

Astragalus membranaceus and Salvia miltiorrhiza Inhibit Tryptase/PAR2-Mediated Mast Cells – Peritoneal Mesothelial Cells Crosstalk to Ameliorate Peritoneal Fibrosis

Yanlin Chen^{1,2}, Yuqing Wu^{1,2}, Chenghua Zhang³, Yuxin Tang^{1,2}, Weiwei Zhu¹, Wei Li^{1,2}, Kun Gao^{1,4}, Enchao Zhou^{1,2,4}, Xufang Wang^{1,4}

¹Department of Nephrology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, People's Republic of China; ²Department of Clinical Medicine, The First School of Clinical Medicine, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, People's Republic of China; ³Department of Nephrology, Changzhou Hospital of Chinese Medicine, Changzhou, Jiangsu, People's Republic of China; ⁴Department of Nephrology, Jiangsu University Key Laboratory of Tonifying Kidney and Anti-Senescence, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, People's Republic of China

Correspondence: Xufang Wang; Enchao Zhou, Department of Nephrology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, People's Republic of China, Email arnold1212@163.com; zhouchao@njucm.edu.cn

Purpose: Mast cells (MCs) act as sentinels of the immune system and are involved in peritoneal fibrosis (PF), but the precise mechanism remains unclear. To investigate this, we focused on *Astragalus membranaceus* and *Salvia miltiorrhiza* (AS), a combination known for its anti-inflammatory properties. This study aimed to elucidate the effect of AS on MCs and its potential to alleviate PF.

Methods: The major chemical components of AS were identified by UPLC-Q-TOF-MS/MS. Subsequently, we employed network pharmacology, molecular docking, and molecular dynamics simulations to investigate the key targets and mechanisms of AS in the treatment of PF. In vivo, a mouse PF model was established by daily intraperitoneal injection of high-glucose (4.25%) peritoneal dialysis fluid (PDF) for 4 weeks. Following AS treatment, the mechanism was explored through transcriptomics analysis, immunohistochemistry (IHC), immunofluorescence (IF), and Western blotting (WB). In vitro, an activated MC model was established by stimulating murine P815 mast cells with LPS. The activation of P815 cells was assessed using transcriptomics analysis, ELISA, and transmission electron microscopy (TEM). Finally, a fibrosis model was further generated by stimulating human peritoneal mesothelial cells (HMrSV5) with tryptase, and the effects of AS were assessed by WB.

Results: UPLC-Q-TOF-MS/MS identified 40 active components in AS. Network pharmacology and molecular docking indicated favorable predicted binding affinities of the key constituents calycosin and tanshinone IIA to MAPK1 and NF-κB1, with binding energies ranging from -9.1 to -6.8 kcal/mol. In vivo experiments demonstrated that high-glucose PDF increased peritoneal tryptase levels by approximately 3.2-fold, whereas AS treatment reduced tryptase release by 71.5%, respectively (baseline-corrected). Correspondingly, AS significantly inhibited MC degranulation and suppressed activation of the PAR2/MAPK/NF-κB pathway. In vitro, AS decreased LPS-induced tryptase and TNF-α secretion in P815 cells by 71.6% and 58.2%, respectively, and attenuated tryptase-induced fibrosis in HMrSV5 cells, accompanied by reduced phosphorylation of MAPK/NF-κB signaling proteins.

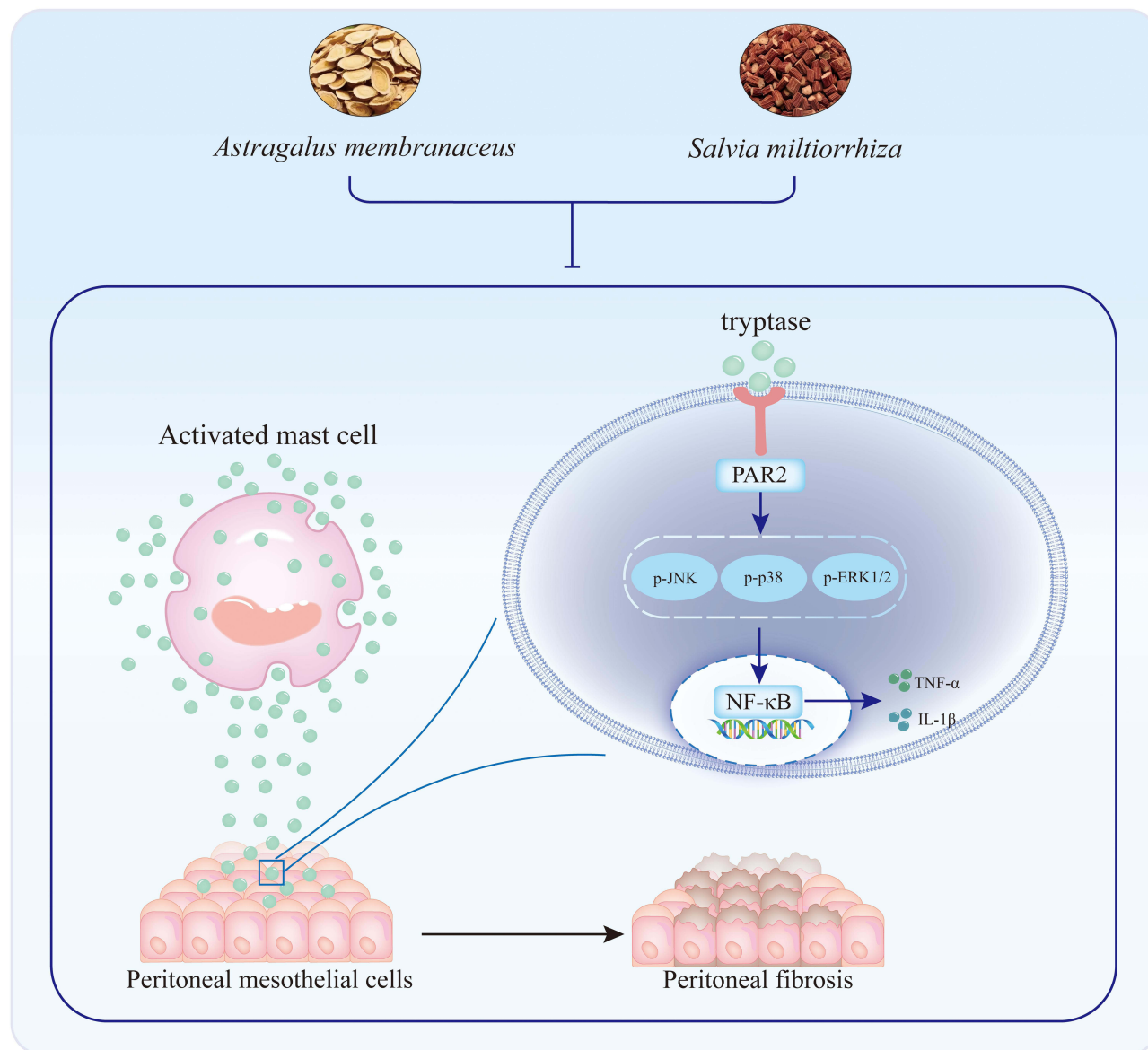
Conclusion: This study demonstrates that the MC tryptase/PAR2 signaling axis contributes to PDF-induced peritoneal fibrotic responses. AS alleviates PF at least in part by inhibiting tryptase-mediated MC-PMC crosstalk and downstream PAR2/MAPK/NF-κB activation.

Keywords: *Astragalus membranaceus* and *Salvia miltiorrhiza*, mast cells, peritoneal mesothelial cells, peritoneal fibrosis, protease-activated receptor 2, tryptase

Introduction

Peritoneal dialysis (PD) is a widely used long-term renal replacement therapy for patients with end-stage renal disease (ESRD). However, patients undergoing PD often experience a shorter duration of therapy compared to those undergoing

Graphical Abstract



hemodialysis (HD). The primary reasons for discontinuation of PD include a high risk of infections, inadequate clearance of small molecules, and structural changes in the peritoneum. Among these, peritoneal fibrosis (PF) is the leading cause of ultrafiltration failure and PD discontinuation.¹ Previous research has identified key factors in the development of PF, including mesothelial-to-mesenchymal transition (MMT) of peritoneal mesothelial cells (PMCs), chronic inflammation, and angiogenesis.² Exposure to high-glucose PD solutions induces inflammatory cell activation results in the secretion of pro-inflammatory cytokines, interleukins, and fibrotic mediators, which are implicated in chronic peritoneal inflammation, and the MMT of PMCs. Nonetheless, the precise contributions of specific inflammatory cell subsets to PF progression remain incompletely characterized.

Escherichia coli is a leading pathogen in peritoneal dialysis-associated peritonitis in Southern China.³ Lipopolysaccharide (LPS), a major component of the outer membrane of *E. coli*, is released upon bacterial death or lysis and interacts with peritoneal tissues.⁴ Combined exposure to LPS and high glucose can induce injury in PMCs, leading to reduced viability,

disruption of intercellular junctions, and ultimately the initiation of MMT.⁵ Furthermore, the co-administration of LPS and high-glucose PDF promotes peritoneal neovascularization.⁵ These factors collectively contribute to the development of PF. Additionally, LPS is a known activator of mast cells (MCs).⁶ Studies have demonstrated that LPS can disrupt intestinal mucosal integrity via MC activation, thereby mediating damage to the intestinal barrier; notably, this effect on intestinal permeability is abolished in MC-deficient models.⁷ LPS has also been shown to induce neuroinflammation through activated MCs.⁸ However, whether and how MCs contribute mechanistically to LPS-associated peritoneal fibrotic responses remains to be fully defined.

MC accumulation and infiltration in the peritoneum of PD patients have been increasingly recognized in recent reviews, with suggested contributions to both inflammatory amplification and fibrotic remodeling.^{9,10} Consistently, elevated MC numbers have also been observed in the fibrotic peritoneum of 5/6 nephrectomy rats.¹¹ Previous studies have demonstrated that LPS and interleukin-33 (IL-33) activate MCs, leading to the release of proteases such as trypsin and cytokines including tumor necrosis factor- α (TNF- α). MC-derived proteases and cytokines have been implicated in renal fibrosis and intestinal barrier disruption. Cromolyn (Cro), a classical MC stabilizer, can suppress MC-induced pro-inflammatory cytokine production and has been reported to attenuate MC-associated neuroinflammation and cognitive impairment.⁸ Collectively, these findings support a pathogenic role of MC activation in diverse inflammatory and fibrotic conditions; however, the pathway-specific contribution of MCs to PDF-associated PF remains insufficiently defined.

MC-derived trypsin has been demonstrated to activate protease-activated receptor 2 (PAR2).¹² As a member of the G protein-coupled receptor family, PAR2 has been extensively studied for its role in inflammation and fibrosis. PAR2 activation promotes renal interstitial fibrosis through the mitogen-activated protein kinase (MAPK)–nuclear factor kappa B (NF- κ B) signaling pathway and increases mesenchymal marker expression in renal epithelial cells.¹³ These findings underscore the critical role of PAR2 in fibrotic mechanisms. In addition, the selective PAR2 antagonist N1-3-methylbutyryl-N4-6-aminohexanoyl-piperazine (ENMD-1068) has been reported to inhibit PAR2 activation, reduce collagen expression, and attenuate hepatic stellate cell activation by blocking transforming growth factor- β 1 (TGF- β 1)/Smad signaling.¹⁴

According to TCM theory, long-term PD is often associated with systemic imbalance related to chronic disease progression. PF is increasingly recognized as a multifactorial process involving inflammation, fibrosis, and barrier dysfunction, for which multi-target therapeutic strategies are considered promising. *Astragalus membranaceus* and *Salvia miltiorrhiza* are frequently used in combination due to their complementary anti-inflammatory and anti-fibrotic properties. Previous studies have shown that *Astragalus membranaceus* and its active extract astragaloside IV can inhibit PF by regulating the Janus Kinase (JAK)/signal transducer and activator of 107 transcription (STAT) pathway and attenuating macrophage-derived exosomal activity.^{15,16} Calycosin, a major isoflavone constituent of *Astragalus membranaceus*, has also been reported to suppress PF through inhibition of the AR/TGF- β 1 pathway.¹⁷ *Salvia miltiorrhiza* exhibits protective effects in PF through multiple mechanisms; its extracts have been shown to inhibit PMC MMT via modulation of the TGF- β /Smad pathway,¹⁸ while sodium tanshinone IIA protects PMCs from oxidative injury by suppressing the ASK1-p38 pathway.¹⁹ In addition, salvianolic acid A ameliorates PF by targeting GSK3 β -regulated Nrf2 and NF- κ B signaling.²⁰

In recent years, multi-target herbal formulations have emerged as a promising strategy for complex fibrotic diseases such as PF. Although substantial evidence supports the anti-inflammatory and anti-fibrotic activities of *Astragalus membranaceus* and *Salvia miltiorrhiza* individually, the synergistic mechanisms of their combination (AS) in regulating MMT and PF remain insufficiently explored.

Materials and Methods

Chemicals and Reagents

Cromolyn (HY-B1619) and ENMD-1068 (HY-124748) were acquired from MedChem Express (USA). LPS (L2880) was purchased from Sigma-Aldrich (USA), and trypsin was obtained from Novoprotein (Suzhou, China). Antibodies against phospho-p38 MAPK (Thr180/Tyr182) (4511), p38 MAPK (8690), phospho-p44/42 MAPK (extracellular signal-regulated kinase 1/2, ERK1/2) (Thr202/Tyr204) (4370), ERK1/2 (4695), phospho-NF- κ B p65 (3033), NF- κ B p65 (8242), phospho-SAPK/JNK (Thr183/Tyr185) (4668), trypsin (19523), and HRP-conjugated anti-mouse or anti-rabbit secondary antibodies were purchased from Cell Signaling Technology (USA). Antibodies against PAR2 (ab180953), phospho-Smad3

(S423/S425) (ab52903), Zonula occludens-1 (ZO-1) (ab221547), and collagen I (ab270993) were purchased from Abcam (UK). Antibodies against fibronectin (15613-1-AP), vimentin (22031-1-AP), E-cadherin (20874-1-AP), α -SMA (14395-1-AP), vascular endothelial growth factor-A (VEGFA) (19,003-1-AP), TGF- β 1 (21,898-1-AP), JNK (51153-1-AP), and GAPDH (60004-1-Ig) were purchased from Proteintech (Wuhan, China).

