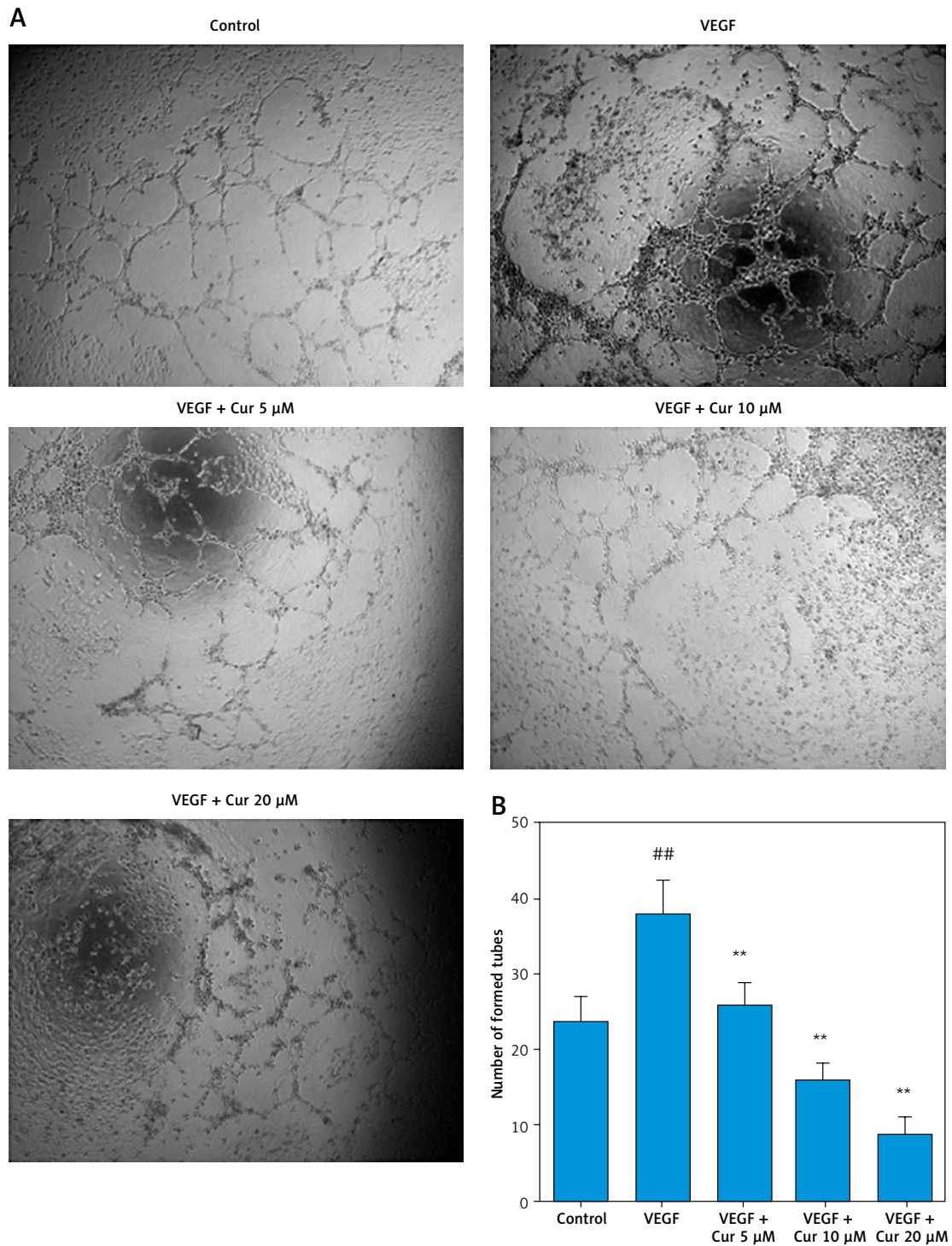
Figure 2. Curcumin suppresses tube formation *in vitro*. HUVECs were treated with curcumin (0–20 µM) *in vitro* in the presence or absence of VEGF (10 ng/ml) for 24 h, then harvested to be seeded in a 96-well plate precoated with 40 µl (10 mg/ml) growth factor-reduced Matrigel (2 × 10⁴ cells/well). After 2–3 h incubation, the capillary tube structures were observed and the images were captured with an inverted phase-contrast microscope. **A** – Representative images (200×) of tube formation. **B** – Tube-like structures were evaluated using ImageJ software. Quantitative data are presented as mean ± SD (*n* = 3). Comparison with control, ##*p* < 0.01; comparison with VEGF group, **$*p* < 0.01

