

Retrospective Investigation, Network Pharmacology and Experimental Validation of *Salvia miltiorrhiza* and Its Main Active Component Tanshinone IIA in the Treatment of Acute Pancreatitis

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Abstract

Purpose

To investigate the efficacy and mechanism of *Salvia miltiorrhiza*(SM) and its active component tanshinone IIA (TSA) against acute pancreatitis (AP).

Methods

We retrospectively analyzed data from 204 patients with acute pancreatitis, divided into conventional western medicine group (104 cases) and *Salvia miltiorrhiza* combined with conventional western medicine group (100 cases). The core targets and pathways of tanshinone IIA were screened via network pharmacology, verified by molecular docking and in vivo mouse experiments.

Results

Salvia miltiorrhiza combination treatment significantly reduced hospitalization costs, pneumonia incidence, abdominal pain duration, white blood cell counts and triglyceride levels. Network pharmacology identified 168 potential targets of tanshinone IIA, with the PI3K-AKT signaling pathway as the most enriched pathway. In vivo experiments confirmed tanshinone IIA alleviated pancreatic injury and inflammatory infiltration via inhibiting the PI3K/AKT/Bcl2 pathway.

Conclusions

Salvia miltiorrhiza has favorable clinical benefits for acute pancreatitis. Its main active component tanshinone IIA exerts pancreatic protective effects via regulating the PI3K/AKT/Bcl2 signaling pathway. Trial registration: International Traditional Medicine Clinical Trial Registry, ITMCTR2025000954. Retrospectively registered on 25 April 2025.

1. Introduction

Acute pancreatitis (AP) is an inflammatory disorder of the pancreas, initiated by premature trypsinogen activation resulting from diverse causative factors. The autodigestive effects of pancreatic enzymes can induce necrosis within the gland and potentially lead to systemic multiple organ dysfunction. Globally, AP affects about 34 people per 100,000 each year, and this rate continues to increase.[1, 2] About 15–20% of cases advance to moderately severe or severe AP, with associated mortality climbing to 20–40% when organ failure develops.[3] Due to the multifactorial pathogenesis of AP, no specific pharmacologic treatment has received worldwide approval for preventing or managing this condition.[4] Thus, discovering and confirming effective drugs remains a pressing need.

As a traditional Chinese medicine (TCM), *Salvia miltiorrhiza* (SM) has been extensively studied for its efficacy in treating cardiovascular disease, diabetes, and liver disease.[5–8] Preliminary clinical evidence suggests that SM injection may support the recovery of damaged pancreatic tissue.[9] Tanshinone IIA (TSA) is a purified, structurally defined lipophilic active monomer isolated from SM. The Compound SM Injection used in the clinical cohort is a standardized herbal preparation approved by the National Medical Products Administration (NMPA) with unified quality control standards. TSA, SM's major lipid-soluble constituent, possesses anti-inflammatory, anticoagulant, antithrombotic, and neuroprotective qualities, primarily mediated through TLR/NF- κ B and MAPK/NF- κ B signaling.[10] Additionally, TSA has shown protective effects in AP models, reducing oxidative damage via the Nrf2/ROS axis.[11] Further investigation into the protective mechanisms of TSA in AP is therefore warranted.

Network pharmacology has become a widely used approach for deciphering the mechanistic basis of traditional medicines.[12, 13] In this study, we evaluated the efficacy of SM and its main active component TSA in the treatment of AP. A comprehensive strategy that integrates retrospective clinical analysis, pharmacological prediction through network-based methods, and experimental validation was employed to clarify the fundamental mechanisms of action.

2. Materials and Methods

2.1 Retrospective Investigation

The retrospective clinical study was approved by the Ethics Review Committee of Fujian Tingzhou Hospital (Approval No. : tzyy20240633, approved on June 15, 2024) and was performed in accordance with the ethical standards laid down in the 2013 revised version of the Declaration of Helsinki. The requirement for written informed consent was waived by the Ethics Review Committee due to the retrospective nature of the study. This retrospective clinical study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

2.1.1 Study setting and data source

We retrospectively selected patients with AP from our hospital between January 2019 and January 2023. The patients were divided into two groups based on different treatment protocols: the Western medicine (WM) treatment group and the *Salvia miltiorrhiza* combined with conventional Western medicine (SM&WM) treatment group.

2.1.2 Study patients

The inclusion criteria for the study were as follows: 1) participants must be aged 18 years or older; 2) a diagnosis aligning with the recognized criteria the 2012 revised Atlanta classification criteria for acute pancreatitis; and 3) presentation within a 72-hour window following the onset of symptoms. Exclusion criteria were: 1) AP resulting from trauma, surgery, or tumor; 2) severe extracorporeal comorbidities

including cardiopulmonary ailments; 3) being pregnant or nursing; 4) diagnosed malignant tumors; and 5) previous allergic reactions to the study medication.

2.1.3 Therapeutic protocol

Both groups of patients with AP were treated according to established clinical guidelines, which included fluid resuscitation, pain management, nutritional support, prevention of infectious complications, and surgical intervention when necessary. The SM&WM group received a combined treatment with Compound SM Injection (Chengdu, Sichuan, Shenghe Pharmaceutical Co, Ltd, National Medical Products Administration (NMPA) Approval No. Z51021303, specification: 10ml) in addition to the aforementioned therapies. This treatment was administered by mixing 20ml of Compound SM Injection with 250ml of 5% glucose injection via intravenous infusion, once daily.

2.1.4 Outcome measures

Primary outcomes were total hospitalization duration, overall medical costs, mortality, and length of intensive care unit (ICU) stay. Secondary outcomes covered time until abdominal pain resolved, bowel function recovery, occurrence of organ failure (respiratory, circulatory, or renal), local complications, signs of infection, and alterations in complete blood count parameters.

2.2 Network Pharmacology Analysis

2.2.1 Targets Screening of TSA

The predicted targets were initially retrieved from various databases, including TCMSP (<http://old.tcmsp-e.com>), NCBI (<http://www.ncbi.nlm.nih.gov>), and PubChem (<http://pubchem.ncbi.nlm.nih.gov>), by searching for "Tanshinone IIA". Protein interactions with TSA were identified using the STITCH database (<http://stitch.embl.de>), with the species restricted to "Homo sapiens". The two-dimensional (2D) structure sdf files of TSA, downloaded from PubChem, were then imported into the SwissTargetPrediction database (<http://www.swisstargetprediction.ch>), also limited to "Homo sapiens", to acquire the predicted targets. Subsequently, the protein names of the predicted targets obtained from the five databases were standardized to official gene symbols using the Uniprot database (<https://www.uniprot.org/>). Finally, the screened targets were obtained by merging the data and removing duplicates.

2.2.2 Targets Screening of AP

The predicted targets for AP were collected from various resources by searching with the term "acute pancreatitis" and restricting the species to "Homo sapiens". Data sources included NCBI, DisGeNet (<http://www.disgenet.org>), GeneCards (<https://www.genecards.org/>), OMIM (<http://omim.org>), and HPO (<http://hpo.jax.org/app/>). To obtain the final set of screened targets for AP, the potential targets were merged, and duplicate entries were removed.