Preparation of AS Extract

The herbal materials, consisting of 30 g *Astragalus membranaceus* and 15 g *Salvia miltiorrhiza*, were obtained from the Chinese medicine pharmacy at the Affiliated Hospital of Nanjing University of Chinese Medicine and met Chinese Pharmacopoeia quality standards. Botanical identity was verified by a licensed pharmacognosist, and all herbs were sourced from a single batch to minimize variability. Batch numbers are listed in [Supplementary Table 1](#).

Briefly, the *Astragalus membranaceus* and *Salvia miltiorrhiza* at a 2:1 ratio were soaked in water (3 cm above the herbs) for 1 hour, boiled for 60 minutes, and filtered through gauze to obtain the whole decoction. Next, the filtered decoction underwent centrifugation at 4000 $\times$ g for 20 minutes at 4°C, followed by a subsequent centrifugation of the resultant supernatant at 8000 $\times$ g for 20 minutes at 4°C. The final supernatant was gathered and underwent freeze-drying. Finally, the collected supernatant was pre frozen at -80°C for 24 hours and subsequently vacuum-dried using a Freezone-12L freeze-dryer (Labcon, USA) for 48 hours, resulting in the production of AS freeze-dried powder.

The mouse dosage was estimated using the traditional body surface area (BSA) conversion coefficient (9.1), a method commonly applied in pharmacological experimental methodology for herbal medicine studies. The human clinical dose is 45 g (30 g *Astragalus membranaceus* and 15 g *Salvia miltiorrhiza*) per 60 kg body weight. The equivalent mouse dose was calculated as follows: 45 g/60 kg $\times$ 9.1 = 6.82 g/kg. This value was defined as the high-dose AS group, and a half dose (3.41 g/kg) was used as the low-dose group.

The selected dose range is consistent with previously reported gram-per-kilogram dosing of TCM formulas in animal models of PF and other fibrotic or inflammatory diseases.^{21,22} During treatment, mice were monitored for body weight, and no overt signs of toxicity or treatment-related mortality were observed.

UPLC-Q-TOF-MS/MS Analysis of AS Extract Components

The primary constituents of AS were identified using a 5600 QTOF mass spectrometer (AB Sciex, USA) coupled with an ACQUITY UPLC H-Class system (Waters, USA) and an AcclaimTM RSLC 120 C18 column (2.1 $\times$ 100 mm, 1.8 μ m; Thermo Fisher Scientific, USA). The column temperature was maintained at 40 °C. Mobile phase A consisted of 0.1% formic acid in water (v/v), while mobile phase B was acetonitrile. The gradient elution program was set as follows: 5% B (0–1.5 min), 5–10% B (1.5–2.5 min), 10–40% B (2.5–14.0 min), 40–95% B (14.0–24.0 min), 95% B (24.0–27.0 min), 95–5% B (27.0–27.1 min), and 5% B (27.1–30.0 min). The injection volume was 4 μ L at a flow rate of 0.4 mL/min. Mass spectrometry was performed in both positive and negative ion modes under the following conditions: ion source temperature, 550 °C; nebulizing gas (GS1), 60 psi; auxiliary gas (GS2), 60 psi; curtain gas, 35 psi; ion spray voltage, $\pm$ 5500 V; and scan range, m/z 50–1500.

Network Pharmacology Analysis

The active ingredients of AS were detected and identified using UPLC-Q-TOF-MS/MS, yielding a total of 40 bioactive compounds. Potential targets of these components were retrieved from four databases: TCMSP, SwissTargetPrediction, TargetNet, and SEA. The collected target information was standardized and annotated using the UniProt database to obtain the final set of AS-related targets. Subsequently, “peritoneal fibrosis” was used as a search term to collect disease-associated targets from multiple databases, including OMIM, GeneCards, PharmGKB, DrugBank, TTD, and DisGeNET. After integration and deduplication, the final disease target set was obtained.

To further analyze interactions between drug and disease targets, a drug–active ingredient–target network was constructed and visualized using Cytoscape (version 3.10.2). The intersecting targets were imported into the STRING database to generate a protein-protein interaction (PPI) network and then re-imported into Cytoscape for topological analysis. Targets were ranked by degree value, and the top 20 were selected as core targets.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed on the intersecting targets using R (version 4.2.1) with the packages *clusterProfiler*, *org.Hs.egdb*, and *ggplot2* to identify relevant biological functions and signaling pathways.

Molecular Docking Analysis

The 2D structures of small-molecule ligands were obtained from the PubChem database and imported into ChemOffice to generate their 3D conformations, which were saved in mol2 format. Crystal structures of target proteins with high resolution were retrieved from the RCSB PDB database and used as receptors for molecular docking. Protein structures were processed using PyMOL to remove water molecules, phosphate groups, and other non-essential components, and the cleaned structures were saved in PDB format.

Molecular docking was performed using AutoDock Vina (version 1.2.3) to evaluate ligand-protein interactions. Both protein and ligand structures were prepared using AutoDock Tools (MGLTools). The optimal binding conformation was determined based on the lowest binding energy score. Docking results were visualized using Discovery Studio 2019.

Molecular Dynamics Analysis

All molecular dynamics (MD) simulations were performed using GROMACS 2022. The receptor protein and ligand were parameterized with the AMBER14SB and GAFF2 force fields, respectively, using *pdb2gmx* and the AutoFF server. The system was solvated in a TIP3P water box with 1 nm padding and neutralized by the addition of counterions. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method with a 1 nm cutoff. All bonds were constrained using the LINCS algorithm. Integration was performed using the leap-frog Verlet scheme with a 1 fs timestep.

Energy minimization consisted of 3000 steps of steepest descent followed by 2000 steps of conjugate gradient, with sequential restraints applied to the solute, ions, and the full system. Production simulations were conducted in the NPT ensemble at 310 K for 100 ns. Trajectories were analyzed using built-in GROMACS tools (*rms*, *rmsf*, *hbond*, *gyrate*, and *sasa*) to calculate the root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bonds, radius of gyration (Rg), and solvent-accessible surface area (SASA).

Animal Experiment

Male C57BL/6 mice, eight weeks old and weighing 20–25 g, were acquired from Charles River Laboratories (license number SCXK (Zhe) 2024–001, Beijing). The animals were maintained under conventional settings, featuring a 12-hour light/dark cycle, with free access to food and water. The experimental protocol was approved by the Experimental Animal Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (approval number: 2024DW-029-01) and performed in compliance with the ARRIVE guidelines.

All mice had a one-week acclimatization period with adaptive feeding prior to the study. Subsequently, they were randomly allocated into six groups ($n = 6$ per group). (1) Control (Ctrl) group: normal saline (NS) intraperitoneal injection (i.p.) + NS intragastric administration (ig.); (2) PDF group: high-glucose PDF (0.01 mL/kg/day) (4.25%, Baxter) i.p. + NS ig.; (3) AS-L group: high-glucose PDF (0.01 mL/kg/day) i.p. + AS (3.41 g/kg/day) ig.; (4) AS-H group: high-glucose PDF (0.01 mL/kg/day) i.p. + AS (6.82 g/kg/day) ig.; (5) Cro group: high-glucose PDF (0.01 mL/kg/day) i.p. + Cro (75 mg/kg, three times per week) i.p.; (6) ENMD group: high-glucose PDF (0.01 mL/kg/day) i.p. + ENMD-1068 (25 mg/kg, twice per week) i.p.

After four weeks, mice were euthanized by intraperitoneal injection of an overdose of sodium pentobarbital (60 mg/mL) at a volume of 0.067–0.083 mL according to body weight (20–25 g). Death was confirmed after at least one minute by cessation of respiration, followed by cervical dislocation. Peritoneal tissues and serum samples were then collected for subsequent analyses.

Toluidine Blue Staining

Paraffin-embedded peritoneal tissue sections were dewaxed and rehydrated, then immersed in 0.5% toluidine blue solution (BIOSSCI, Wuhan, China) for 30 min. After rinsing, the sections were differentiated with 0.5% glacial acetic

acid under microscopic observation. The sections were subsequently air-dried. For clearing and mounting, sections were immersed in xylene for 5 min, removed, briefly air-dried, and mounted with a neutral mounting medium. All sections were examined and imaged using a light microscope.

MCs were considered degranulated when exhibiting one or more of the following features: loss of purple staining, indistinct cellular morphology, altered cell shape, or the presence of numerous extracellular granules surrounding the cell.⁸

Immunohistochemistry (IHC)

Immunohistochemistry was performed to evaluate the expression of collagen I, fibronectin, vimentin, α -SMA, E-cadherin, TGF- β 1, p-p38, p-ERK1/2, NF- κ B, and PAR2. Paraffin-embedded mouse peritoneal sections were subjected to deparaffinization, rehydration, antigen retrieval, and endogenous peroxidase blocking. After blocking for 30 min, sections were incubated with primary antibodies overnight at 4 °C, followed by incubation with appropriate secondary antibodies for 1 h at room temperature. Signal visualization was achieved using DAB substrate, and nuclei were counterstained with hematoxylin. After dehydration and mounting, all sections were examined and imaged using a light microscope.

All IHC analyses were conducted using samples from three independent biological replicates, and each experiment was performed at least three times.

Immunofluorescence (IF)

The procedure was similar to that used for IHC, except that fluorescent secondary antibodies were applied (ZO-1: Alexa Fluor 594; VEGFA and p-Smad3: Alexa Fluor 488) and incubated in the dark for 1 h. Nuclei were counterstained with DAPI for 5 min, and the slides were mounted using an anti-fade mounting medium. Images were captured using a fluorescence microscope.

Cell Culture

P815 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM (Gibco, China) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Gibco, China) at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were passaged when reaching 70–80% confluence. For stimulation experiments, cells were treated with LPS (L2880, Sigma-Aldrich, USA) and/or the mast cell stabilizer cromolyn (HY-B1619, MedChem Express, USA), as indicated.

Human peritoneal mesothelial cells (HMrSV5) were obtained from LAMI-Bio (Shanghai, China) and cultured in DMEM (Gibco, China) supplemented with 10% FBS (VivaCell, China) and 1% penicillin/streptomycin (Gibco, China) under the same conditions. Cells were passaged at 70–80% confluence. For stimulation, cells were treated with tryptase (CP82, Novoprotein, China), as indicated.

Cell Viability

P815 cells (1.5×10^4 cells/well) and HMrSV5 cells (1.0×10^4 cells/well) were seeded into 96-well plates and cultured until reaching 70–80% confluence. Cells were then treated with the indicated agents for the indicated time periods, and cellular morphology was observed and recorded using an inverted phase-contrast microscope (Olympus, Japan). After treatment, the medium was removed and replaced with 100 μ L of serum-free medium containing 10 μ L of Cell Counting Kit-8 (CCK-8; APEX BIO, USA). Following incubation at 37 °C for 1–2 h, absorbance at 450 nm was measured using a microplate reader (ELx800, BioTek, USA).

Lentivirus-Mediated CRISPR/Cas9 Knockout of PAR2

PAR2 knockout in HMrSV5 cells was achieved using a lentivirus-mediated CRISPR/Cas9 system. Briefly, cells were trypsinized, counted, and seeded into culture dishes at a standardized density. When cultures reached 70% confluence, the medium was replaced with serum-free DMEM, and cells were transduced with lentiviral particles at a multiplicity of infection (MOI) of 100. The sgPAR2 group was transduced with a LentiCRISPR v2-Puro vector targeting PAR2, whereas the sgCon group received a non-targeting control virus; an untreated group served as a blank control.

At 24h post-transduction, the medium was replaced with selection medium containing 2 µg/mL puromycin, and selection was continued for 7–10 days. Surviving cells were collected after complete death of cells in the negative control group. Knockout efficiency was validated by Western blotting (WB).

TEM

P815 cells (1.5×10^6) were collected by centrifugation to obtain a cell pellet and fixed in 2.5% glutaraldehyde (BIOSSCI, Wuhan, China) for 2 h at room temperature, followed by fixation at 4 °C overnight. The pellet was gently resuspended to ensure adequate penetration of fixative. After rinsing, samples were post-fixed in 1% osmium tetroxide in the dark at room temperature for 2 h. The pellet was dehydrated through a graded ethanol series and acetone, infiltrated and embedded in resin, and then polymerized. Ultrathin sections were cut, stained, and observed using a transmission electron microscope (HITACHI HT7800, Japan) for image acquisition.

Transcriptomic Analysis

Total RNA was isolated and purified from peritoneal tissues and P815 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNA concentration and purity were assessed using the NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). cDNA libraries were constructed and sequenced on the Illumina NovaSeq™ 6000 platform (LC Bio Technology Co., Ltd., Hangzhou, China).

Fastp was used for raw read filtering to remove adapter sequences, low-quality bases, and undetermined nucleotides under default parameters. High-quality reads were aligned against the reference genome using HISAT2. Gene expression levels were quantified using StringTie, and differential expression was determined based on the thresholds $|\log_2(\text{Fold Change})| > 1$ and $p\text{-value} < 0.05$, indicating significant differences between groups.

Western Blot Analysis

HMrSV5 cells and peritoneal tissues were lysed in RIPA buffer (P0013B, Beyotime, Shanghai, China) supplemented with a protease and phosphatase inhibitor cocktail (P1045, Beyotime). After lysis on ice, samples were sonicated; tissue homogenization was aided using stainless-steel beads. Protein concentrations were determined using a BCA assay kit (Epizyme, Shanghai, China). Equal amounts of protein were separated by SDS-PAGE and transferred onto 0.22-µm PVDF membranes. Membranes were blocked with 5% skim milk for 1 h and incubated with primary antibodies overnight at 4 °C. After washing, membranes were incubated with appropriate secondary antibodies for 1 h at room temperature. Protein bands were visualized using enhanced chemiluminescence, and band intensities were quantified using Image J (version 1.54). All WB analyses were performed using samples from three independent biological replicates.

ELISA Analysis

Tryptase levels in mouse peritoneal tissue were measured using an ELISA kit (CSB-E14326m, CUSABIO, China). In addition, serum levels of TNF-α (E-EL-M3063, Elabscience, China), TGF-β (E-EL0162, Elabscience, China), and IL-1β (EM0118, Fine Test, China) were determined.