ing the weight loss, diarrhea, and bloody stool of the DSS-induced colitis mice (Figure 6 C). In the serum of mice with ulcerative colitis, TNF-α content was significantly elevated, and curcumin inhibited TNF-α expression in serum (Figure 6 D). Similar results were observed for IL-6 and IL-1β content in serum (Figures 6 E, F). The results of H&E staining indicated that, compared with the
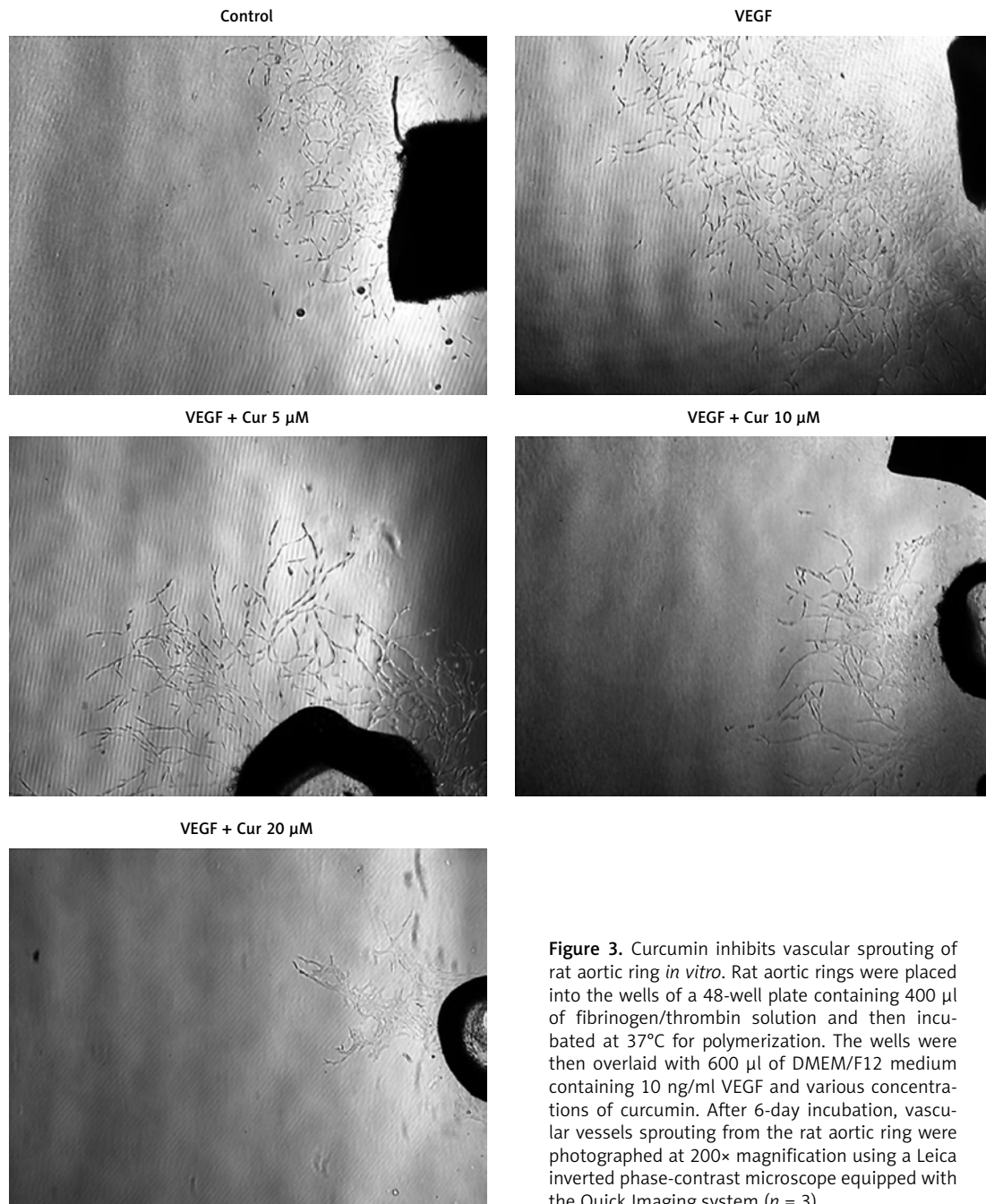
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Control

VEGF

VEGF + Cur 5 µM

VEGF + Cur 10 µM

VEGF + Cur 20 µM

Figure 3. Curcumin inhibits vascular sprouting of rat aortic ring *in vitro*. Rat aortic rings were placed into the wells of a 48-well plate containing 400 µl of fibrinogen/thrombin solution and then incubated at 37°C for polymerization. The wells were then overlaid with 600 µl of DMEM/F12 medium containing 10 ng/ml VEGF and various concentrations of curcumin. After 6-day incubation, vascular vessels sprouting from the rat aortic ring were photographed at 200× magnification using a Leica inverted phase-contrast microscope equipped with the Quick Imaging system (*n* = 3)

blank group, the colon of control group mice exhibited severe mucosal damage, inflammatory cell infiltration, and gland structure damage (Figure 6 G). However, curcumin partially reversed colonic mucosal injury, eliminated inflammatory cell infiltration, and alleviated destruction of glands (Figure 6 G). The further immunohistochemical staining results showed that expression of STAT3 and VEGF was markedly elevated in the tissue of colitis colon, whereas it was decreased in the groups with curcumin treatment (Figures 6 H, I, K, L). Also, CD31 expression analysis suggested that curcumin inhibited colonic angiogenesis in mice (Figures 6 J, M). Overall, the above results suggested that curcumin exerted therapeutic effect on ulcerative colitis and the molecular mechanism was related to inhibition of the VEGF-activated JAK/STAT3 pathway.