2.2.3 Construction of Protein-Protein Interaction (PPI) Networks

The predicted targets associated with TSA and AP were examined utilizing the Venn diagram function provided by the "venn" package in R software. The common targets that were identified were subsequently uploaded to the STRING database version 11.5 (<http://string-db.org>) to facilitate the construction of a PPI network, specifically focusing on "Homo sapiens" and employing a confidence score threshold greater than 0.9. The resultant PPI data were exported as TSV files and subsequently visualized using Cytoscape version 3.8.0. Furthermore, a topological analysis was conducted via the CytoHubba plugin, which enabled the computation of betweenness centrality, shortest path, and degree centrality for the target proteins. Nodes exhibiting degree values equal to or exceeding the median value of 7.05 were designated as potential candidate targets. The top 40 targets were then resubmitted to the STRING database with the same parameters as before. CYP3A4, which shows no interactions, was excluded. The resulting PPI data were saved as TSV files and visualized in Cytoscape 3.8.0.

2.2.4 Performance Analysis of GO and KEGG Pathway Enrichment

The analysis of Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment for potential therapeutic targets was performed using the "clusterProfiler" R package (www.r-project.org). The results were illustrated through bubble plots, employing a significance criterion set at a p-value of less than 0.05. The GO enrichment analysis delineated the top ten terms across biological processes (BP), cellular components (CC), and molecular functions (MF). In contrast, the KEGG enrichment analysis pinpointed the fifteen most enriched pathways. Among the pathways identified through the KEGG enrichment analysis, the PI3K-AKT pathway (hsa04151) was specifically selected. Following this, a subnetwork encompassing targets associated with the PI3K-AKT pathway was extracted from the overarching network.

2.2.5 Molecular Docking

The initial structural representation of TSA was acquired from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov>), and its three-dimensional structure was subsequently generated using Chem3D software. Following this, protein domain pdb format files for TP53 (ID: 5O1D), AKT1 (ID: 1UNQ), MYC (ID: 6G6K), HDAC1 (ID: 5ICN), CTNNB1 (ID: 7AFW), RELA (ID: 1NFI), CCND1 (ID: 6P8H), and Bcl2 (ID: 8HTS) were retrieved from the Protein Data Bank (PDB) (<http://www.rcsb.org>). To identify active pockets, AutoDockTools version 1.5.6 was employed to convert the pdb format files of the active compounds and principal proteins into pdbqt format. Subsequently, the Vina script was executed to compute the molecular binding energies, and the results of the docking simulations were presented. Additionally, the Discovery Studio 2019 software was used to identify docking sites and assess the flexible binding LibDockScore. The resultant molecular docking outputs were imported into PyMOL.

software for the visualization of the docking analyses. All database retrievals were conducted in March 2025.

2.3 Experimental Validation

The research adhered to the Principles of Laboratory Animal Care as outlined in NIH Publication No. 85Y23 (revised 1996). The animal experimental protocol was approved by the Science and Technology Commission of Yangzhou University Medical College (Approval No.: YXYLL-2023-144, approved on March 10, 2023). All experimental procedures were performed in strict accordance with the 3Rs principles (Replacement, Reduction, Refinement) for laboratory animal welfare. This study complied with the ARRIVE guidelines and both national and institutional guidelines for the care and use of laboratory animals, with all procedures designed to minimize animal suffering. Humane endpoints were predefined: mice would be euthanized early if they presented with severe distress, body weight loss > 20%, or inability to access food and water, to minimize unnecessary suffering.

2.3.1 Animals

Male ICR mice, each weighing between 25–30 g, were sourced from the Model Animal Center at a university-affiliated model animal center in Nanjing Jiangsu Province, China. These mice were kept in specific pathogen-free environments, provided with standard laboratory feed and water, and were maintained at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under a 12-hour light and dark cycle prior to the initiation of the experiment.

2.3.2 AP Model Preparation and Drugs Intervention

Mice were randomly divided into three groups: the control group, the Caerulein-induced AP group (Cae), and the TSA-treated (25 mg/kg) Cae-induced AP group (TSA).[14] Each group consisted of 6 mice. To induce the AP model, Cae (100 $\mu\text{g}/\text{kg}$; Nanjing Peptide Co. Ltd., Cat. Pep03263) [15] was intraperitoneally injected at one-hour intervals for a total of 10 injections. TSA (Cat. HY-N1370-100 mg, MCE Co. Ltd., New Jersey, USA) was dissolved in phosphate-buffer saline (PBS) and injected intraperitoneally at the second hour before Cae injection. The control group was administered an equivalent volume of PBS. All mice were euthanized 12 hours following the initial administration of Cae, as illustrated in Fig. 5a.

2.3.3 Sample Collection

Following the administration of an intraperitoneal injection of sodium pentobarbital at a dosage of 50 mg/kg, mice from each experimental group were euthanized. Orbital venous blood samples were subsequently harvested to assess serum levels of amylase and lipase, in accordance with the manufacturer's instructions. The Amylase Kits (Catalog Number 100000060) were procured from Biosino Bio-Technology, located in Beijing, China, while the Lipase Kits (Catalog Number A054-1-1) were sourced from Nanjing Jiancheng Corporation in Nanjing, Jiangsu China. Pancreatic tissue specimens were obtained and promptly preserved in a 4% phosphate-buffered formaldehyde solution, subsequently

embedded in paraffin blocks to facilitate hematoxylin and eosin staining. The leftover pancreatic tissue samples were maintained at a temperature of -80°C for subsequent analyses.

2.3.4 Histopathological Analysis

Pancreatic tissues were fixed in 4% paraformaldehyde and subsequently embedded in paraffin. Prior to hematoxylin-eosin staining, the paraffin-embedded tissue underwent dewaxing and dehydration processes. Stained tissues were examined using a light microscope. Histopathological scoring of the pancreatic tissues was conducted by two pathologists independently and in a blinded manner, following the methods outlined in our previous study.

2.3.5 Immunohistochemistry (IHC)

The preparation of tissue slides involved several critical steps, including dewaxing, dehydration, antigen retrieval, and the inhibition of endogenous peroxidase activity alongside the application of normal goat serum for blocking. Subsequently, the slides were incubated overnight at a temperature of 4°C with a rabbit monoclonal antibody targeting F4/80 (dilution of 1:100; GB113373, Servicebio, Wuhan, China) and an anti-Myeloperoxidase (MPO) antibody (dilution of 1:200; ab208670, Abcam, Cambridge, UK). Following this, an HRP-conjugated anti-rabbit secondary antibody was introduced and allowed to incubate at room temperature for a duration of 20 minutes. After the incubation period, the slides underwent DAB staining followed by hematoxylin staining. The specimens that were stained were subsequently analyzed under a light microscope (BX53, Olympus, Tokyo, Japan). The areas exhibiting positive staining were evaluated in a semi-quantitative manner utilizing Image J 1.x software.