After 12 h of drug treatment, culture supernatants from P815 cells were collected to quantify tryptase and TNF-α secretion. Tryptase (CSB-E14326m, CUSABIO, China) and TNF-α (E-EL-M3063, Elabscience, China) were measured using the corresponding ELISA kits according to the manufacturers' instructions.

Statistical Analysis

Normality was assessed using the Shapiro–Wilk test. Normally distributed data are presented as mean ± SD, and comparisons between two groups were performed using Student's *t*-test. For comparisons among multiple groups, one-way ANOVA followed by Dunnett's post hoc test was applied. Non-normally distributed data are presented as median (interquartile range) and were compared using the Mann–Whitney *U*-test. Categorical data are presented as percentages and were compared using Fisher's exact test. All analyses were performed using GraphPad Prism 9, and statistical significance was set at $p < 0.05$.

Fold change (FC) was calculated as the ratio of the mean value in the experimental group to that in the control group. The baseline-corrected inhibition/restoration rate was calculated as $(\text{Model} - \text{Treatment}) / (\text{Model} - \text{Control}) \times 100\%$, representing the percentage reversal toward control levels (applicable to both increased and decreased endpoints).

Results

Network Pharmacology Study of AS in the Treatment of PF

Astragalus membranaceus and *Salvia miltiorrhiza* have been reported to suppress PF.^{17,19} We therefore used UPLC-Q-TOF-MS/MS to characterize the major constituents of their combination (AS) and identified 40 components, including calycosin, formononetin, cryptotanshinone, and tanshinone IIA (Figure 1A and B; [Supplementary Table 2](#)).

To explore potential targets of AS in PF, we performed network pharmacology analysis and identified 611 putative AS-related targets and 2579 PF-related targets from public databases. Intersection analysis yielded 577 overlapping targets (Figure 2A). Among the active components, calycosin and tanshinone IIA showed higher network connectivity and were therefore prioritized for subsequent analyses, whereas MAPK1 and NF- κ B1 were linked to a greater number of compounds (Figure 2B). The PPI network constructed from the 577 targets contained 551 nodes and 9726 edges (Figure 2C), and centrality analysis highlighted MAPK1 and NF- κ B1 as core nodes (Figure 2D).

GO enrichment suggested an association with inflammatory response regulation (Figure 2E), and KEGG enrichment indicated potential involvement of MAPK-related signaling (Figure 2F and G). Collectively, these network-based analyses suggest that MAPK/NF- κ B signaling may represent a candidate pathway underlying AS-associated anti-fibrotic effects. These analyses are hypothesis-generating and were used to prioritize candidate compounds, targets, and pathways for subsequent validation. To further assess the structural feasibility of the predicted compound-target interactions, molecular docking and molecular dynamics simulations were subsequently performed.

Molecular Docking and Molecular Dynamics Analysis of AS in PF

To assess potential compound-target interactions suggested by network pharmacology, molecular docking was performed to evaluate the predicted binding of the prioritized AS components (calycosin and tanshinone IIA) to the major targets MAPK1 and NF- κ B1. As shown in Figure 3A, calycosin showed docking scores of -8.1 kcal/mol (MAPK1) and -9.1 kcal/mol (NF- κ B1), whereas tanshinone IIA showed docking scores of -6.8 kcal/mol (MAPK1) and -7.1 kcal/mol (NF- κ B1). Overall, both compounds yielded favorable docking scores (all < -5.0 kcal/mol).

To further examine the stability of these predicted protein-ligand complexes, molecular dynamics simulations were conducted. Analyses of RMSD, Rg, SASA, hydrogen bonding, and RMSF indicated that the MAPK1-calycosin, MAPK1-tanshinone IIA, NF- κ B1-calycosin, and NF- κ B1-tanshinone IIA complexes showed stable interaction patterns over the simulation period, with relatively consistent hydrogen-bond interactions (Figure 3B–F). These computational findings support the structural feasibility of interactions between calycosin/tanshinone IIA and MAPK1/NF- κ B1, but should be interpreted as hypothesis-generating and require further experimental validation.

AS Attenuates PDF-Induced PF by Suppressing MC Activation

To investigate how AS ameliorates PF, we established a mouse model by daily intraperitoneal injection of high-glucose (4.25%) PDF for 4 weeks. PDF induced peritoneal injury and fibrosis, evidenced by loosened peritoneal structure, partial mesothelial exfoliation, increased peritoneal thickness, inflammatory cell infiltration, and collagen I deposition (Figure 4A).

We next characterized the infiltrating cells. Compared with controls, the PDF group showed increased MC number with features of activation/degranulation, as indicated by toluidine blue staining and tryptase-positive MC staining (Figure 4B). Consistently, peritoneal tryptase levels increased by 3.2-fold ($p < 0.01$) in the PDF group (Figure 4C). AS markedly attenuated this increase, reversing 71.5% ($p < 0.01$) of the PDF-induced elevation toward control levels (baseline-corrected), whereas Cro reversed 91.9% ($p < 0.01$). Together, these data indicate that high-glucose PDF triggers MC activation and tryptase release, supporting a role for MC-derived tryptase in MC-PMC crosstalk.

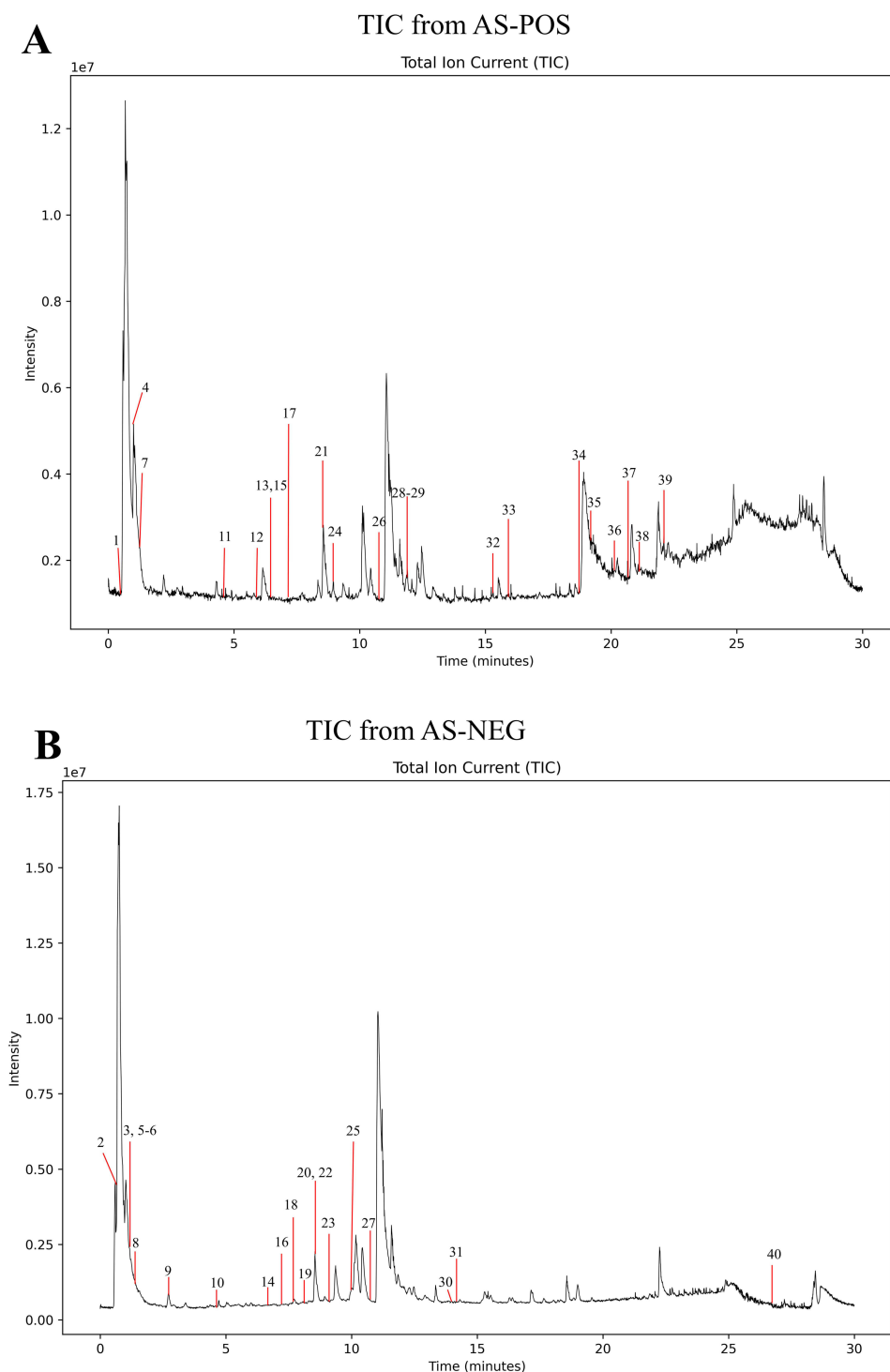


Figure 1 Identification of AS characterization and analysis of active ingredients. **(A and B)** UPLC-Q/TOF-MS total ion chromatograms of AS recorded in the positive and negative ion mode.

AS Suppresses LPS-Induced Activation of P815 Cells

To elucidate the impact of AS on MC activation *in vitro*, P815 cells were first exposed to increasing concentrations of AS (0–8mg/mL) for 12h, and cell viability was assessed using a CCK-8 assay. As shown in [Figure 5A](#), 2mg/mL was selected for subsequent experiments. P815 cells were then stimulated with graded concentrations of LPS to establish an activation model, with maximal tryptase and TNF- α release observed at 1 μ g/mL LPS ([Figure 5B](#)).

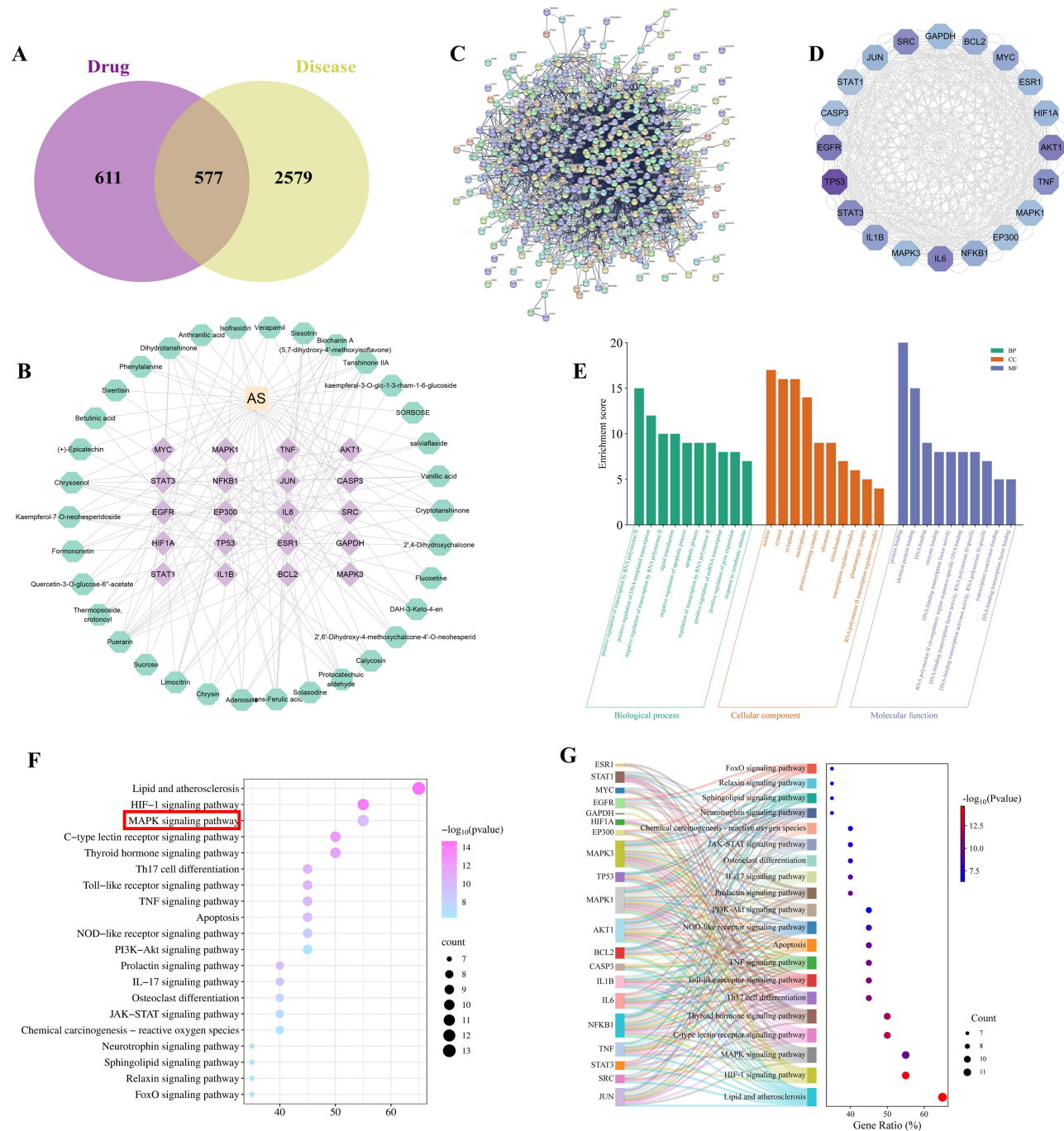


Figure 2 Network pharmacology study of AS in the treatment of PF. **(A)** Venn diagram showing AS constituent-related and PF-related targets. **(B)** AS-Ingredients-Target network **(C)** PPI network analysis of anti-PF targets of AS. **(D)** Top 20 genes in the PPI network, ranked by degree values. **(E)** GO functional enrichment analysis of the anti-PF targets of AS. **(F and G)** KEGG enrichment analysis of the anti-PF targets of AS. The red box indicates the MAPK signaling pathway.

For intervention studies, P815 cells were pretreated with AS (2 mg/mL) or Cro (10 mg/mL) for 30 min prior to LPS exposure. Compared with the control group, LPS stimulation increased tryptase and TNF- α secretion by approximately 2.2-fold and 2.1-fold, respectively (both $p < 0.01$) (Figure 5C). AS pretreatment reversed 71.62% and 58.2% of the LPS-induced increases in tryptase and TNF- α levels toward control levels, respectively, whereas Cro reversed 58.98% and 59.0% (all $p < 0.01$) (Figure 5C). These findings indicate that AS significantly suppresses LPS-induced P815 cell activation.