Discussion

According to the hypothesis, we firstly used the tools of multiple databases to analyze the possible targets of curcumin against UC, and the top ten proteins – AKT1, EGFR, PPARG, ALB, STAT3, CASP3, BCL2, IGF1, PLAU, SRC – were screened. Among these proteins, STAT3 is closely involved in inflam-
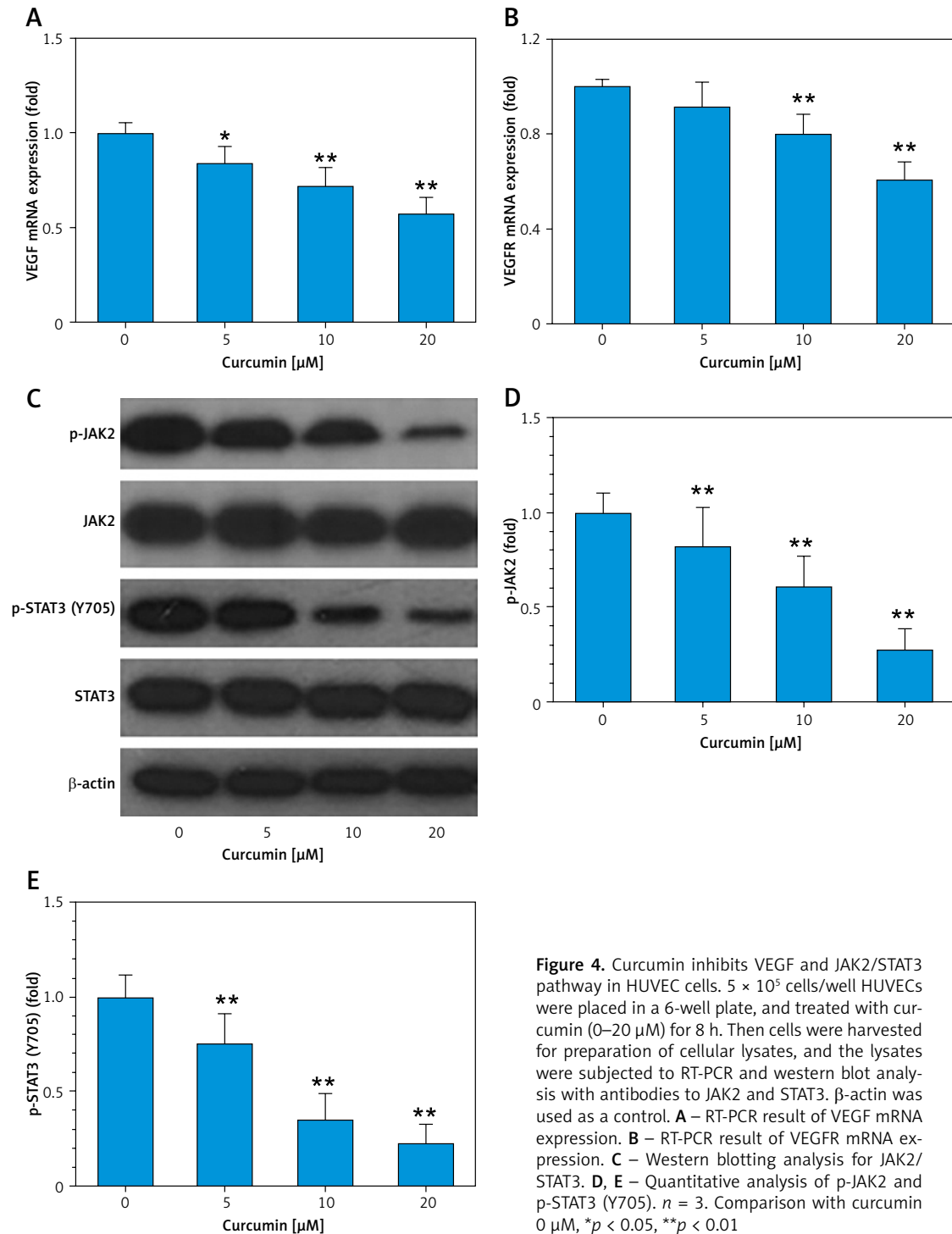
Figure 4. Curcumin inhibits VEGF and JAK2/STAT3 pathway in HUVEC cells. 5×10^5 cells/well HUVECs were placed in a 6-well plate, and treated with curcumin (0–20 μM) for 8 h. Then cells were harvested for preparation of cellular lysates, and the lysates were subjected to RT-PCR and western blot analysis with antibodies to JAK2 and STAT3. β-actin was used as a control. **A** – RT-PCR result of VEGF mRNA expression. **B** – RT-PCR result of VEGFR mRNA expression. **C** – Western blotting analysis for JAK2/STAT3. **D**, **E** – Quantitative analysis of p-JAK2 and p-STAT3 (Y705). $n = 3$. Comparison with curcumin 0 μM, *$p < 0.05$, **$p < 0.01$