2.3.6 Western Blotting

Western blotting was conducted on pancreatic tissue samples. The total protein concentrations were quantified utilizing the bicinchoninic acid assay in accordance with the manufacturer's instructions. Protein separation was achieved via 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Following separation, the proteins were transferred to a polyvinylidene fluoride membrane, which was subsequently blocked with a 5% solution of defatted milk or bovine serum diluted in TBS + Tween-20, and left at room temperature for 2 hours. The membranes were then incubated overnight at 4°C with primary antibodies including anti-Bcl2 (1:500, Cat. sc7382, Santa Cruz, CA, USA), anti-AKT (1:1000, Cat. cst4691T, Cell Signaling Technology, Danvers, Massachusetts, USA), anti-phospho-AKT (1:1000, Cat. cstS473, Cell Signaling Technology, Danvers, Massachusetts, USA), and β -actin (1:1000, Cat. sc47778, Santa Cruz, CA, USA). Following this incubation, the membranes were washed three times with TBST, with each wash lasting 15 minutes. The membranes were then incubated with a secondary antibody, IgG HRP (Horseradish peroxidase), diluted 1:5000 in 5% (w/v) skim milk in TBST, at room temperature for an additional 2 hours. Subsequently, the membranes underwent three more washes with TBST for 15 minutes each. The detection of protein bands was performed using the ECL Plus chemiluminescent system, and the intensity of the images was analyzed using ImageJ 1.x software.

2.3.7 Statistical Analysis

Statistical evaluations were carried out utilizing GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA) alongside SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were represented as mean $\pm$ standard deviation or median (interquartile range), whereas categorical variables were conveyed in terms of percentages. The assessment of continuous variables was performed using either Student's t-test or the Mann-Whitney U test, while categorical data were examined via the chi-square test or Fisher's exact test. A *P*-value of less than 0.05 was established as the threshold for statistical significance, with significance levels indicated as * for *P* < 0.05, ** for *P* < 0.01, and *** for *P* < 0.001.

3. Results

3.1 Retrospective Investigation

3.1.1 Patient screening and enrollment

Between January 2019 and January 2023, an initial cohort of 292 patients was identified from the AP database. Among the total participants, 204 individuals met the defined inclusion criteria and were subsequently included in the analysis. This cohort consisted of 104 patients assigned to the WM group and 100 patients allocated to the SM&WM group (Fig. 1).

3.1.2 Baseline characteristics

The two groups of patients were generally well-balanced. No statistically meaningful discrepancies were noted among the groups regarding age, sex, smoking history, alcohol history, medical history, etiology, time of onset, BMI, mean arterial pressure, Multislice Computed Tomography (MSCT) scores, Systemic Inflammatory Response Syndrome (SIRS) scores, Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, Bedside Index of Severity in Acute Pancreatitis (BISAP) scores, Sequential Organ Failure Assessment (SOFA) scores and other severity scores. Detailed baseline characteristics of the participants were shown in Table 1.

Table 1
Baseline characteristics of patients with acute pancreatitis.

Parameters	WM group (n = 104)	SM&WM group (n = 100)	t/x ²	P
Age, years	44.66 ± 14.285	47.10 ± 14.477	-1.210	0.228
Sex, male	71(68.3%)	63(63%)	0.628	0.428
Smoking	39(37.5%)	30(30.0%)	1.281	0.258
History of alcohol intake (%)	52(50%)	41(41%)	1.665	0.197
Medical history (%)				
Diabetes mellitus	36(34.6%)	39(39%)	0.422	0.516
Hypertension	27(26%)	24(24%)	0.105	0.746
Fatty liver disease	41(39.4%)	41(41%)	0.053	0.818
Etiology, (%)			0.851	0.837
Biliary	16(15.4%)	14(14.0%)		
Hypertriglyceridemia	73(70.2%)	75(75.0%)		
Alcohol	7(6.7%)	6(6.0%)		
Others	8(7.7%)	5(5.0%)		
BMI	25.669 ± 3.426	25.135 ± 2.984	1.189	0.236
Mean arterial pressure	103.756 ± 13.308	101.503 ± 11.515	1.296	0.198
Clinical severity scores				
MSCT	2(2–4)	2(2–4)		0.861
SIRS	2(1–2)	2(1–3)		0.725
APACHE II	3(1–5)	3(1–5)		0.906
BISAP	1(0–1)	1(0–1)		0.493
SOFA	1(0–1)	1(0–2)		0.405
Marshall	1(0–1)	1(0–1)		0.890
Onset hours(hours)	15(7–23)	12(6–22)		0.271
Note: Continuous variables are presented as mean ± SD or median (interquartile range), and compared using Student's t-test or Mann-Whitney U test. Categorical variables are expressed as percentages and compared using χ^2 test or Fisher's exact test.				

Parameters	WM group (n = 104)	SM&WM group (n = 100)	t/ χ^2	P
Laboratory markers on the first day				
Hematocrit (L/L)	0.43 $\pm$ 0.047	0.42 $\pm$ 0.051	0.361	0.719
White blood cell counts($\times 10^9$ /L)	13.757 $\pm$ 4.445	13.901 $\pm$ 4.166	-1.543	0.124
Albumin(g/L)	42.653 $\pm$ 4.172	42.214 $\pm$ 3.377	0.827	0.409
Lactate dehydrogenase(IU/L)	236(204.5-293.5)	223(183.5–262.0)		0.068
Serum glucose(mmol/L)	9.7(7.0-13.05)	9.45(7.35-13.0)		0.915
Blood urinary nitrogen (mmol/L)	4.77(3.97–5.56)	4.64(3.54–5.57)		0.439
Creatinine(mmol/L)	75.30(63.15–87.9)	79.85(67.2–92.6)		0.056
Calciumions(mmol/L)	2.407 $\pm$ 0.167	2.397 $\pm$ 0.178	0.699	0.692
Triglycerides(mmol/L)	14.19(4.44–19.09)	15.87(9.73–19.47)		0.310
Note: Continuous variables are presented as mean $\pm$ SD or median (interquartile range), and compared using Student's t-test or Mann-Whitney U test. Categorical variables are expressed as percentages and compared using χ^2 test or Fisher's exact test.				

3.1.3 Primary outcome and Secondary outcomes

For the primary outcomes, the SM&WM group showed significantly lower hospitalization costs ($P = 0.041$). No notable differences were observed between the two groups in terms of total hospital stay, ICU admission, or mortality. Among secondary outcomes, the SM&WM group had a lower incidence of pneumonia ($P = 0.043$) and a shorter duration of abdominal pain ($P = 0.029$).

The disparities in local complications, including pseudocysts, necrosis, and fluid accumulation, were not statistically significant. Likewise, the investigation revealed no considerable differences in the incidence of pleural effusion, respiratory failure, circulatory failure, renal failure, or the duration required for bowel recovery. Additionally, the analysis indicated that there were no statistically meaningful differences observed in the utilization of non-invasive positive pressure ventilation (NIPPV), inotropic medications, or continuous renal replacement therapy (CRRT). Comparison of laboratory results on the third day after treatment indicated that the SM&WM group had significantly reduced white blood cell counts ($P = 0.007$) and triglyceride levels ($P < 0.001$) (Table 2).