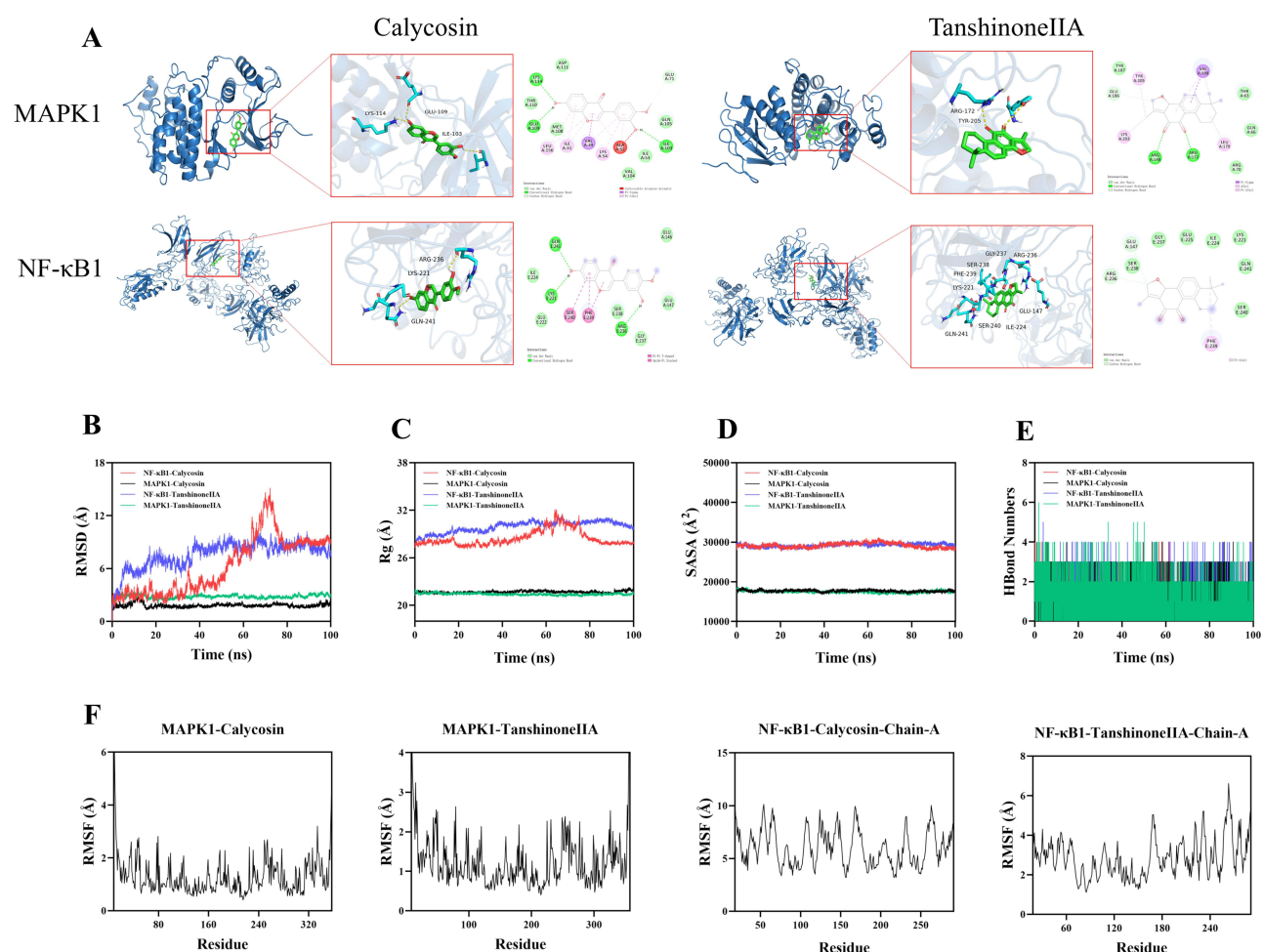


Figure 3 Molecular docking and molecular dynamics analysis of AS in PF. **(A)** Molecular docking of calycosin and tanshinone IIA with MAPK1 and NF-κB1. **(B)** Root mean square deviation (RMSD) of the protein-ligand complex over time. **(C)** Radius of gyration (Rg) of the protein-ligand complex over time. **(D)** Solvent-accessible surface area (SASA) of the protein-ligand complex over time. **(E)** Number of hydrogen bonds in the protein-ligand complex over time. **(F)** Root mean square fluctuation (RMSF) of the protein-ligand complex.

In addition, MC degranulation is a key marker of activation. TEM results showed that control group P815 cells retained intact cytosolic granules and liposomes, with structurally preserved plasma membranes. In contrast, LPS-stimulated cells exhibited significant degranulation, including irregular granule morphology and partial-to-complete luminal vacuolization. Notably, both AS and Cro treatments significantly suppressed degranulation and reduced vesicular excretion (Figure 5D).

To further explore the mechanism by which AS inhibits P815 cell activation, transcriptomic sequencing was performed on control and LPS-treated P815 cells. Volcano plot analysis identified 406 differentially expressed genes (DEGs), including 192 upregulated and 214 downregulated genes (Figure 5E). Several inflammation- and fibrosis-related genes were significantly altered, such as *Nfkbiz* ($\log_2\text{FC} = 1.87$, $p \text{ value} = 3.19 \times 10^{-18}$), *Nlrp3* ($\log_2\text{FC} = 1.24$, $p \text{ value} = 4.37 \times 10^{-6}$), *Il6* ($\log_2\text{FC} = 1.13$, $p \text{ value} = 6.27 \times 10^{-4}$), *Tnf* ($\log_2\text{FC} = 1.16$, $p \text{ value} = 3.13 \times 10^{-11}$) (Figure 5F). KEGG pathway analysis indicated significant enrichment of cytokine-cytokine receptor interaction, MAPK, JAK-STAT, and TNF signaling pathways (Figure 5G). These results suggest that LPS activation of P815 cells is accompanied by enhanced inflammatory transcriptional programs.

AS Inhibits Peritoneal PAR2 Activation and MMT Induced by MC Activation

Upon activation, MCs secrete tryptase, which influences neighboring cells and promotes inflammatory injury. Previous studies have identified PAR2 as a downstream target of tryptase in fibrotic processes.²³ Consistently, IHC staining

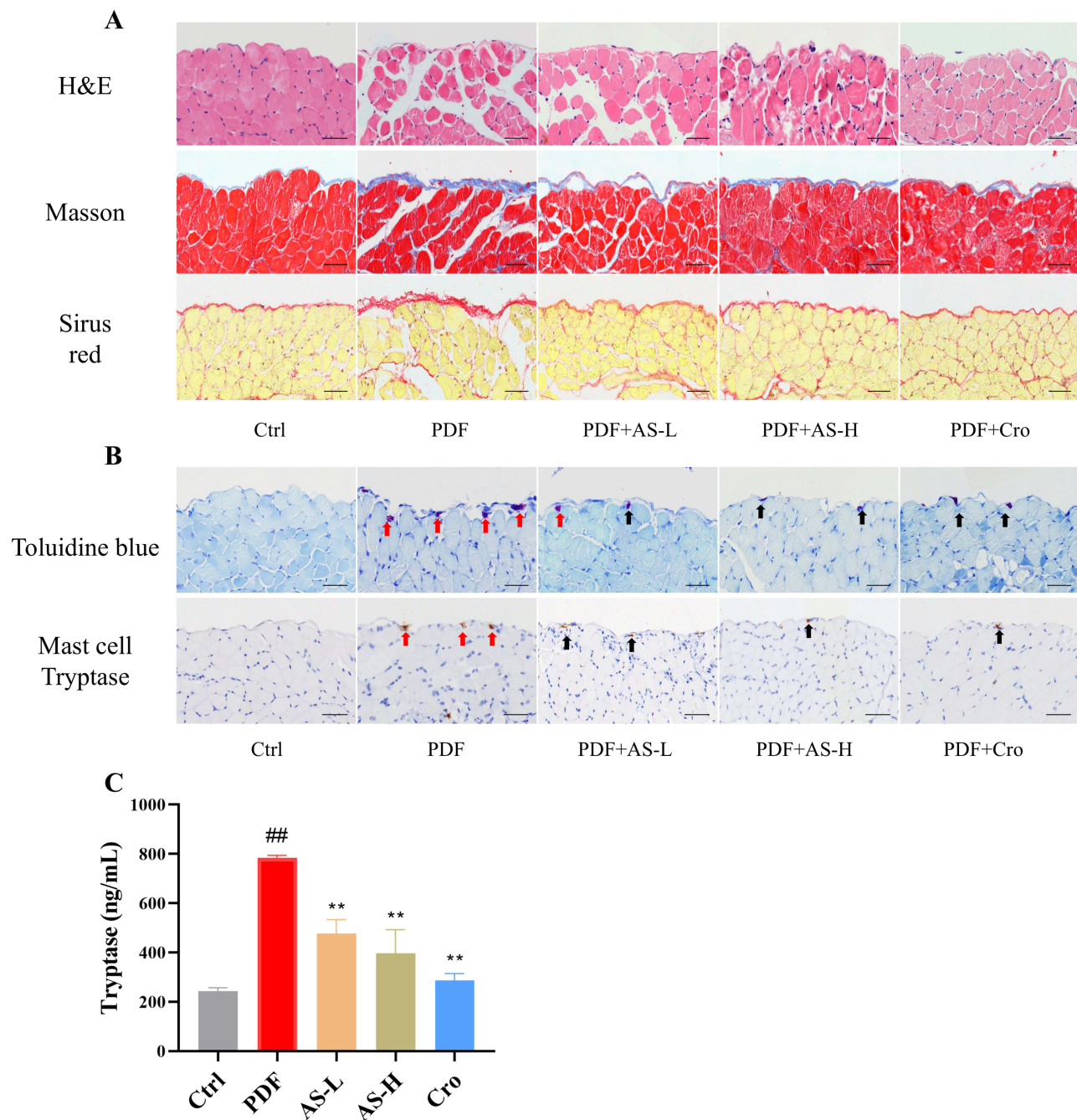


Figure 4 AS attenuates PDF-induced PF by suppressing MC activation. **(A)** H&E, Masson's trichrome, and Sirius red staining of parietal peritoneal tissues in each group ($n = 4$; scale bar = 50 μm). **(B)** Toluidine blue staining for mast cells and immunostaining for tryptase in parietal peritoneal tissues ($n = 4$; scale bar = 50 μm). **(C)** Tryptase levels were quantified by ELISA ($n = 3$). $^{##} p < 0.01$ vs. the Ctrl group; $^{**} p < 0.01$ vs. the PDF group.

revealed markedly increased PAR2 expression in PMCs of the PDF group (Figure 6A). WB analysis showed that PAR2 protein levels were elevated by approximately 3.47-fold ($p < 0.01$) compared to the control group. Treatment with AS and Cro reduced PAR2 expression by 57.5% ($p < 0.05$) and 66.3% ($p < 0.01$), respectively (Figure 6B and C). A similar inhibitory trend was observed with the PAR2 antagonist ENMD (83.0%, $p < 0.01$) (Figure 6B and C). These findings support the involvement of the tryptase/PAR2 axis in MC-PMC crosstalk.

MMT is a recognized mechanism underlying PF. IHC analysis showed that PDF induced an MMT-like protein profile in PMCs, which was partially reversed by AS treatment (Figure 6D). Consistently, WB analysis demonstrated that PDF