mation. Combined with curcumin's potent anti-inflammatory activity [11, 27], STAT3 is regarded as a mediator of angiogenesis regulating UC. Central to angiogenesis regulation is vascular endothelial growth factor (VEGF), a master cytokine that promotes endothelial cell proliferation, migration, and vascular permeability [28]. VEGF-A, the most studied isoform, is upregulated by hypoxia and promotes sprouting angiogenesis by activating receptors such as VEGFR-2, triggering downstream signaling pathways including PI3K/AKT and MAPK [28]. In tumors, VEGF overexpression drives aberrant vascular networks, facilitating metastasis and immune evasion [29].

In UC, chronic inflammation triggers excessive and disorganized blood vessel formation through several interconnected mechanisms [30]. Tissue hypoxia in inflamed mucosa upregulates hypoxia-inducible factor-1α (HIF-1α), leading to VEGF overexpression. VEGF drives immature, hyperper-
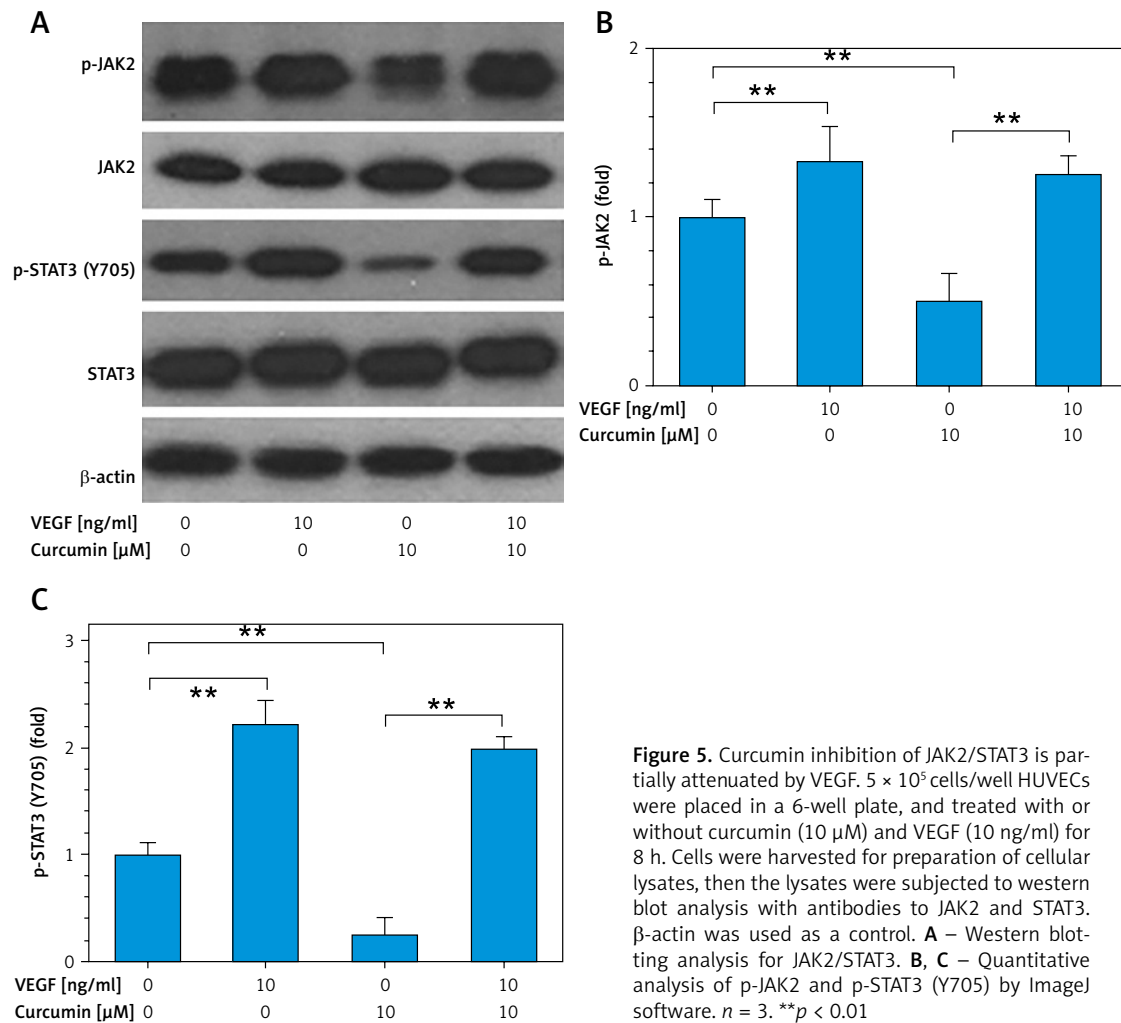
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Figure 5. Curcumin inhibition of JAK2/STAT3 is partially attenuated by VEGF. 5×10^5 cells/well HUVECs were placed in a 6-well plate, and treated with or without curcumin (10 μM) and VEGF (10 ng/ml) for 8 h. Cells were harvested for preparation of cellular lysates, then the lysates were subjected to western blot analysis with antibodies to JAK2 and STAT3. β-actin was used as a control. **A** – Western blotting analysis for JAK2/STAT3. **B**, **C** – Quantitative analysis of p-JAK2 and p-STAT3 (Y705) by ImageJ software. $n = 3$. $**p < 0.01$