Table 2
Primary outcome and Secondary outcomes

Primary outcome	WM group (n = 104)	SM&WM group (n = 100)	t/x ²	P
Hospitalization costs (¥RMB/person)	6400.339 ± 2116.42	5765.63 ± 2277.38	2.060	0.041
Length of stay (days)	7(5–8)	6(5–8)		0.104
ICU admission (%)	4(3.8%)	3(3%)		0.522
Mortality (%)	3(2.9%)	2(2%)		0.519
Secondary outcomes				
Pseudocyst (%)	1(1%)	1(1%)		0.741
Necrotic material accumulation (%)	3(2.9%)	1(1%)		0.326
Fluid accumulation (%)	13(12.5%)	12(12%)	0.012	0.913
Incidence of pneumonia (%)	25(24.0%)	13(13.0%)	4.098	0.043
Pleural effusion (%)	9(8.7%)	6(6.0%)	0.527	0.468
Respiratory failure (%)	12(11.5%)	10(10.0%)	0.125	0.450
Circulatory failure (%)	3(2.9%)	2(2.0%)		0.519
Renal failure (%)	3(2.9%)	3(3.0%)		0.640
NIPPV (%)	10(9.6%)	7(7%)	0.457	0.499
NIPPV (days)	3(2–5)	2(1–4)		0.846
CRRT (%)	3(2.9%)	1(1%)		0.622
CRRT (days)	2(1–4)	1(1–3)		0.333
Inotropic agents (%)	4(3.8%)	3(3%)		0.522
Inotropic agents (days)	2(1–5)	2(1–3)		0.732
Duration of abdominal pain (days)	4(3–5)	3(3–4)		0.029
Bowel movement recovery time (days)	2(1–2)	2(1–2)		0.746
Laboratory markers on the third day				
Hematocrit(L/L)	0.39 ± 0.047	0.37 ± 0.049	1.136	0.134
White blood cell counts(×10⁹ /L)	9.314 ± 3.287	8.167 ± 2.677	2.711	0.007
Albumin(g/L)	35.795 ± 5.406	34.744 ± 4.369	1.326	0.184

Primary outcome	WM group (n = 104)	SM&WM group (n = 100)	<i>t</i> / <i>x</i> ²	<i>P</i>
Lactate dehydrogenase(IU/L)	196(169–229)	197(170–216)		0.389
Serum glucose(mmol/L)	8.0(6.6–11.2)	8.5(6.5–10.5)		0.944
Blood urinary nitrogen(mmol/L)	3.71(3.08–4.13)	3.91(2.98–4.96)		0.154
Creatinine(mmol/L)	77.020 ± 15.267	79.626 ± 13.411	-1.226	0.224
Calciumions(mmol/L)	2.279 ± 0.181	2.249 ± 0.154	0.214	0.214
Triglycerides(mmol/L)	6.15(2.37–8.10)	3.37(1.49–5.17)		< 0.001

3.2 Network Pharmacology Analysis

3.2.1 Predicted Targets of TSA and AP

By implementing predetermined screening criteria, a total of 193 predicted targets for TSA were identified from various databases, including PubChem (13), STITCH (9), Swiss (121), NCBI (29), and TCMSP (38) (Fig. 2a). In addition, a total of 7468 AP-associated targets were identified through screening databases such as DisGeNet (86), NCBI (323), OMIM (68), HPO (8), and Genecards (7449) (Fig. 2b).

3.2.2 Common Targets and PPI Network Analysis

Through the construction and analysis of Venn diagrams, we identified a total of 168 potential therapeutic targets of TSA in AP (Fig. 2c). These targets were then imported into STRING to generate a PPI network. Subsequently, the PPI network was visualized by using Cytoscape, resulting in two distinct networks. The first network consisted of 119 nodes and 420 edges (Fig. 2d), while the second network consisted of 39 nodes and 197 edges (Fig. 2e, Fig. 2f). Notably, TP53, AKT1, NF-κB, and Bcl2 were identified in both PPI network maps.

3.2.3 GO Function and KEGG Pathway Enrichment Analyses

GO and KEGG enrichment analyses were conducted to clarify the biological functions and pathways linked to the identified candidate targets. The GO enrichment analysis revealed a predominant association of BP with "regulation of apoptotic signaling pathway", while MF were closely linked to "p53 binding" and "NF-κB binding" (Fig. 3a). The KEGG enrichment analysis identified the top one pathway as "PI3K-Akt signaling pathway" (Fig. 3b). The PI3K-AKT pathway (hsa04151) was visualized using the "pathview" package in R software, with the candidate targets highlighted in red (Fig. 3c). P53, NF-κB, and Bcl2 were identified as the downstream targets of AKT.

3.2.4 Molecular Docking Verification

Molecular docking analyses were conducted to assess the interactions between TSA and the potential target proteins, namely TP53, AKT1, MYC, HDAC1, CTNNB1, RELA, CCND1, and Bcl2. The calculated binding energies for these interactions were -8.2 kcal/mol, -6.3 kcal/mol, -5.8 kcal/mol, -6.8 kcal/mol, -6.7 kcal/mol, -6.7 kcal/mol, -7.4 kcal/mol, and -7.2 kcal/mol, respectively. Figure 4 displays the 2D and 3D diagrams illustrated the binding hydrogen bonds. The stability of the conformational binding between target proteins and ligands (active compounds) was assessed based on the binding affinity, categorized into strong interactions (<-6.0 kcal/mol), moderate interactions (-6.0~-5.0 kcal/mol), and weak interactions (-5.0~-4.0 kcal/mol).[14, 16] The results indicated strong interactions between TSA and the top 7 proteins ranked within the PPI network and the Bcl2.

3.3 Experiments Validation

3.3.1 TSA Alleviated Cae-induced AP

We assessed the therapeutic effect of TSA on pancreatic injury in AP mice by conducting histopathological scoring and measuring serum amylase and lipase levels. Histologically, the AP group displayed significant morphological damage characterized by necrosis and inflammatory expansion, while the treatment group exhibited a marked reduction in pancreatic tissue injury (Fig. 5b and Fig. 5c). A notable reduction in serum levels of amylase and lipase was recorded in the TSA treatment cohort in comparison to the Cae group (Fig. 5d). To evaluate the extent of inflammatory infiltration, macrophages and neutrophils were labeled using F4/80 and MPO antibodies, respectively. The findings indicated a significantly lower presence of infiltrated macrophages and neutrophils in the TSA treatment group compared to the Cae group (Fig. 5e and Fig. 5f). All these results indicated that TSA treatment effectively diminished the inflammatory infiltration and pancreatic injury in AP.