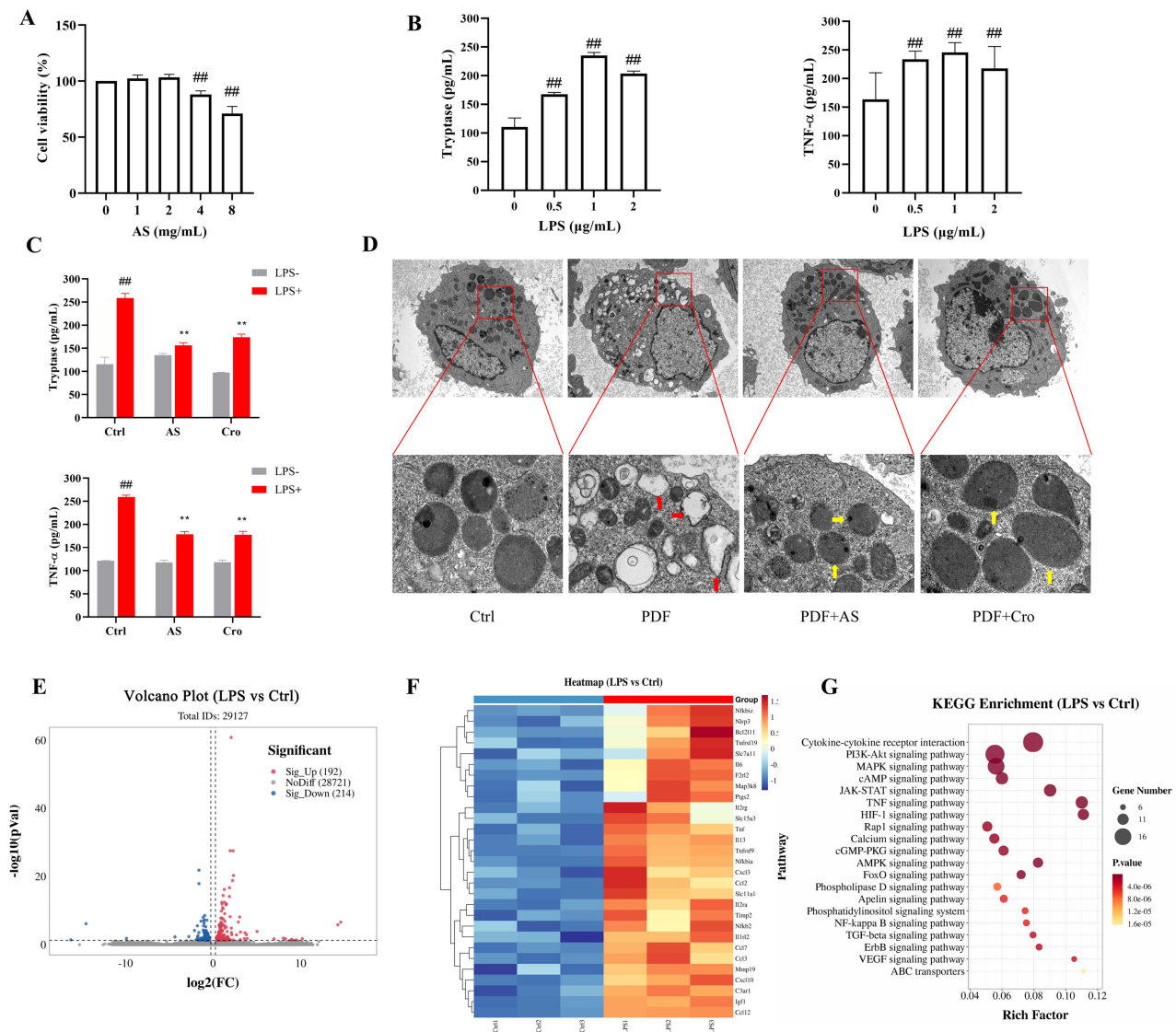


Figure 5 AS suppresses LPS-induced activation of P815 cells. **(A)** The viability of P815 cells stimulated by AS at varying concentrations (0–8 mg/mL) for 12 h was measured using the CCK-8 assay ($n = 3$). **(B)** The levels of tryptase and TNF- α in the supernatant of LPS-induced P815 cells at varying concentrations (0–2 μ g/mL) were measured using ELISA ($n = 3$). **(C)** Tryptase and TNF- α concentrations in the supernatants of LPS-stimulated P815 cells treated with AS and Cro were quantified using ELISA ($n = 3$). **(D)** The granule morphology of P815 cells was observed by TEM (scale bars = 1 μ m and 2 μ m) ($n = 3$). Red arrows mark LPS-induced activated P815 cells with piecemeal degranulation and irregular/emptied granules; yellow arrows mark inhibited degranulation after treatment, with more regular granules and reduced granule extrusion. **(E)** Volcano plot illustrating DEGs in P815 cells **(F)** Heatmap illustrating DEGs in P815 cells. Blue indicates downregulated genes, and red indicates upregulated genes. **(G)** KEGG enrichment analysis between the LPS and Ctrl groups ($n = 3$). ### $p < 0.01$ vs. the Ctrl group; ** $p < 0.01$ vs. the LPS group.

increased Vimentin and α -SMA by 5.8 and 9.7-fold, respectively, while decreasing E-cadherin to 0.38-fold of control (all $p < 0.01$) (Figure 6E and F). AS partially reversed these changes, reducing the PDF-induced elevations by 78.1% and 84.0%, and restoring E-cadherin by 47.6% toward control levels (all $p < 0.01$) (Figure 6E and F). In addition, AS reduced activation of the TGF- β 1/Smad3 pathway (Figure 6G). Collectively, these findings indicate that AS suppresses PAR2 activation and MMT in PDF-induced PF, supporting a regulatory role of the tryptase/PAR2 pathway in MC-PMC interactions.

AS Attenuates Tryptase-Induced Expression of HMrSV5 PAR2 and MMT

Previous in vivo studies have demonstrated that MC activation contributes to high-glucose PDF-induced peritoneal injury through tryptase release. In vitro, LPS activation also induces P815 cell degranulation and tryptase release, but the role of

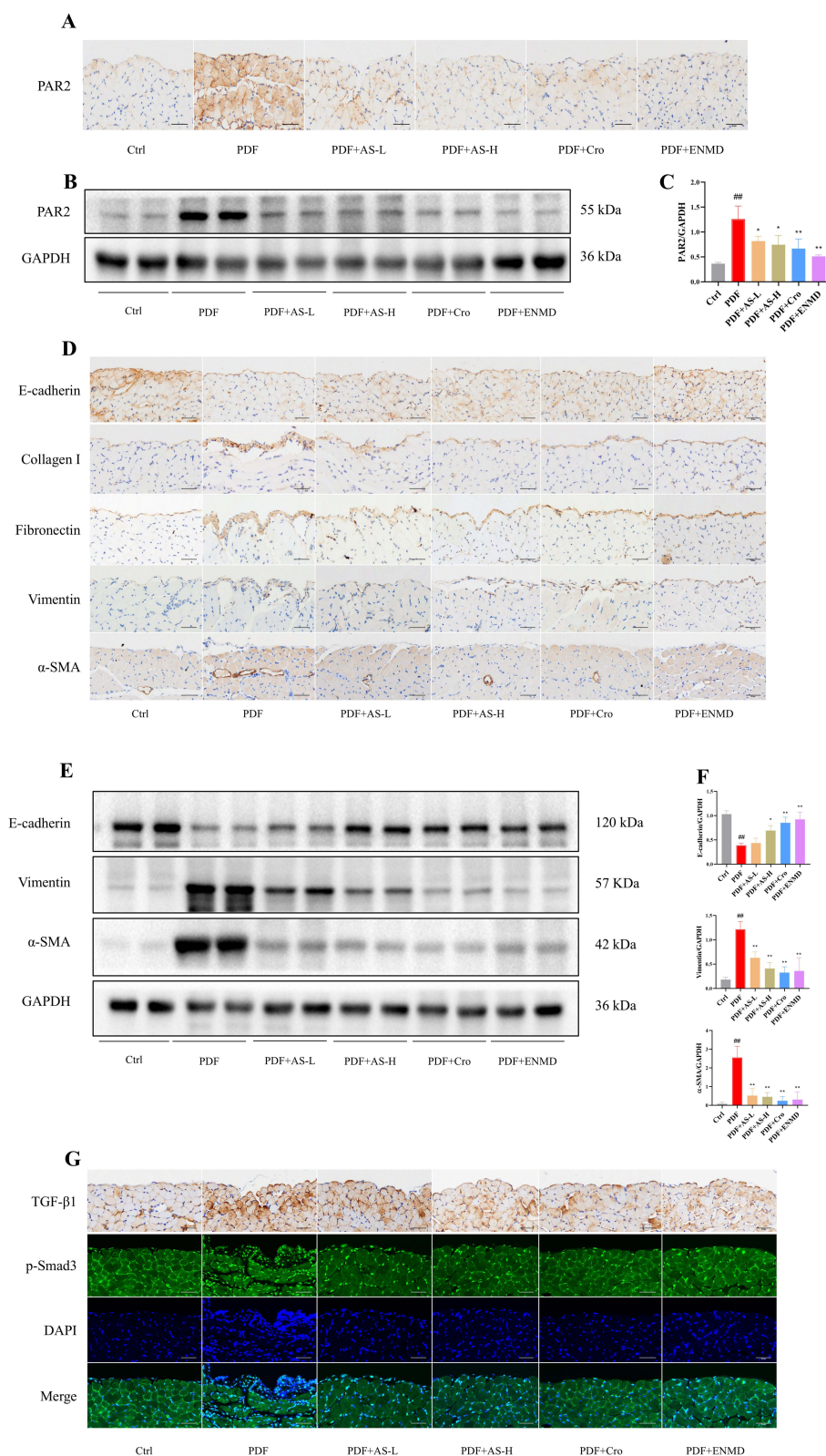


Figure 6 AS inhibits peritoneal PAR2 activation and MMT induced by MC activation. **(A)** IHC staining of PAR2 in peritoneal tissue (n = 4, scale bar 50 μ m). **(B)** WB analysis of PAR2 protein expression in peritoneal tissue. **(C)** Semi-quantitative analysis of the immunoblotting data (n = 3). **(D)** Representative IHC for E-cadherin, Collagen I, Fibronectin, Vimentin, and α -SMA in peritoneal tissue (n = 4, scale bar 50 μ m). **(E)** WB analysis of the protein expression levels of E-cadherin, Vimentin, and α -SMA in peritoneal tissue. **(F)** Semi-quantitative analysis of the immunoblotting data (n = 3). **(G)** Representative IF for TGF- β 1 and IF for p-Smad3 (green), with DAPI for nuclear visualization (blue) in peritoneal tissue (n = 4; scale bar = 50 μ m). ### $p < 0.01$ vs. the Ctrl group; * $p < 0.05$ vs. the PDF group, ** $p < 0.01$ vs. the PDF group.

trypsin in inducing fibrosis in PMCs remains unclear. To address this, we used trypsin to stimulate HMrSV5 cells and further investigate the mechanism in PMCs.

HMrSV5 cells were treated with varying concentrations of trypsin (0–16 ng/mL) and AS (0–8 mg/mL) for 24 h, with safe concentrations determined by CCK-8 assay (Figure 7A). A cellular fibrosis model was established by stimulating HMrSV5 cells with trypsin (8 ng/mL). WB analysis showed that trypsin increased PAR2 expression by 3.8-fold and aggravated MMT marker dysregulation (Figure 7B and C). Compared with controls, E-cadherin decreased to 0.26-fold, whereas mesenchymal markers (Vimentin and α -SMA) increased by 3.07–5.46-fold (all $p < 0.01$) (Figure 7B and C). Trypsin also induced fibrotic-like morphological changes, which were alleviated by AS (Figure 7D). AS partially normalized MMT markers, restoring 40.8% of the E-cadherin decrease and reversing 40.0–80.0% of the Vimentin/ α -SMA increases toward control levels (all $p < 0.01$) (Figure 7E and F). Notably, AS attenuated PAR2 upregulation, reversing 82.3% of the PAR2 increase toward control levels ($p < 0.01$) (Figure 7E and F). These findings support that AS inhibits trypsin-induced PF by modulating PAR2-associated MMT in PMCs.

Transcriptomic Analysis Reveals Mechanisms by Which AS Ameliorates PF

To investigate the molecular mechanisms underlying the protective effects of AS *in vivo*, RNA sequencing (RNA-seq) was performed on mouse peritoneal tissues. DEGs between the PDF and control groups identified 251 upregulated and 71 downregulated genes (Figure 8A). GO enrichment analysis indicated that the differentially expressed genes were enriched in biological processes related to inflammatory response, signal transduction, oxidative stress, and transcriptional regulation (Figure 8B). KEGG analysis further suggested activation of the MAPK pathway following high-glucose PDF exposure (p value < 0.01) (Figure 8C).

We next compared the AS group with the PDF group, identifying 34 upregulated and 119 downregulated genes (Figure 8D). Heatmap analysis showed that AS partially reversed the gene expression changes induced by high-glucose PDF (Figure 8E). Consistent with these results, GSEA demonstrated that MAPK signaling was significantly suppressed by AS treatment (NES = -1.25 , p value = 0.012) (Figure 8F). In addition, several pathways associated with immune activation and fibrosis, including Fc epsilon RI, VEGF, Toll-like receptor, AGE-RAGE, and Nod-like receptor signaling, were attenuated by AS (Figure 8G). Notably, Fc epsilon RI signaling is involved in MC activation, suggesting that AS may modulate MC-associated pathways at the transcriptomic level.

AS Ameliorates PF by Modulating the PAR2/MAPK/NF- κ B Pathway

Based on the above findings, we evaluated MAPK/NF- κ B pathway activation in mouse peritoneal tissues. IHC analysis showed increased nuclear p65 expression together with enhanced p-p38 and p-ERK staining in the PDF group, all of which were attenuated by AS treatment (Figure 9A). WB analysis further demonstrated that high-glucose PDF markedly elevated phosphorylation of p65, JNK, p38, and ERK1/2, whereas AS treatment significantly reduced these phosphorylated protein levels by 69.0%, 89.2%, 90.0%, and 90.6%, respectively (all $p < 0.05$) (Figure 9B and C). In addition, AS reduced serum TNF- α , TGF- β 1, and IL-1 β levels by 82.7%, 76.4%, and 70.0%, respectively (all $p < 0.01$) (Figure 9D). These data suggest that AS mitigates PF-associated inflammatory signaling, at least in part, through suppression of MAPK/NF- κ B activation.

To corroborate the *in vivo* findings, we assessed MAPK/NF- κ B pathway activation in a trypsin-stimulated HMrSV5 fibrosis model. Trypsin markedly increased phosphorylation of MAPK/NF- κ B pathway proteins (JNK, p38, ERK1/2, and p65) (Figure 10A and B), whereas AS treatment significantly reduced p-JNK, p-p38, p-ERK1/2, and p-p65 levels by 83.6%, 62.9%, 81.5%, and 81.7%, respectively (all $p < 0.01$) (Figure 10C and D).

To further define the role of PAR2, we abrogated PAR2 expression in HMrSV5 cells using lentivirus-mediated CRISPR/Cas9. PAR2 knockdown efficiency was confirmed by WB, showing an approximately 55% reduction in PAR2 protein expression compared with the sgCtrl group ($p < 0.01$) (Figure 10E and F). PAR2 deletion reduced phosphorylation of MAPK/NF- κ B pathway proteins in a manner comparable to AS treatment (Figure 10G and H). Moreover, PAR2 deletion attenuated trypsin-induced MMT, as shown by decreased Vimentin and α -SMA and restored E-cadherin expression (Figure 10I and J). Collectively, these findings indicate that AS alleviates trypsin-induced MMT in PMCs by modulating the PAR2/MAPK/NF- κ B pathway.