meable vessel formation [23]. These vessels exhibit poor barrier function, facilitating leukocyte extravasation and edema, and further aggravating mucosal injury [23]. Notably, VEGF is not merely a bystander; its levels correlate with disease severity and internal inflammation [31].

Recent studies highlighted the interplay between VEGF and STAT3, a transcription factor activated by reactive oxygen species (ROS) or cytokine signaling [32]. ROS-mediated STAT3 activation enhances VEGF expression, forming a feedforward loop that sustains angiogenesis [33]. For instance, 27-hydroxycholesterol (27HC), a tumor-promoting metabolite, induces STAT3 phosphorylation via ROS, leading to VEGF autocrine signaling and endothelial recruitment in both estrogen receptor-positive and -negative cancers [33]. Inhibiting STAT3 or ROS effectively suppresses VEGF-driven angiogenesis, underscoring their therapeutic potential [34]. Together, VEGF and STAT3 represent pivotal targets for modulating angiogenesis in diseases, with ongoing research exploring dual-pathway inhibitors to combat pathological vascularization. Thus,

targeting pathological angiogenesis offers novel avenues for UC treatment.

The present study demonstrated that curcumin improved DSS-induced UC in mice. Further results showed that the therapeutic effects of curcumin on UC are related to inhibition of angiogenesis. Among the potential mechanisms, inhibition of the VEGF-mediated JAK2/STAT3 pathway was demonstrated to be involved in this process. Curcumin can inhibit several types of cancers [35, 36] and improve acute renal injury [37] and liver injury [38] via inhibition of the JAK2/STAT3 pathway. These findings suggest that JAK2/STAT3 is a key regulatory molecule mediating the pharmacologic activities of curcumin.

Aberrant angiogenesis is not merely a secondary phenomenon but a central pathological pillar in UC, driving ischemia, inflammation, and impaired tissue repair. Targeting specific pathways through localized VEGF inhibition holds transformative potential. Future therapies should aim to normalize vascular function rather than indiscriminately inhibit angiogenesis, thereby promoting true mucosal healing. Combining these

vascular-targeted strategies with conventional immunomodulation offers a promising roadmap for overcoming therapeutic resistance in complex UC cases. Furthermore, based on its extensive activity, we inferred that curcumin likely combated UC by synergistically modulating inflammation, oxidative stress, immune responses, and microbiota dysbiosis. Simultaneously targeting multiple proteins related to UC may achieve a better curative effect than a single protein target.
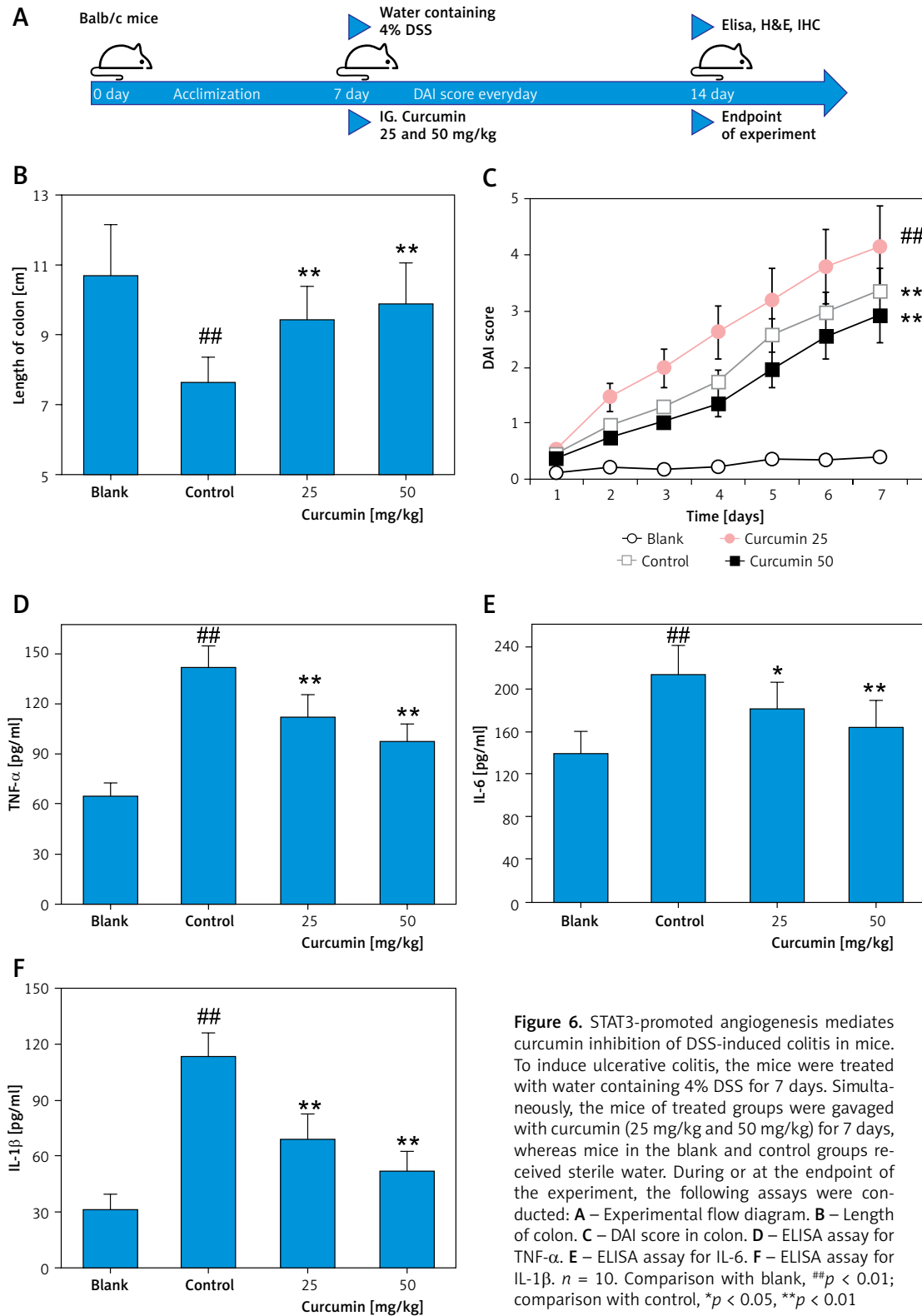