3.3.2 TSA Treatment Suppressed the Expression of p-AKT/Bcl2

We conducted additional research to explore the possible role of the PI3K/AKT signaling pathway in the protective effects mediated by TSA. Our findings revealed that the expression levels of p-AKT were notably elevated in the Cae group, while a significant decrease was observed in the TSA treatment group. A similar trend was also observed for the downstream signaling molecule Bcl2 (Fig. 5g and Fig. 5h). These findings suggested that TSA can modulate apoptosis in pancreatic tissue by targeting the PI3K/AKT/Bcl2 signaling pathway.

4. Discussion

AP involves a multifaceted pathological progression characterized by pancreatic autodigestion, inflammatory activation, oxidative stress, and acinar cell apoptosis and necrosis.[17, 18] Currently, there

remains a lack of effective pharmaceutical interventions for the management or prevention of AP.[19] Previous studies have suggested that TCM may alleviate certain complications of AP.[20]

SM, a widely used medicinal herb in TCM, contributes to microcirculation improvement, oxygen-free radical elimination, modulation of inflammatory lipid metabolites, maintenance of intestinal barrier function, and reduction in leukocyte count, triglyceride levels, and inflammatory mediators.[21–23] Experimental studies indicate that SM attenuates lung injury in severe AP models by modulating apoptosis.[24] Our clinical findings demonstrate that SM injection significantly reduced hospitalization expenses ($P = 0.041$), lowered pneumonia incidence ($P = 0.043$), shortened abdominal pain duration ($P = 0.029$), and markedly decreased white blood cell counts ($P = 0.007$) and triglyceride concentrations ($P < 0.001$).

TSA, recognized as the principal bioactive constituent of SM, has been the subject of significant research focusing on its anti-inflammatory effects. Prior research reports that TSA alleviates aortic endothelial injury in rat AP models.[25] Liu and Shen indicated that TSA significantly improves AP-associated lung injury through regulating cytokine production and reducing inflammatory cell accumulation.[26] Additionally, TSA was found to ameliorate trinitrobenzene sulfonic acid (TNBS)-induced pancreatic damage and relieve mechanical allodynia in rats via inhibition of the HMGB1-TLR4 pathway and its downstream signals.[27] In this study, we confirmed that TSA administration notably alleviated cerulein-induced pancreatic injury and reduced inflammatory cell infiltration.

Apoptosis is crucial for processes such as cellular regeneration, the regulation of immune responses, and the development of embryos.[28] Kaiser et al.[29] demonstrated a negative correlation between apoptosis levels and AP severity, wherein inhibiting apoptosis exacerbated pancreatic inflammation. Enhancing apoptosis may therefore alleviate AP, as it exerts a protective effect in this context.[30] Earlier investigations have shown that formulations such as Xiaochaihu Tang inhibit MAPK3 protein expression,[31] while Qingyi Tang downregulates p-p65 and upregulates p-ERK1/2 and c-Fos.[16] Furthermore, both Dachengqi Tang and Chaiqin Chengqi Tang suppress the PI3K-AKT pathway,[32, 33] collectively promoting acinar cell apoptosis to mitigate AP.

Our network pharmacology analysis predicted that TSA regulates apoptotic activity through the PI3K-AKT signaling cascade, identifying Bcl2 as a key target. The Bcl-2 protein family, comprising pro-apoptotic (e.g., BAD) and anti-apoptotic (e.g., Bcl-2, Bcl-xL, MCL-1) members, crucially regulates cell survival/death dynamics.[34] Anti-apoptotic proteins localize to mitochondrial, endoplasmic reticulum, and nuclear membranes, preserving organelle integrity by counteracting pro-apoptotic factors.³⁵ Phosphorylation of BAD at Ser136 by AKT facilitates its binding to 14-3-3 proteins, thereby releasing anti-apoptotic proteins like Bcl-2 and Bcl-xL and inhibiting cell death.[36, 37]

In vivo experiments further revealed that TSA inhibits AKT phosphorylation within the PI3K-AKT pathway and downregulates Bcl2 expression, promoting acinar cell apoptosis and reducing pancreatic damage. Existing evidence indicates that PI3K-AKT inhibition reduces NF- κ B activation, enhances P53 function, induces pro-apoptotic gene expression, and ultimately attenuates pancreatic injury.[38, 39] For example,

abdominal paracentesis drainage was shown to decrease AKT phosphorylation and NF- κ B activity,[40] and resveratrol upregulates P53 to induce apoptosis and ameliorate AP in mice.[41] Concordantly, our network analysis suggests that TSA may interact with P53 and NF- κ B to regulate apoptotic pathways, underpinning its pancreas-protective effects.

Our study has several limitations that should be noted. First, the retrospective clinical analysis was conducted in a single center with a relatively small sample size, which may lead to potential selection bias; multi-center, large-sample prospective studies are needed to further verify the clinical efficacy of SM in AP treatment. Second, we only validated the protective effect of TSA in a Cae-induced AP mouse model, and further verification in other AP models (such as sodium taurocholate-induced severe AP) is required to confirm the universality of the mechanism. Third, the upstream and downstream regulatory details of the PI3K/AKT/Bcl2 pathway in TSA-mediated pancreatic protection need to be further explored through in vitro cell experiments.

Abbreviations

AP

Acute pancreatitis

APACHE II

Acute Physiology and Chronic Health Evaluation II

BISAP

Bedside Index of Severity in Acute Pancreatitis

BP

Biological Processes

Cae

Caerulein

CC

Cellular Components

CRRT

Continuous Renal Replacement Therapy

GO

Gene Ontology

ICU

Intensive Care Unit

IHC

Immunohistochemistry

KEGG

Kyoto Encyclopedia of Genes and Genomes

MF

Molecular Functions

MPO

Myeloperoxidase

MSCT

Multislice Computed Tomography

NIPPV

Non-Invasive Positive Pressure Ventilation

PBS

Phosphate-Buffer Saline

PPI

Protein-Protein Interactions

SIRS

Systemic Inflammatory Response Syndrome

SM

Salvia miltiorrhiza

SOFA

Sequential Organ Failure Assessment

TCM

Traditional Chinese Medicine

TSA

Tanshinone IIA

WM

Western medicine

Declarations

Ethics approval

The retrospective clinical study was approved by the Ethics Review Committee of Fujian Tingzhou Hospital (Approval No. : tzyy20240633, June 15, 2024) and was performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

Consent to participate

The requirement for written informed consent was waived by the Ethics Review Committee of Fujian Tingzhou Hospital due to the retrospective design of the study.

Consent to publish

Not applicable. No identifiable personal information of participants is included in this manuscript.

Competing interests

The authors declare no competing interests related to this work.