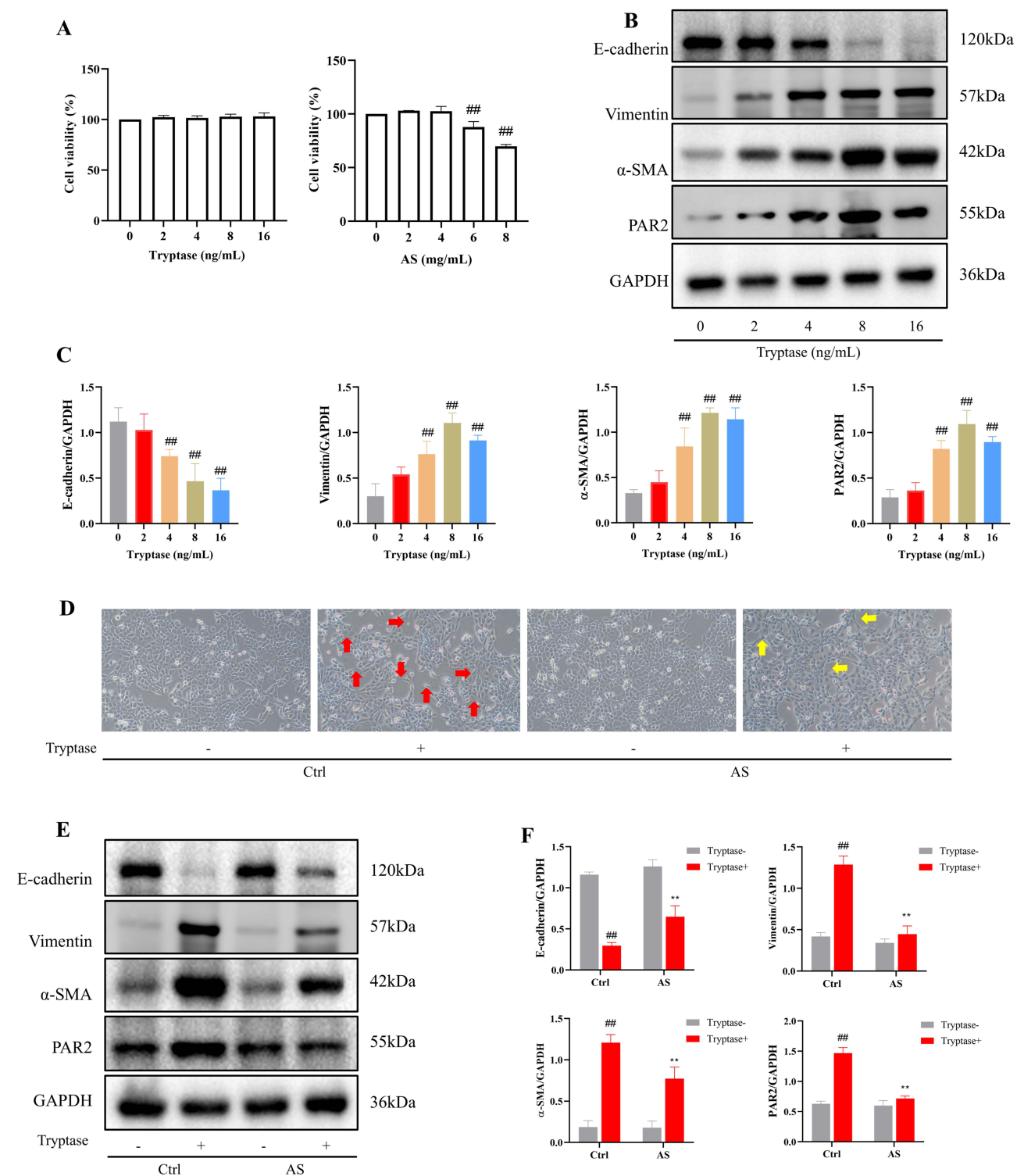


Figure 7 AS attenuates tryptase-induced expression of HMrSV5 PAR2 and MMT. **(A)** Effects of varying concentrations of tryptase and AS on HMrSV5 cell viability ($n = 3$). **(B)** WB analysis of PAR2 and MMT-related protein expression in HMrSV5 cells treated with increasing concentrations of tryptase. **(C)** Semi-quantitative analysis of PAR2 and MMT-related protein levels ($n = 3$). **(D)** Representative images showing that AS ameliorated tryptase-induced fibrotic morphological changes in HMrSV5 cells. Red arrows indicate tryptase-induced spindle-shaped, fibroblast-like transition of HMrSV5 cells; yellow arrows indicate that AS alleviated this spindle-shaped change. **(E)** WB analysis of PAR2 and MMT-related protein expression in tryptase-stimulated HMrSV5 cells with AS treatment. **(F)** Semi-quantitative analysis of PAR2 and MMT-related protein levels ($n = 3$). $^{##} p < 0.01$ vs. the Ctrl group; $^{**} p < 0.01$ vs. the Tryptase group.

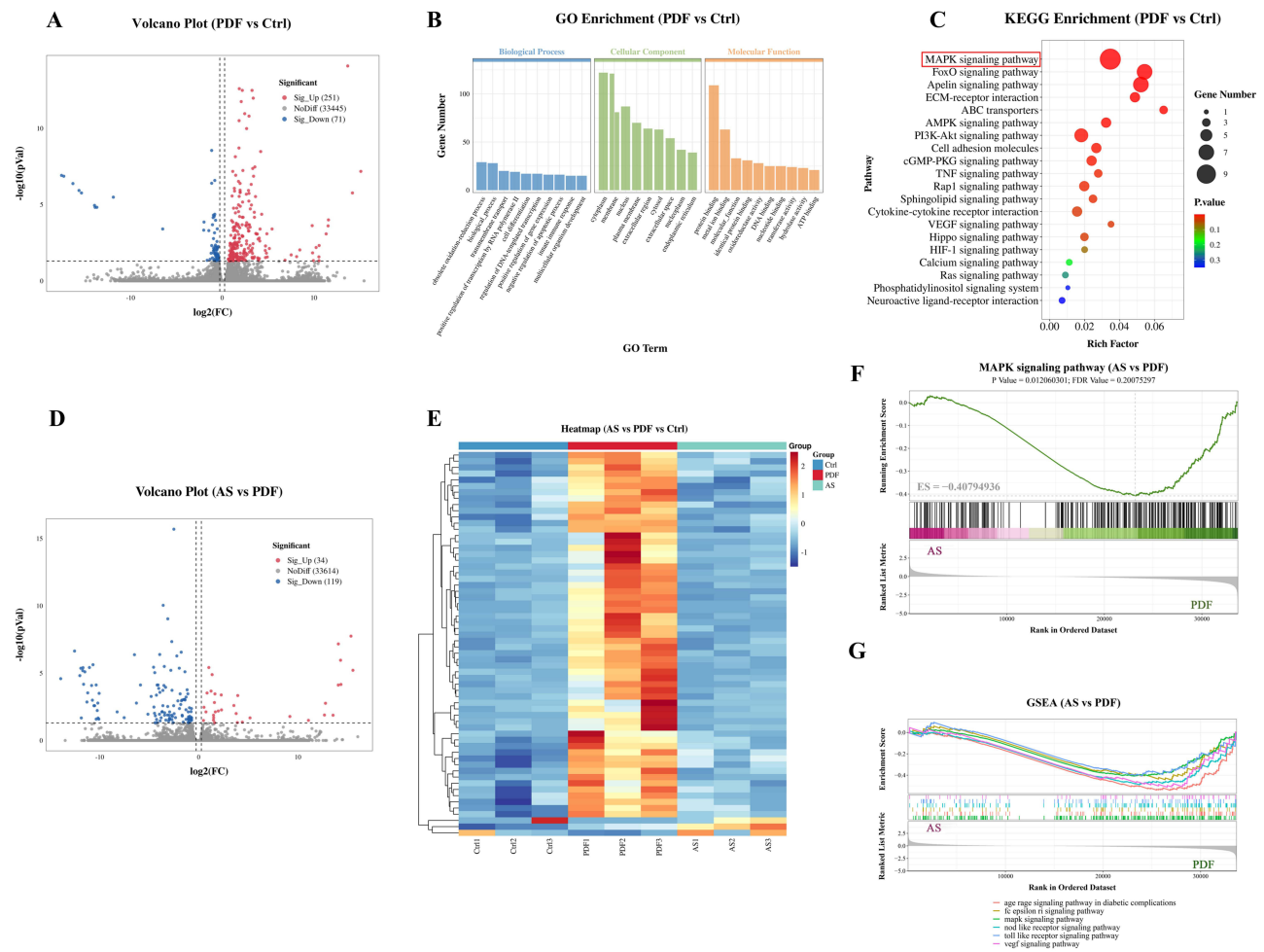


Figure 8 Transcriptomic analysis reveals mechanisms by which AS ameliorates PF. **(A)** Volcano plot of DEGs in peritoneal tissues (PDF vs Ctrl). **(B)** GO enrichment analysis (PDF vs Ctrl). **(C)** KEGG pathway enrichment analysis (PDF vs Ctrl). The red box indicates the MAPK signaling pathway. **(D)** Volcano plot of DEGs in peritoneal tissues (AS vs PDF). **(E)** Heatmap of DEGs in peritoneal tissues (Ctrl vs PDF vs AS). Blue indicates downregulated genes, and red indicates upregulated genes. **(F)** GSEA of the MAPK pathway in peritoneal tissues (AS vs PDF; $n = 3$). **(G)** GSEA of inflammation- and fibrosis-related pathways (AS vs PDF; $n = 3$).

AS Ameliorates Neoangiogenesis and Junctional Integrity

PF is a complex process. Besides the MAPK/NF- κ B pathways, we also evaluated other mechanisms involved in fibrosis, such as neoangiogenesis and cell junction integrity.^{24,25} Given that VEGFA and ZO-1 are key regulators of these processes, we examined the effects of AS and MCs on their expression in the mouse peritoneum. Fluorescence microscopy revealed a marked elevation of VEGFA expression in the PDF group, which was substantially reduced by AS treatment (Figure 11A). Moreover, high-glucose PDF significantly downregulated ZO-1 protein expression of in PMCs, indicating disruption of peritoneal barrier integrity. AS treatment restored ZO-1 expression in peritoneal tissue (Figure 11B). Collectively, these findings suggest that AS inhibits MC activation, thereby attenuating peritoneal neovascularization, preserving tight junction integrity, reducing inflammation, and ultimately alleviating PF.

Discussion

PF commonly develops in patients undergoing long-term PD and remains a major complication limiting technique survival.²⁶ Chronic inflammation is a central driver of PF; however, the contribution of MCs to PDF-induced peritoneal injury remains incompletely defined. In this study, we provide mechanistic evidence that MC activation promotes PF through tryptase-dependent PAR2 signaling in PMCs, and that AS attenuates PF by suppressing MC degranulation and

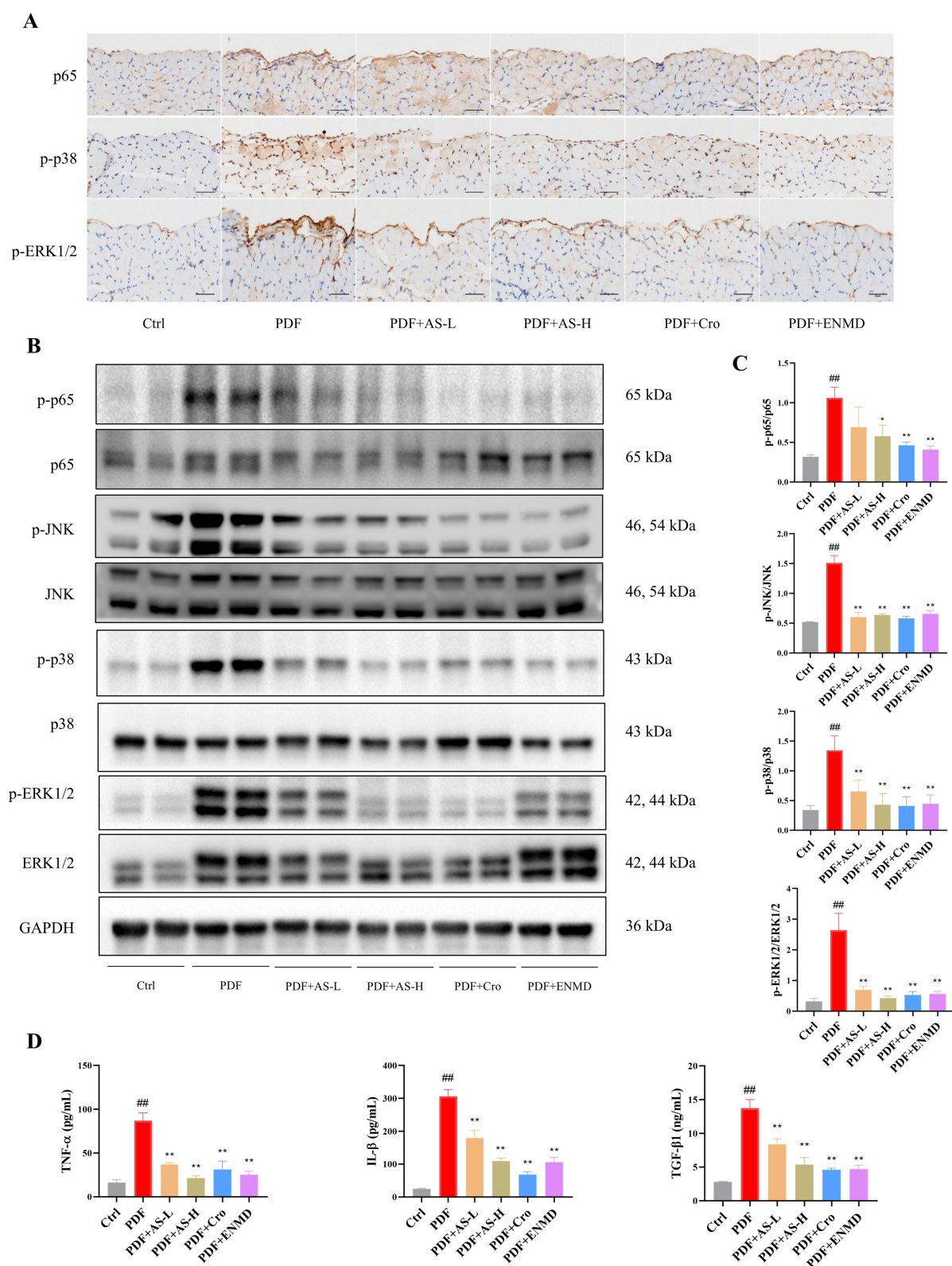


Figure 9 AS ameliorates PF in vivo by modulating the PAR2/MAPK/NF- κ B pathway. **(A)** IHC staining of NF- κ B, p-p38, and p-ERK1/2 in mouse peritoneal tissues from each group ($n = 4$; scale bar = 50 μ m). **(B)** WB analysis of p-p65, p-JNK, p-p38, and p-ERK1/2 protein expression in mouse peritoneal tissues. **(C)** Semi-quantitative analysis of the immunoblotting data ($n = 3$). **(D)** Serum concentrations of TNF- α , TGF- β 1, and IL-1 β were quantified by ELISA ($n = 3$). ## $p < 0.01$ vs. the Ctrl group; # $p < 0.05$ vs. the PDF group; * $p < 0.01$ vs. the PDF group; ** $p < 0.05$ vs. the PDF group.