Figure 6. STAT3-promoted angiogenesis mediates curcumin inhibition of DSS-induced colitis in mice. To induce ulcerative colitis, the mice were treated with water containing 4% DSS for 7 days. Simultaneously, the mice of treated groups were gavaged with curcumin (25 mg/kg and 50 mg/kg) for 7 days, whereas mice in the blank and control groups received sterile water. During or at the endpoint of the experiment, the following assays were conducted: **A** – Experimental flow diagram. **B** – Length of colon. **C** – DAI score in colon. **D** – ELISA assay for TNF-α. **E** – ELISA assay for IL-6. **F** – ELISA assay for IL-1β. $n = 10$. Comparison with blank, $^{\#\#}p < 0.01$; comparison with control, $^*p < 0.05$, $^{**}p < 0.01$

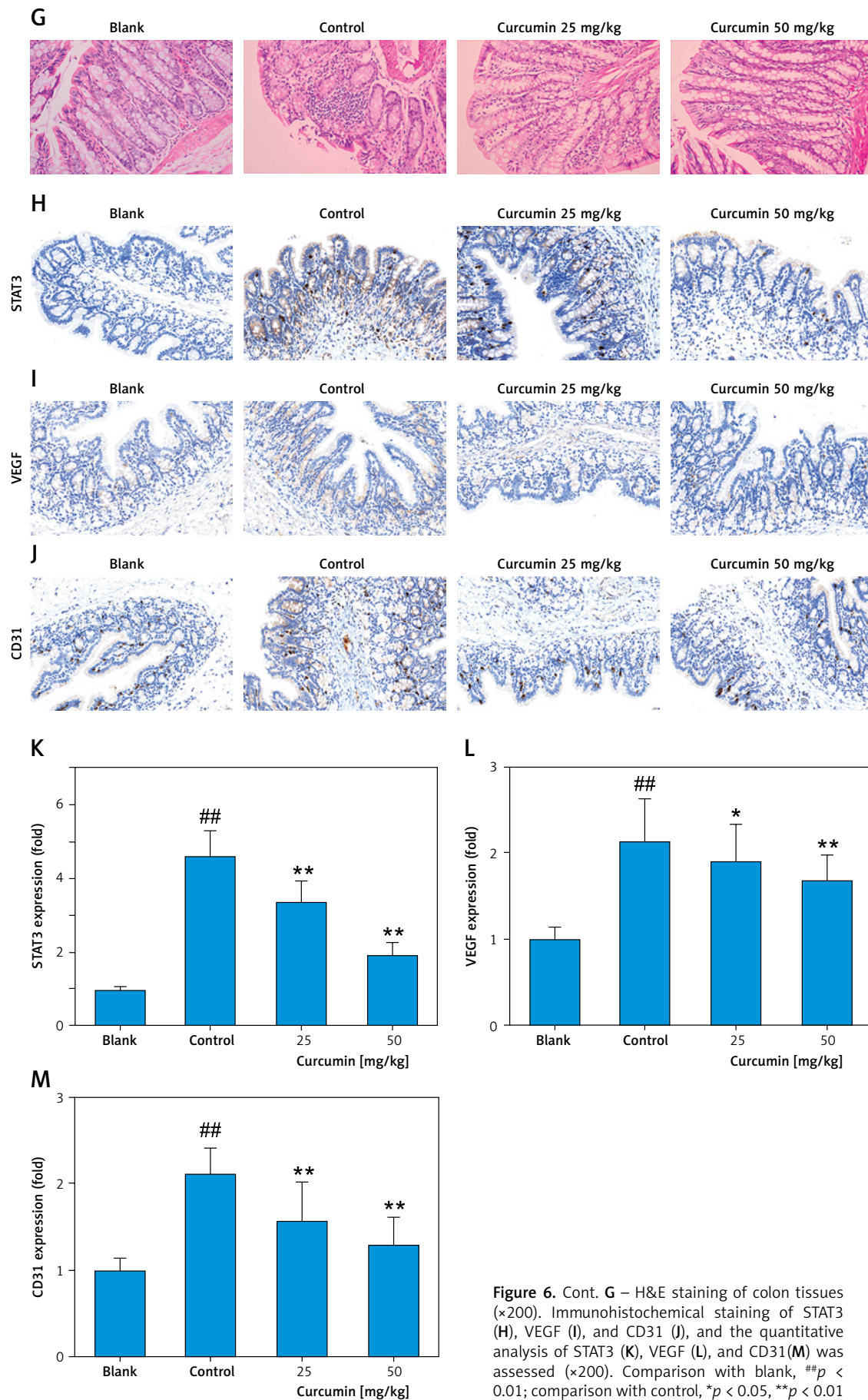Su Xu, Zixuan Zhou, Aoxun Zhu, Yurong Guo, Shengnan Jin, Maozhen Zhang, Yanping Hao