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Author Contribution

WWC and GTL conceived and designed the experiments. XJC collected the clinical data. FC, WYW and WST performed the experiments. XJC, PY and WYW implemented the molecular docking analysis. CCY, XWD and QTZ were responsible for the quality control of the experiments. WYW prepared the TSA lyophilized powder. XJC analyzed the data. XJC and WYW wrote the manuscript. WWC and PY revised the manuscript. All authors contributed to the article have read and approved the final manuscript.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure1

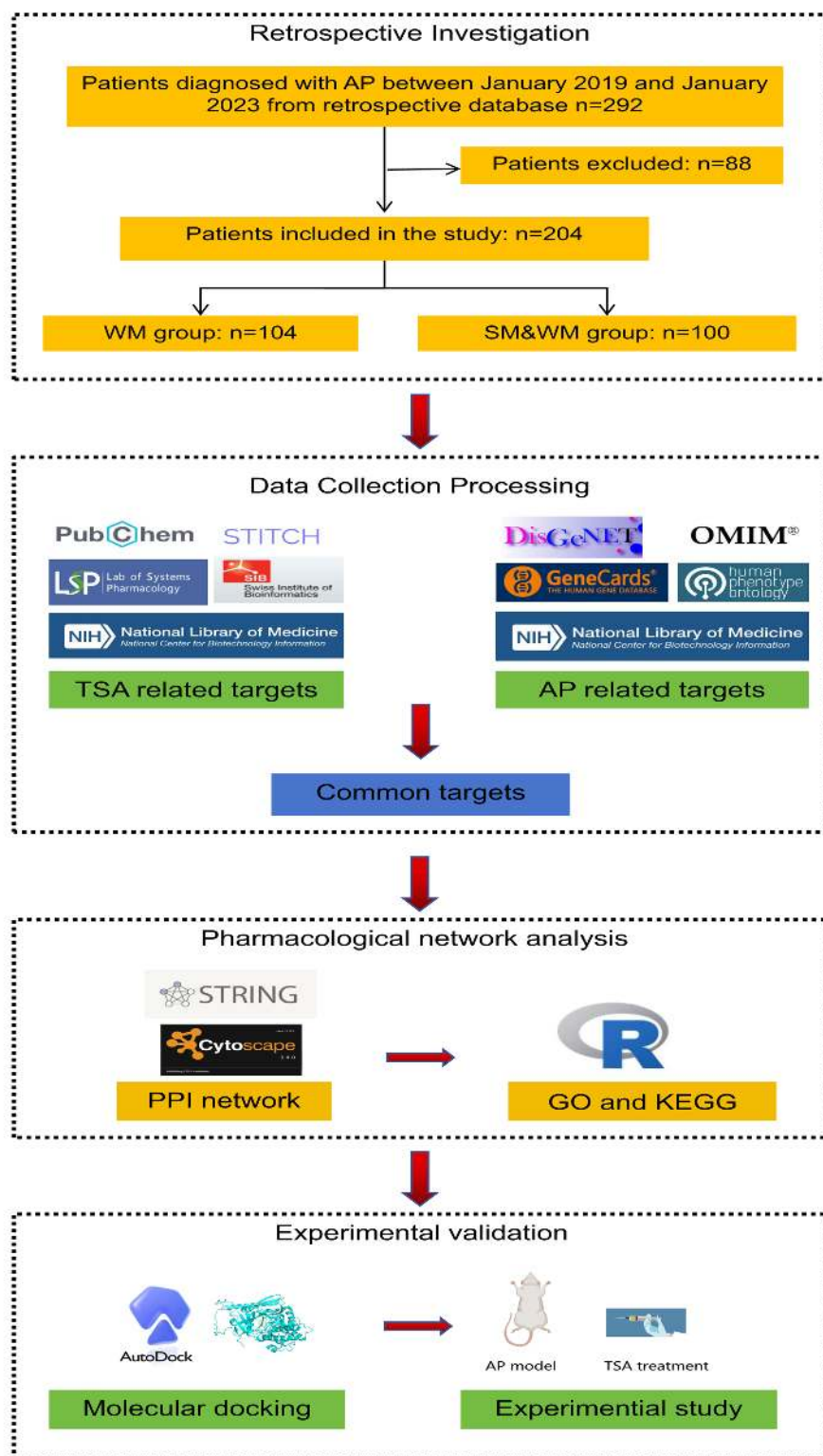


Figure 1

Schematic diagram of *Salvia miltiorrhiza* and its main active component tanshinone IIA for acute pancreatitis treatment