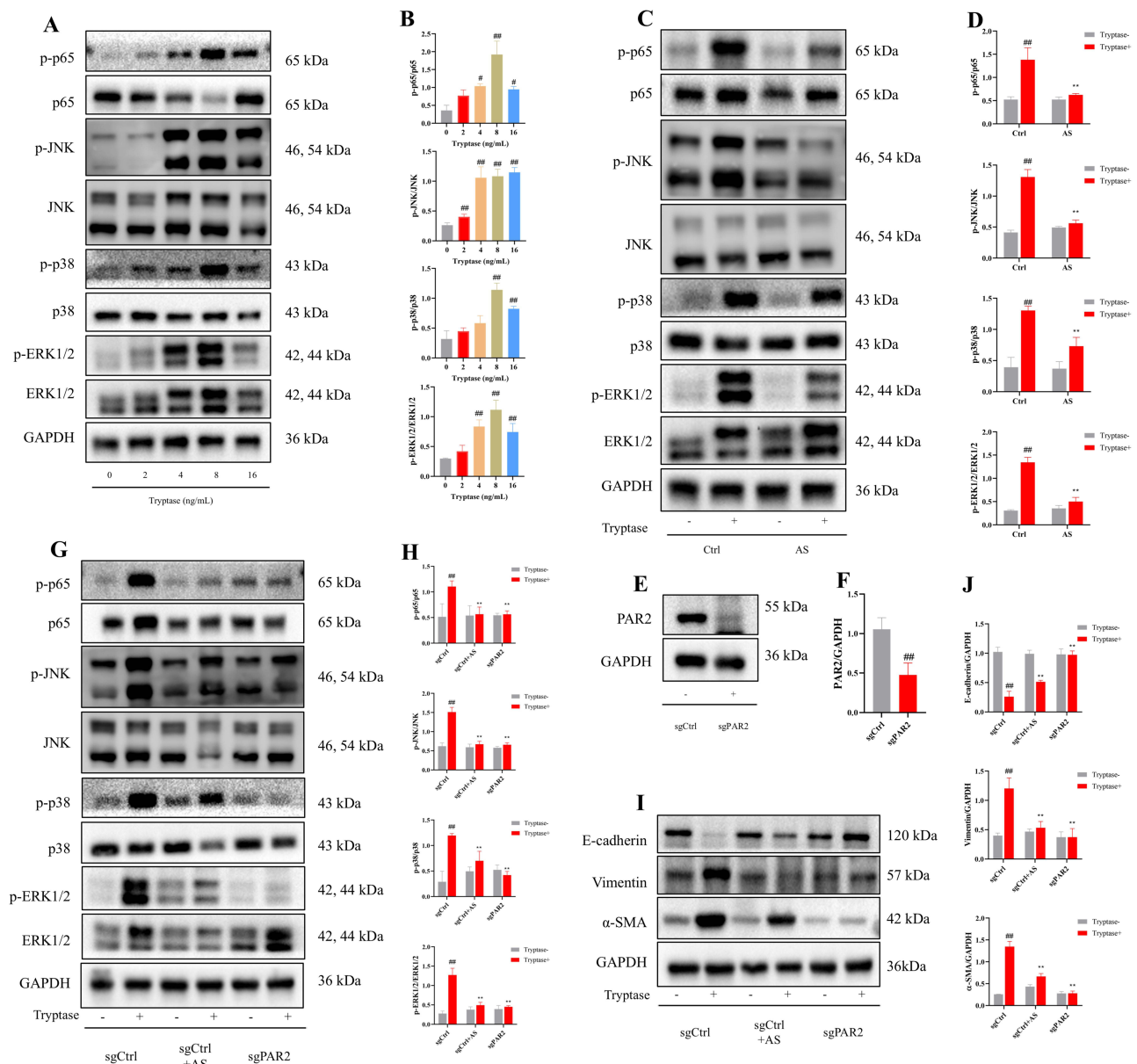


Figure 10 AS suppresses tryptase-induced PAR2/MAPK/NF-κB activation in vitro. (A) WB analysis of MAPK/NF-κB pathway-related protein expression in HMrSV5 cells treated with varying concentrations of tryptase. (B) Semi-quantitative analysis of the immunoblotting data (n = 3). # $p < 0.05$ vs. the Ctrl group; ### $p < 0.01$ vs. the Ctrl group. (C) WB analysis of MAPK/NF-κB pathway-related protein expression in tryptase-induced HMrSV5 cells with AS treatment. (D) Semi-quantitative analysis of the immunoblotting data (n = 3). ### $p < 0.01$ vs. the Ctrl group; ** $p < 0.01$ vs. the Tryptase group. (E) WB analysis of PAR2 protein expression following CRISPR/Cas9-mediated knockout. (F) Semi-quantitative analysis of PAR2 protein levels. ### $p < 0.01$ vs. the sgCtrl group. (G) WB analysis of MAPK/NF-κB pathway-related protein expression in tryptase-induced HMrSV5 cells treated with AS and compared with PAR2 sgRNA. (H) Semi-quantitative analysis of the immunoblotting data (n = 3). ### $p < 0.01$ vs. the sgCtrl group; ** $p < 0.01$ vs. the sgCtrl+Tryptase group. (I) WB analysis of MMT-associated protein expression in tryptase-induced HMrSV5 cells treated with AS and compared with PAR2 sgRNA. (J) Semi-quantitative analysis of MMT-associated protein levels (n = 3). ### $p < 0.01$ vs. the sgCtrl group; ** $p < 0.01$ vs. the sgCtrl+Tryptase group.

downstream PAR2/MAPK/NF-κB activation. In addition, AS reduced VEGFA expression and restored ZO-1, supporting broader protective effects on neangiogenesis and peritoneal barrier integrity.

AS consists of *Astragalus membranaceus* and *Salvia miltiorrhiza*. Astragaloside IV has been reported to counteract PMC MMT induced by LPS-activated macrophages via modulation of Foxc1/β-catenin signaling through inhibition of macrophage-derived exosomal miR-204-5p.¹¹ Tanshinone IIA, a key constituent of *Salvia miltiorrhiza*, mitigates PF by suppressing TGF-β1 and connective tissue growth factor expression.²⁷ Despite these reported anti-inflammatory and anti-

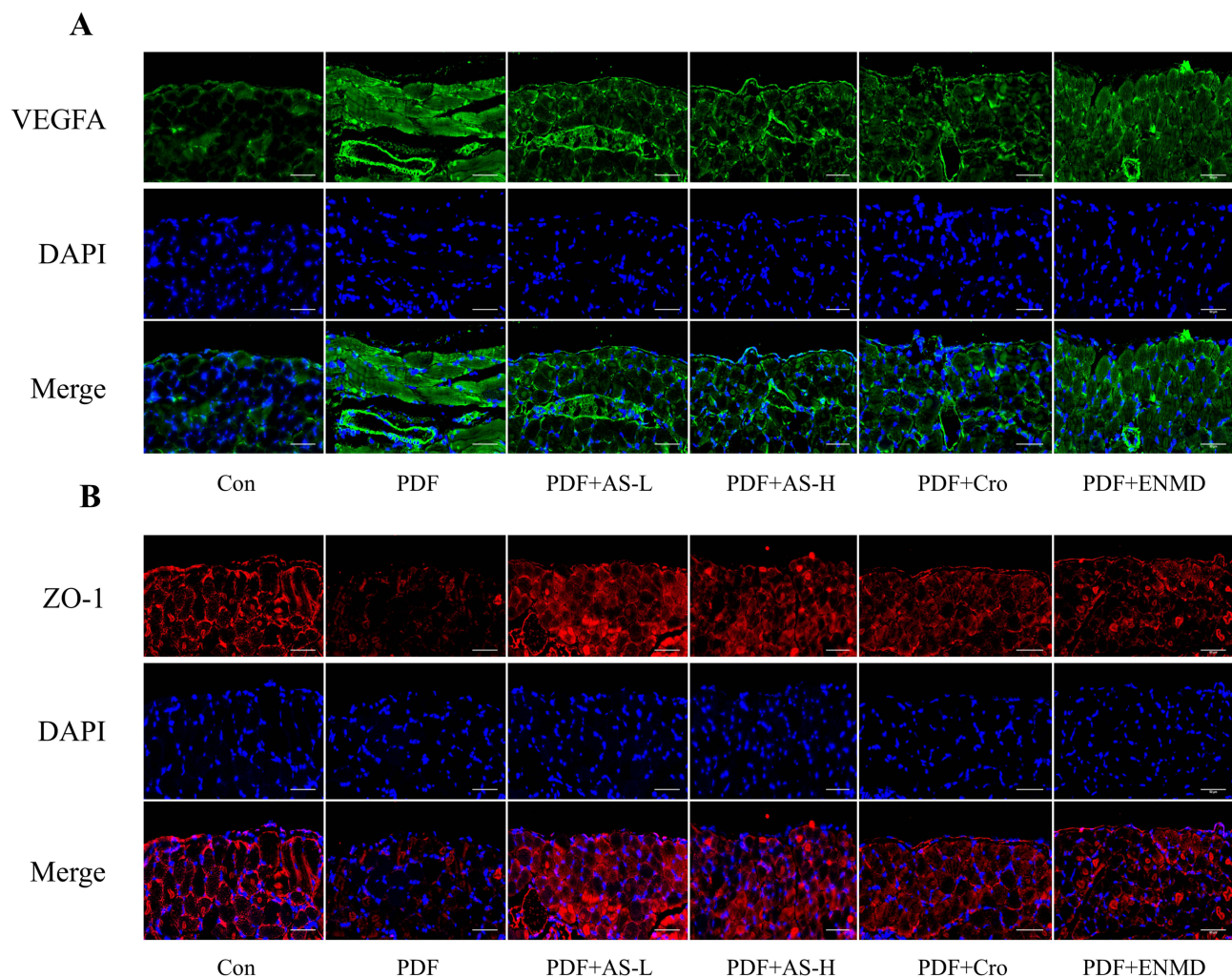


Figure 11 AS ameliorates neoangiogenesis and junctional integrity. **(A)** IF staining for VEGFA (green) with DAPI nuclear counterstaining (blue) in mouse peritoneal tissues ($n = 3$; scale bar = 50 μm). **(B)** IF staining for ZO-1 (red) with DAPI nuclear counterstaining (blue) in mouse peritoneal tissues ($n = 3$; scale bar = 50 μm).

fibrotic activities, the role of AS in regulating MC-driven peritoneal inflammation and fibrosis has not been systematically explored.

MC-derived mediators, including tryptase, are known contributors to inflammatory and fibrotic responses across multiple organs, and tryptase-PAR2 signaling has been implicated in pulmonary and renal fibrosis.^{13,28} Although MC accumulation has been observed in fibrotic peritoneum,^{9,10} their precise mechanistic role in PF has remained insufficiently clarified. Our *in vivo* and *in vitro* data consistently demonstrate that AS inhibits MC infiltration, activation, and degranulation, supporting a direct regulatory effect on this upstream inflammatory cell population. We further identified tryptase as a key messenger underlying MC-PMC crosstalk, with PAR2 functioning as a central mediator in PMCs. While PAR2 has been associated with renal interstitial fibrosis,^{13,29} its involvement in PD-related PF has been less explored. Our findings indicate that tryptase induces PAR2 activation and subsequent mesenchymal transition in HMrSV5 cells, whereas AS attenuates this signaling cascade.

PF is a multifactorial process involving MMT, inflammation, tissue injury, neoangiogenesis, and disruption of epithelial barrier integrity. MCs appear to participate in several of these events, and our data suggest that AS may modulate these processes through both direct cellular effects and indirect MC-mediated mechanisms. More prominently, PAR2 robustly engaged the MAPK/NF- κB axis, a central regulator of inflammatory and fibrotic signaling. Activation of this pathway has been associated with cytokine release and fibrosis in multiple organs, including diabetic nephropathy and renal injury.^{30,31} Consistent with these reports, phosphorylation of MAPK and NF- κB signaling proteins has also been observed in PF and is linked to PMC MMT and peritoneal inflammation.^{32,33} In our study, tryptase markedly increased phosphorylation of JNK,

p38, ERK1/2, and p65 in HMrSV5 cells, whereas AS treatment substantially suppressed this activation, indicating effective inhibition of this inflammatory-fibrotic signaling cascade. Additionally, TGF- β 1/Smad signaling, another recognized driver of PMC mesenchymal transition, was also attenuated by AS, further supporting its multi-target regulatory potential.³⁴

Beyond local peritoneal injury, chronic PD-associated inflammation has been reported to influence distant vascular beds and contribute to systemic vascular alterations.^{9,35} In this broader context, the anti-inflammatory effects of AS observed here may extend beyond localized fibrosis control, as suppression of MC activation and cytokine release could potentially reduce systemic inflammatory burden. Although clinical validation is required, these findings support potential translational relevance in PD management.

In parallel with its anti-inflammatory activity, AS also influenced vascular remodeling and epithelial integrity, both of which are critical in PF progression. Neoangiogenesis has been demonstrated to contribute to PF advancement, with peritoneal vascularization closely correlating with fibrosis severity.² VEGF is a key regulator of angiogenesis, and its level in peritoneal effluent increases with PF duration.³⁶ Inhibition of neoangiogenesis has been shown to ameliorate PF.³⁷ Activated MCs release mediators including VEGF, and PAR2 has been reported to promote VEGF production via ERK activation.³⁸ ZO-1, a crucial tight-junction protein, is essential for maintaining epithelial barrier integrity, and its downregulation during PMC MMT leads to impaired barrier function and enhanced fibrosis.³⁹ In studies of allergic rhinitis, PAR2-mediated downregulation of ZO-1 has been reported to contribute to nasal epithelial barrier dysfunction.⁴⁰ Collectively, these findings suggest that AS alleviates PF partly by suppressing neoangiogenesis and restoring junctional integrity, supporting its potential as a multi-target therapeutic strategy in PF.

Several limitations should be acknowledged. MC-deficient mouse models were not employed, and other inflammatory cell populations were not rigorously excluded; therefore, although MCs are strongly implicated, they are not definitively isolated as the sole drivers of fibrosis. In addition, the present findings are derived from preclinical models, and the use of LPS as a surrogate for infection may not fully recapitulate the clinical PD context. The inherent complexity and batch variability of herbal extracts, as well as the potential synergistic versus individual effects of AS constituents, may further influence translational reproducibility. Future studies incorporating MC-deficient models, dissecting the contributions of individual AS constituents, and validating these mechanisms in human PD cohorts will be important to strengthen mechanistic conclusions and enhance clinical applicability.