Figure 6. Cont. **G** – H&E staining of colon tissues (×200). Immunohistochemical staining of STAT3 (**H**), VEGF (**I**), and CD31 (**J**), and the quantitative analysis of STAT3 (**K**), VEGF (**L**), and CD31(**M**) was assessed (×200). Comparison with blank, $^{\#\#}p <$ 0.01; comparison with control, $^{*}p < 0.05$, $^{**}p < 0.01$

Ethical approval

This study was approved by the Yancheng TCM Hospital affiliated with Nanjing University of Chinese Medicine Institutional Animal Care and Use Committee (SLDW231201).

Conflict of interest

The authors declare no conflict of interest.

References

1. Berre CL, Honap S, Peyrin-Biroulet L. Ulcerative colitis. Lancet 2023; 402: 571-84.
2. Wangchuk P, Yeshi K, Loukas A. Ulcerative colitis: clinical biomarkers, therapeutic targets, and emerging treatments. Trends Pharmacol Sci 2024; 45: 892-903.
3. Du L, Ha C. Epidemiology and pathogenesis of ulcerative colitis. Gastroenterol Clin North Am 2020; 49: 643-54.
4. Jeong JH, Ojha U, Lee YM. Pathological angiogenesis and inflammation in tissues. Arch Pharmacal Res 2021; 44: 1-15.
5. Szade A, Grochot-Przeczek A, Florczyk U, Jozkowicz A, Dulak J. Cellular and molecular mechanisms of inflammation-induced angiogenesis. IUBMB Life 2015; 67: 145-59.
6. Liang Y, Li Y, Lee C, Yu Z, Chen C, Liang C. Ulcerative colitis: molecular insights and intervention therapy. Mol Biomed 2024; 5: 42.
7. Stavsky J, Maitra R. The synergistic role of diet and exercise in the prevention, pathogenesis, and management of ulcerative colitis: an underlying metabolic mechanism. Nutr Metab Insights 2019; 12: 1178638819834526.
8. Ismail AMA, Morsy MM. Effect of baduanjin exercise on lipid profile, blood pressure, and thyroid-stimulating hormone in elderly with subclinical hypothyroidism and mild cognitive impairment: a randomized-controlled trial in women. Geriatr Nurs 2025; 64: 103434.
9. Kotha RR, Luthria DL. Curcumin: biological, pharmaceutical, nutraceutical, and analytical aspects. Molecules 2019; 24: 2930.
10. Weng W, Goel A. Curcumin and colorectal cancer: an update and current perspective on this natural medicine. Semin Cancer Biol 2022; 80: 73-86.
11. Peng Y, Ao M, Dong B, et al. Anti-inflammatory effects of curcumin in the inflammatory diseases: status, limitations and countermeasures. Drug Des Dev Ther 2021; 15: 4503-25.
12. Talpan D, Salla S, Seidelmann N, Walter P, Fuest M. Anti-fibrotic effects of caffeine, curcumin and pirfenidone in primary human keratocytes. Int J Mol Sci 2023; 24: 1461.
13. Wang Y, Tang Q, Duan P, Yang L. Curcumin as a therapeutic agent for blocking NF-κB activation in ulcerative colitis. Immunopharmacol Immunotoxicol 2018; 40: 476-82.
14. Afrin R, Arumugam S, Rahman A, et al. Curcumin ameliorates liver damage and progression of NASH in NASH-HCC mouse model possibly by modulating HMGB1-NF-κB translocation. Int Immunopharmacol 2017; 44: 174-82.
15. Guo J, Zhang YY, Sun M, Xu LF. Therapeutic potential of curcumin in a rat model of dextran sulfate sodium-induced ulcerative colitis by regulating the balance of treg/Th17 cells. Inflammation 2022; 45: 2163-71.
16. Wang Z, Zheng J, Yang S, et al. Curcumin prevents the arsenic-induced neuroimmune injury through JAK2/STAT3 pathway. Chin J Cell Mol Immunol 2024; 40: 1067-74.
17. Zhang X, Zhang H, Wang J, et al. Curcumin attenuates ulcerative colitis via regulation of sphingosine kinases 1/NF-κB signaling pathway. Biofactors 2025; 51: e70001.