Figure2

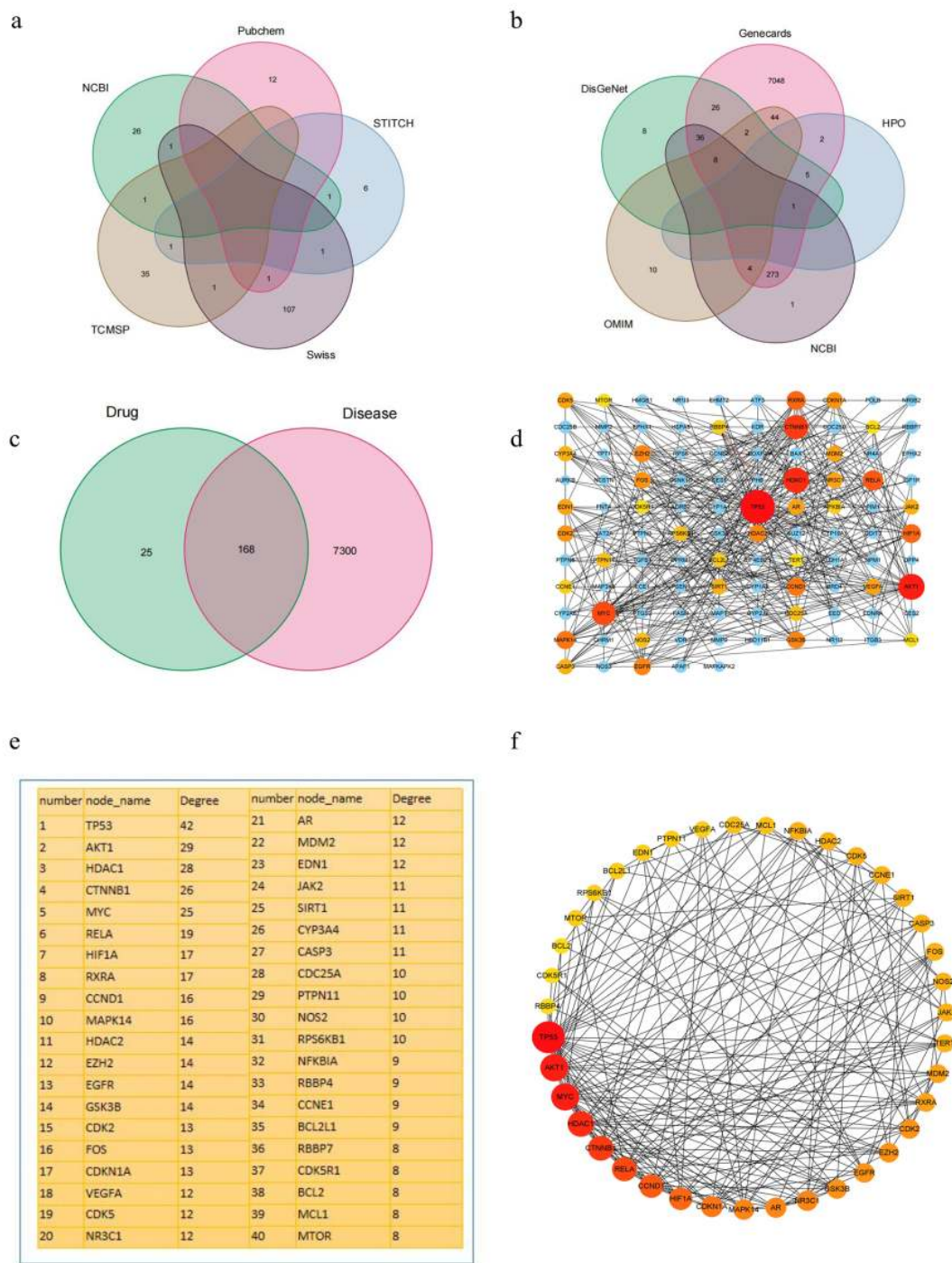


Figure 2

Network targets of tanshinone IIA (TSA) in acute pancreatitis (AP). a: Venn diagram of TSA-related targets; b: Venn diagram of AP-related targets; c: Venn diagram of TSA-related targets overlapping with AP-related targets; d: Protein–protein interaction (PPI)network in the STRING database; e: The top 40 targets with degrees greater than the average value; f: The PPI of the top 39 targets

Figure3

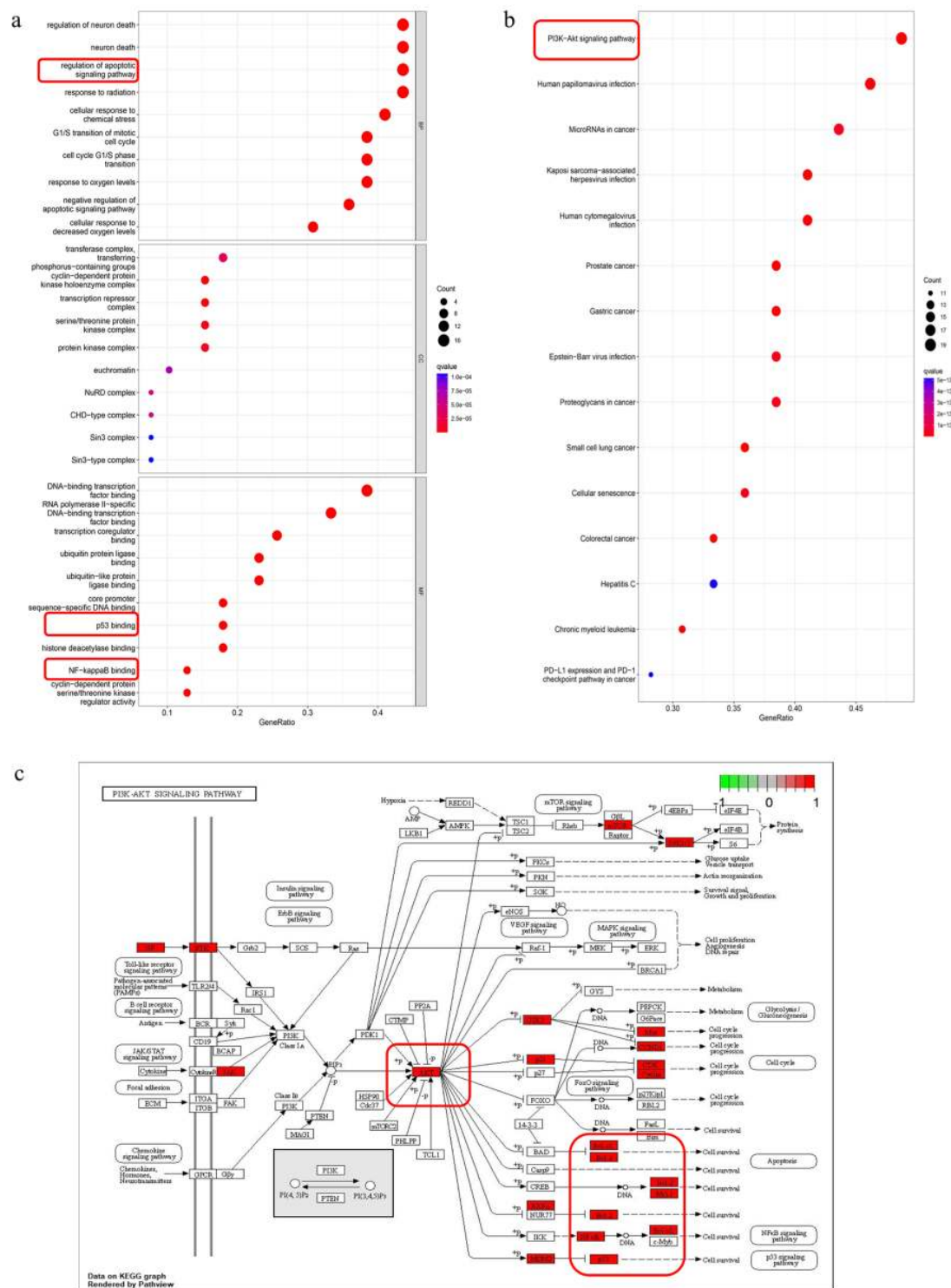


Figure 3

GO function and KEGG pathway analysis. a: GO enrichment analysis of the 39 targets from three aspects: biological process (BP), cellular component (CC) and molecular function (MF); b: KEGG pathway enrichment analysis of the 39 targets. c: The visualized PI3K-AKT pathway (hsa04151)