Conclusion

To our knowledge, this study provides mechanistic evidence that MC-derived tryptase promotes PDF-induced PF by activating PAR2 signaling in PMCs and downstream MAPK/NF- κ B pathways. AS alleviates PF, at least in part, by suppressing MC degranulation and inhibiting tryptase/PAR2-mediated MC-PMC crosstalk, accompanied by reduced inflammatory responses and improved barrier-associated features. Nevertheless, the preclinical nature of this work and extract variability should be considered when translating these findings to clinical PD settings.

ARRIVE Guidelines Statement

The animal experiments were performed in compliance with the ARRIVE guidelines. Mice were euthanized by an intraperitoneal injection of an overdose of sodium pentobarbital (60 mg/mL) at a volume of 0.067-0.083 mL, based on body weight (20-25 g). Death was confirmed after at least one minute by the cessation of respiration, followed by cervical dislocation.

Abbreviations

AS, *Astragalus membranaceus* and *Salvia miltiorrhiza*; Cro, Cromolyn; CKD, chronic kidney disease; Ctrl, control; ERK1/2, extracellular signal-regulated kinase1/2; ENMD-1068, N1-3-methylbutyryl-N4-6-aminohexanoyl-piperazine; HMrSV5, human peritoneal mesothelial cells; MMT, mesothelial-to-mesenchymal transition; MCs, mast cells; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor κ B; PD, peritoneal dialysis; PF, peritoneal fibrosis; PDF, peritoneal dialysis fluid; PAR2, protease-activated receptor 2; PMCs, peritoneal mesothelial cells; TCM, traditional Chinese Medicine; TNF- α , tumor necrosis factor- α ; VEGFA, vascular endothelial growth factor A; ZO-1, zona occludens-1.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The animal experiments were approved by the Experimental Animal Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (approval number: 2024DW-029-01) and complied with the Guidelines for the Ethical Review of Laboratory Animal Welfare (GB/T 35892-2018).

Author Contributions

Yanlin Chen: Writing - original draft, Validation, Methodology, Formal analysis, Data curation. Yuqing Wu: Validation, Methodology, Data curation, Writing - original draft. Chenghua Zhang: Validation, Software, Writing - original draft. Yuxin Tang: Data curation, Formal analysis, Writing - original draft. Weiwei Zhu: Methodology, Writing - review & editing. Wei Li: Validation, Writing - review & editing. Kun Gao: Funding acquisition, Resources, Writing - review & editing. Enchao Zhou: Funding acquisition, Supervision, Writing - review & editing. Xufang Wang: Funding acquisition, Project administration, Writing - review & editing.

All authors 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.

Funding

This study was supported by National Natural Science Foundation of China (82104750, 82474427, 82474473), Jiangsu Province Leading Talents Cultivation Project for Traditional Chinese Medicine (SLJ0319, SLJ0323) and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (SJCX24_0955).

Disclosure

All authors state that they have no conflicts of interest regarding the publication of this paper.

References

1. Zhao H, Zhang HL, Jia L. High glucose dialysate-induced peritoneal fibrosis: pathophysiology, underlying mechanisms and potential therapeutic strategies. *Biomed Pharmacother.* **2023**;165:115246. In eng. doi:10.1016/j.biopha.2023.115246
2. Zhang Z, Jiang N, Ni Z. Strategies for preventing peritoneal fibrosis in peritoneal dialysis patients: new insights based on peritoneal inflammation and angiogenesis. *Front Med.* **2017**;11:349–358. In eng. doi:10.1007/s11684-017-0571-2
3. Wang HH, Huang CH, Kuo MC, et al. Microbiology of peritoneal dialysis-related infection and factors of refractory peritoneal dialysis related peritonitis: a ten-year single-center study in Taiwan. *J Microbiol Immunol Infect.* **2019**;52:752–759. In eng. doi:10.1016/j.jmii.2018.10.013
4. Li H, Wang Y, Meng Q, et al. Comprehensive proteomic and metabolomic profiling of mcr-1-mediated colistin resistance in *Escherichia coli*. *Int J Antimicrob Agents.* **2019**;53:795–804. In eng. doi:10.1016/j.ijantimicag.2019.02.014
5. Margetts PJ, Kolb M, Yu L, et al. A chronic inflammatory infusion model of peritoneal dialysis in rats. *Perit Dial Int.* **2001**;21 Suppl 3:S368–72. In eng.
6. Caslin HL, Abebayehu D, Abdul qayum A, et al. Lactic acid inhibits lipopolysaccharide-induced mast cell function by limiting glycolysis and ATP availability. *J Immunol.* **2019**;203:453–464. In eng. doi:10.4049/jimmunol.1801005
7. Sun T, Wang Y, Hu S, et al. Lipopolysaccharide induces the early enhancement of mice colonic mucosal paracellular permeability mainly mediated by mast cells. *Histol Histopathol.* **2019**;34:191–200. In eng. doi:10.14670/hh-18-039
8. Dong H, Wang Y, Zhang X, et al. Stabilization of brain mast cells alleviates LPS-induced neuroinflammation by inhibiting microglia activation. *Front Cell Neurosci.* **2019**;13:191. In eng. doi:10.3389/fncel.2019.00191
9. Ito Y, Sun T, Tawada M, et al. Pathophysiological mechanisms of peritoneal fibrosis and peritoneal membrane dysfunction in peritoneal dialysis. *Int J Mol Sci.* **2024**. In eng. doi:10.3390/ijms25168607 25
10. Terri M, Trionfetti F, Montaldo C, et al. Mechanisms of peritoneal fibrosis: focus on immune cells-peritoneal stroma interactions. *Front Immunol.* **2021**;12:607204. In eng. doi:10.3389/fimmu.2021.607204
11. Kazama I, Baba A, Endo Y, et al. Mast cell involvement in the progression of peritoneal fibrosis in rats with chronic renal failure. *Nephrology.* **2015**;20:609–616. In eng. doi:10.1111/nep.12489
12. Li Y, Di C, Song S, et al. Choroid plexus mast cells drive tumor-associated hydrocephalus. *Cell.* **2023**;186:5719–38.e28. In eng. doi:10.1016/j.cell.2023.11.001
13. Ha S, Chung KW, Lee J, et al. Renal tubular PAR2 promotes interstitial fibrosis by increasing inflammatory responses and EMT process. *Arch Pharm Res.* **2022**;45:159–173. In eng. doi:10.1007/s12272-022-01375-5
14. Sun Q, Wang Y, Zhang J, et al. ENMD-1068 inhibits liver fibrosis through attenuation of TGF- β 1/Smad2/3 signaling in mice. *Sci Rep.* **2017**;7:5498. In eng. doi:10.1038/s41598-017-05190-7

15. Shan Y, Yu M, Dai H, et al. The role of macrophage-derived exosomes in reversing peritoneal fibrosis: insights from Astragaloside IV. *Phytomedicine*. 2024;129:155683. In eng. doi:10.1016/j.phymed.2024.155683
16. Sun J, Tang L, Shan Y, et al. TMT quantitative proteomics and network pharmacology reveal the mechanism by which asiaticoside regulates the JAK2/STAT3 signaling pathway to inhibit peritoneal fibrosis. *J Ethnopharmacol*. 2023;309:116343. In eng. doi:10.1016/j.jep.2023.116343
17. Sheng L, Sun J, Huang L, et al. *Astragalus membranaceus* and its monomers treat peritoneal fibrosis and related muscle atrophy through the AR/TGF- β 1 pathway. *Front Pharmacol*. 2024;15:1418485. In eng. doi:10.3389/fphar.2024.1418485
18. Yu L, Li J, Wang X, et al. Danshen Injection inhibits peritoneal dialysis fluid-induced endothelial-mesenchymal transition in HMrSV5 cells by regulating the TGF- β /Smad signaling pathway. *Nan Fang Yi Ke Da Xue Xue Bao*. 2024;44:2276–2282. In eng. doi:10.12122/j.issn.1673-4254.2024.12.02
19. Zhou Y, He W, Sun W, et al. Sulfotanshinone IIA sodium ameliorates glucose peritoneal dialysis solution-induced human peritoneal mesothelial cell injury via suppression of ASK1-P38-mediated oxidative stress. *Cell Physiol Biochem*. 2018;46:2434–2444. In eng. doi:10.1159/000489650
20. Zhou F, Yao L, Lu X, et al. Therapeutic targeting of GSK3 β -regulated Nrf2 and NF κ B signaling pathways by salvianolic acid A ameliorates peritoneal fibrosis. *Front Med Lausanne*. 2022;9:804899. In eng. doi:10.3389/fmed.2022.804899
21. Meng X, Sheng L, You Y, et al. Integrated serum metabolomics and network pharmacology reveal molecular mechanism of Qixue Huazheng formula on peritoneal fibrosis. *Front Pharmacol*. 2025;16:1515038. In eng. doi:10.3389/fphar.2025.1515038
22. Xu Q, Liu X, Chen Z, et al. Combination decoction of *Astragalus mongholicus* and *Salvia miltiorrhiza* mitigates pressure-overload cardiac dysfunction by inhibiting multiple ferroptosis pathways. *Front Pharmacol*. 2024;15:1447546. In eng. doi:10.3389/fphar.2024.1447546
23. Bagher M, Larsson-Callerfelt AK, Rosmark O, et al. Mast cells and mast cell tryptase enhance migration of human lung fibroblasts through protease-activated receptor 2. *Cell Commun Signal*. 2018;16:59. In eng. doi:10.1186/s12964-018-0269-3
24. Zhao T, Sun Z, Lai X, et al. Tamoxifen exerts anti-peritoneal fibrosis effects by inhibiting H19-activated VEGFA transcription. *J Transl Med*. 2023;21:614. In eng. doi:10.1186/s12967-023-04470-3
25. Jia M, Qiu H, Lin L, et al. Inhibition of PI3K/AKT/mTOR signalling pathway activates autophagy and suppresses peritoneal fibrosis in the process of peritoneal dialysis. *Front Physiol*. 2022;13:778479. In eng. doi:10.3389/fphys.2022.778479
26. Krediet RT, Struijk DG. Peritoneal changes in patients on long-term peritoneal dialysis. *Nat Rev Nephrol*. 2013;9:419–429. In eng. doi:10.1038/nrneph.2013.99
27. Chunming J, Miao Z, Cheng S, et al. Tanshinone IIA attenuates peritoneal fibrosis through inhibition of fibrogenic growth factors expression in peritoneum in a peritoneal dialysis rat model. *Ren Fail*. 2011;33:355–362. In eng. doi:10.3109/0886022x.2011.559681
28. Xu Z, Cai K, Su SL, et al. Salvianolic acid B and tanshinone IIA synergistically improve early diabetic nephropathy through regulating PI3K/Akt/NF- κ B signaling pathway. *J Ethnopharmacol*. 2024;319:117356. In eng. doi:10.1016/j.jep.2023.117356
29. Grandaliano G, Pontrelli P, Cerullo G, et al. Protease-activated receptor-2 expression in IgA nephropathy: a potential role in the pathogenesis of interstitial fibrosis. *J Am Soc Nephrol*. 2003;14:2072–2083. In eng. doi:10.1097/01.asn.0000080315.37254.a1
30. Vesey DA, Suen JY, Seow V, et al. PAR2-induced inflammatory responses in human kidney tubular epithelial cells. *Am J Physiol Renal Physiol*. 2013;304:F737–50. In eng. doi:10.1152/ajprenal.00540.2012
31. Han X, Wei J, Zheng R, et al. Macrophage SHP2 deficiency alleviates diabetic nephropathy via suppression of MAPK/NF- κ B- dependent inflammation. *Diabetes*. 2024;73:780–796. In eng. doi:10.2337/db23-0700
32. Yu C, Yang N, Wang W, et al. Blocking core fucosylation of epidermal growth factor (EGF) receptor prevents peritoneal fibrosis progression. *Ren Fail*. 2021;43:869–877. In eng. doi:10.1080/0886022x.2021.1918557
33. Yu M, Shin HS, Lee HK, et al. Effect of aldosterone on epithelial-to-mesenchymal transition of human peritoneal mesothelial cells. *Kidney Res Clin Pract*. 2015;34:83–92. In eng. doi:10.1016/j.krcp.2015.03.005
34. Su W, Wang H, Feng Z, et al. Nitro-oleic acid inhibits the high glucose-induced epithelial-mesenchymal transition in peritoneal mesothelial cells and attenuates peritoneal fibrosis. *Am J Physiol Renal Physiol*. 2020;318:F457–f67. In eng. doi:10.1152/ajprenal.00425.2019
35. Elphick EH, Zavvos V, Belcher J, et al. The role of peritoneal interleukin-6 in predicting patient survival on peritoneal dialysis. *Kidney Int Rep*. 2025;10:3164–3173. In eng. doi:10.1016/j.ekir.2025.06.049
36. Zweers MM, Struijk DG, Smit W, et al. Vascular endothelial growth factor in peritoneal dialysis: a longitudinal follow-up. *J Lab Clin Med*. 2001;137:125–132. In eng. doi:10.1067/mlc.2001.112235
37. Kariya T, Nishimura H, Mizuno M, et al. TGF- β 1-VEGF-A pathway induces neoangiogenesis with peritoneal fibrosis in patients undergoing peritoneal dialysis. *Am J Physiol Renal Physiol*. 2018;314:F167–f80. In eng. doi:10.1152/ajprenal.00052.2017
38. Dutra-Oliveira A, Monteiro RQ, Mariano-Oliveira A. Protease-activated receptor-2 (PAR2) mediates VEGF production through the ERK1/2 pathway in human glioblastoma cell lines. *Biochem Biophys Res Commun*. 2012;421:221–227. In eng. doi:10.1016/j.bbrc.2012.03.140
39. Strippoli R, Loureiro J, Moreno V, et al. Caveolin-1 deficiency induces a MEK-ERK1/2-Snail-1-dependent epithelial-mesenchymal transition and fibrosis during peritoneal dialysis. *EMBO Mol Med*. 2015;7:357. In eng. doi:10.15252/emmm.201570010
40. Wang J, Kang X, Huang ZQ, et al. Protease-activated receptor-2 decreased zonula occludens-1 and claudin-1 expression and induced epithelial barrier dysfunction in allergic rhinitis. *Am J Rhinol Allergy*. 2021;35:26–35. In eng. doi:10.1177/1945892420932486