18. Yang M, Wang J, Yang C, Han H, Rong W, Zhang G. Oral administration of curcumin attenuates visceral hyperalgesia through inhibiting phosphorylation of TRPV1 in rat model of ulcerative colitis. Mol Pain 2017; 13: 1744806917726416.
19. Rogers NM, Kireta S, Coates PTH. Curcumin induces maturation-arrested dendritic cells that expand regulatory T cells in vitro and in vivo. Clin Exp Immunol 2010; 162: 460-73.
20. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci 2020; 77: 1745-70.
21. Otrock ZK, Mahfouz RAR, Makarem JA, Shamseddine AI. Understanding the biology of angiogenesis: review of the most important molecular mechanisms. Blood Cells Mol Dis 2007; 39: 212-20.
22. Lopes-Coelho F, Martins F, Pereira SA, Serpa J. Anti-angiogenic therapy: current challenges and future perspectives. Int J Mol Sci 2021; 22: 3765.
23. Li Z, Zeng L, Huang W, Zhang X, Zhang L, Xie Q. Angiogenic factors and inflammatory bowel diseases. Biomedicines 2025; 13: 1154.
24. Zhang L, Wei W, Ai X, et al. Extracellular vesicles from hypoxia-preconditioned microglia promote angiogenesis and repress apoptosis in stroke mice via the TGF-β/Smad2/3 pathway. Cell Death Dis 2021; 12: 1068.
25. Aplin AC, Nicosia RF. The rat aortic ring model of angiogenesis. Methods Mol Biol 2015; 1214: 255-64.
26. Hao L, Alkry LT, Alattar A, et al. Ibrutinib attenuated DSS-induced ulcerative colitis, oxidative stress, and the inflammatory cascade by modulating the PI3K/akt and JNK/NF-κB pathways. Arch Med Sci 2022; 18: 805-15.
27. Kou H, Huang L, Jin M, He Q, Zhang R, Ma J. Effect of curcumin on rheumatoid arthritis: a systematic review and meta-analysis. Front Immunol 2023; 14: 1121655.
28. Ahmad A, Nawaz MI. Molecular mechanism of VEGF and its role in pathological angiogenesis. J Cell Biochem 2022; 123: 1938-65.
29. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat Rev Clin Oncol 2018; 15: 325-40.
30. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. Immunity 2019; 51: 27-41.

31. Scaldaferri F, Vetrano S, Sans M, et al. VEGF-a links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology 2009; 136: 585-95.

32. Hu C, Wu Z, Huang Z, et al. Nox2 impairs VEGF-a-induced angiogenesis in placenta via mitochondrial ROS-STAT3 pathway. Redox Biol 2021; 45: 102051.

33. Zhu D, Shen Z, Liu J, et al. The ROS-mediated activation of STAT-3/VEGF signaling is involved in the 27-hydroxycholesterol-induced angiogenesis in human breast cancer cells. Toxicol Lett 2016; 264: 79-86.

34. Wang L, Astone M, Alam SK, et al. Suppressing STAT3 activity protects the endothelial barrier from VEGF-mediated vascular permeability. Dis Models Mech 2021; 14: dmm049029.

35. Hu Y, Zhou N, Zhu Q. Curcumin inhibits proliferation and invasion of papillary thyroid carcinoma cells by inhibiting the JAK2 / STAT3 pathway. J BUON 2021; 26: 1635-41.

36. Sun Y, Liu L, Wang Y, He A, Hu H, Zhang J, et al. Curcumin inhibits the proliferation and invasion of MG-63 cells through inactivation of the p-JAK2/p-STAT3 pathway. OncoTargets Ther 2019; 12: 2011-21.

37. Zhu S, Zhang C, Weng Q, Ye B. Curcumin protects against acute renal injury by suppressing JAK2/STAT3 pathway in severe acute pancreatitis in rats. Exp Ther Med 2017; 14: 1669-74.

38. Jiang Z, Liu L, Su H, et al. Curcumin and analogues in mitigating liver injury and disease consequences: from molecular mechanisms to clinical perspectives. Phytomedicine 2024; 123: 155234.