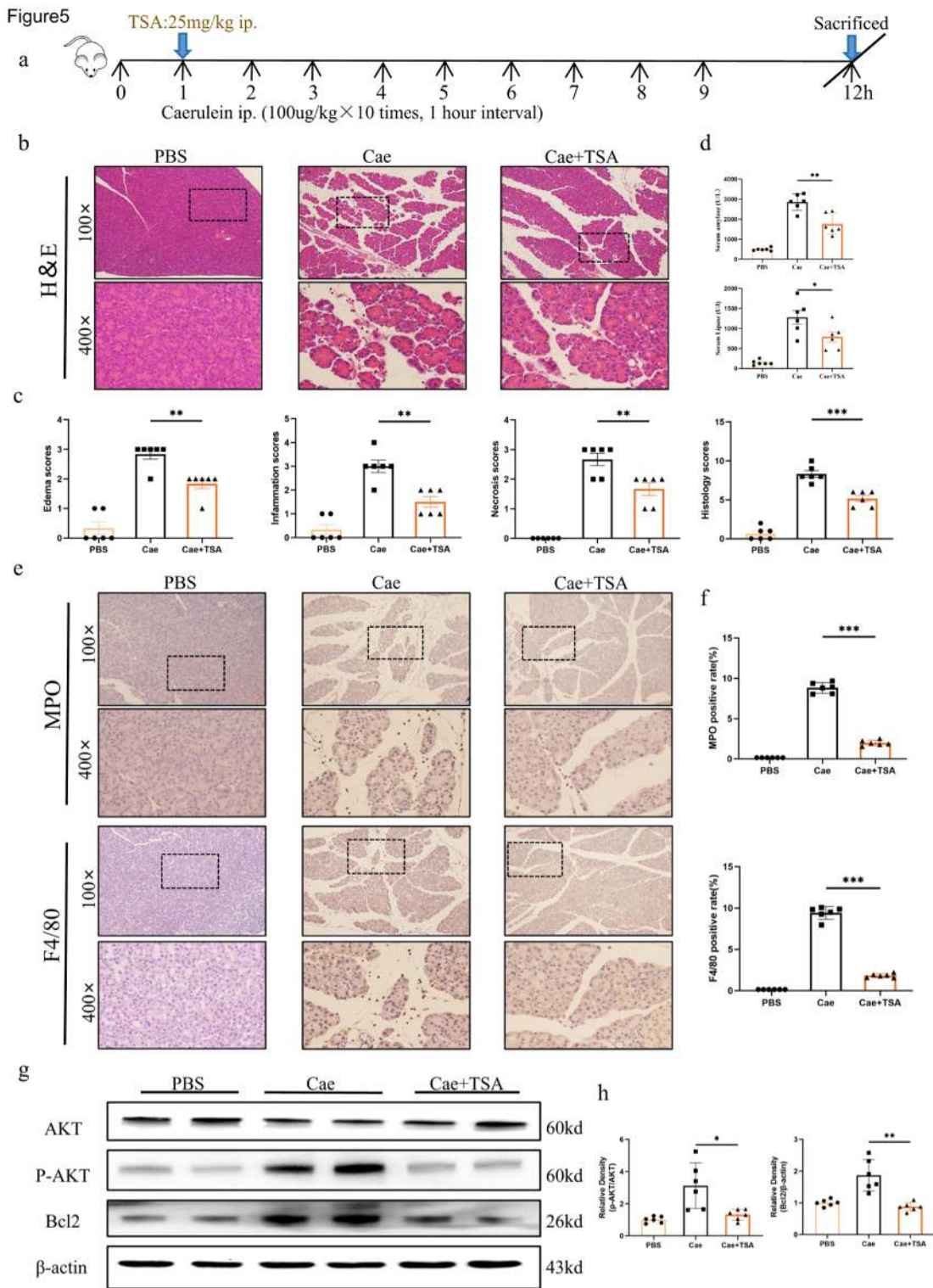


Figure 4

Effects of TSA on Cae-induced AP in mice. a: Schematic illustration of caerulein-induced AP model and TSA administration in mice; b: Representative H&E images of pancreatic sections at magnifications of $\times 100$ and $\times 400$; c: Pancreatic histopathology scores; d: Serum levels of amylase and lipase; e: Representative immunohistochemical images of MPO and F4/80 expression in the pancreas at magnifications of $\times 100$ and $\times 400$; f: Quantitative analysis of MPO-positive neutrophils and F4/80-positive

macrophages in pancreatic tissue; g: Representative western blotting images for p-AKT, AKT, Bcl2 and β -actin; h: Relative expression levels of corresponding proteins. All data were presented as mean $\pm$ SD (n=6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Scale bar = 100 μ m for $\times 100$ magnification, 25 μ m for $\times 400$ magnification

Figure4

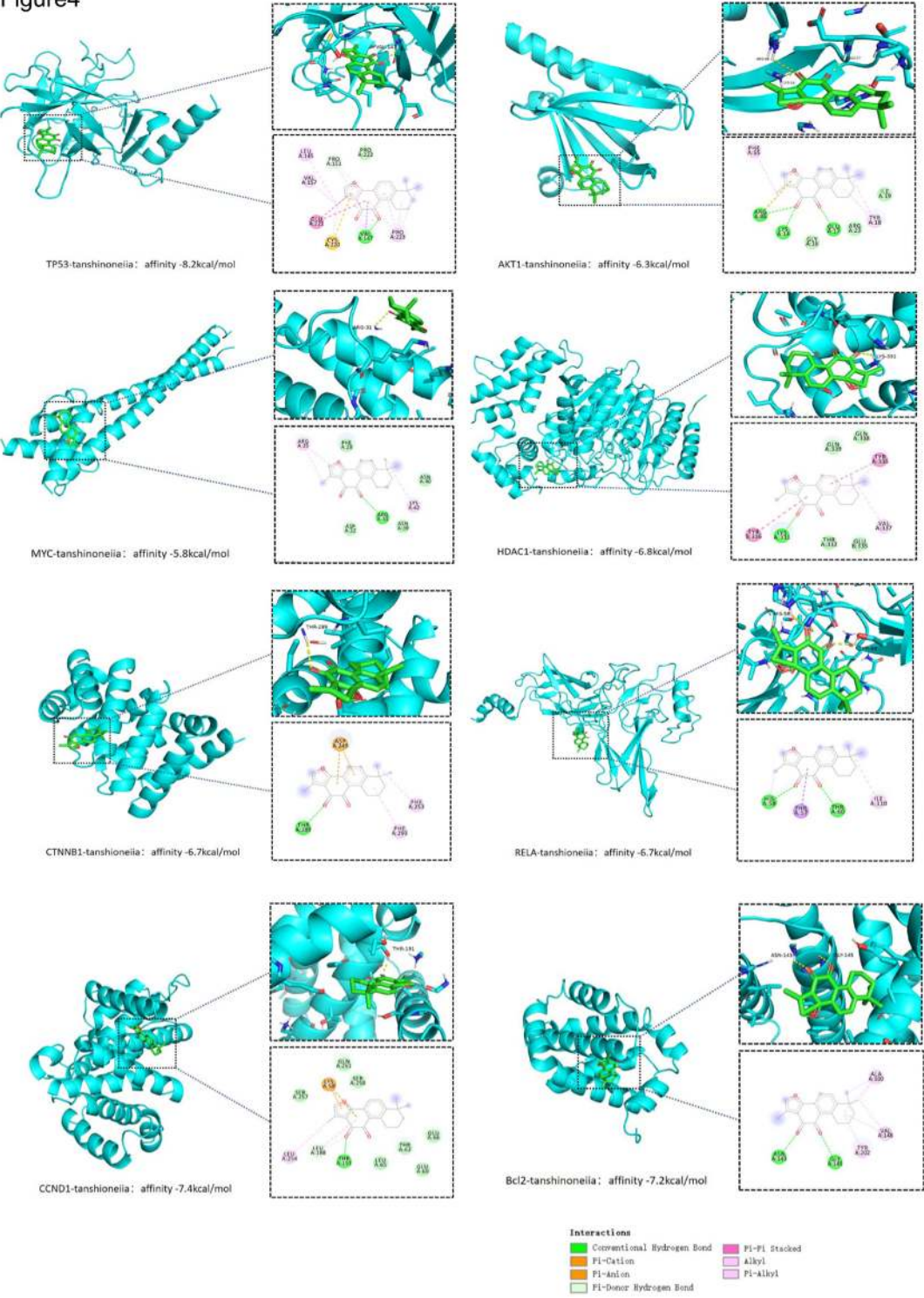


Figure 5

Molecular docking models of the TSA binding to crucial therapeutic targets