Research Square

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Exploring The Mechanism Of Duhuo In Treating Rheumatoid Arthritis

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Abstract

Background

Angelicae Pubescentis Radix (Duhuo) is a key herb in traditional Chinese medicine for alleviating rheumatic pain, with demonstrated efficacy in treating rheumatoid arthritis (RA). Its multi-component nature aligns with the complex pathogenesis of RA, yet the precise molecular mechanisms remain incompletely understood.

Objective

We aimed to investigate the effect of Duhuo in treating RA and to delve into its underlying mechanisms. This study provides experimental evidence for the pharmacological basis and action mechanisms of Duhuo in treating RA.

Methods

Network pharmacology identified Duhuo's primary active constituents and core RA targets. A constituent-target-disease network was constructed to reveal multi-constituent synergies. Molecular docking validated binding affinities between active constituents and RA targets. In vitro validation used TNF-α-induced MH7A fibroblast-like synovial cells to assess effects on proliferation, apoptosis, and target gene expression.

Results

Duhuo and RA shared 85 intersecting genes, enriched in 612 Gene Ontology (GO) biological processes and 148 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Molecular docking confirmed strong affinities between Duhuo constituents and target proteins. Cell experiments showed Duhuo inhibits MH7A cell proliferation and induces apoptosis via the PIK3CA/JAK2/PIK3CG pathway.

Conclusions

The active constituents in Duhuo inhibit the expression of target genes JAK2、PIK3CA and PIK3CG in RA-affected cells, thereby suppressing proliferation of synovial fibroblasts and inducing apoptosis.

1. Introduction

Rheumatoid arthritis (RA) is a debilitating autoimmune disease recognized by the World Health Organization as one of the top ten most disabling conditions worldwide. Its prevalence in adults is

approximately 0.5%–1%[1], with women affected 2–3 times more frequently than men. RA's complex pathogenesis involves synovial hyperplasia, inflammatory cell infiltration, and pannus formation[2], leading to progressive cartilage and bone destruction. Key mechanisms include aberrant activation of pathways such as NF-κB, MAPK, and JAK-STAT, alongside imbalances in pro-inflammatory (e.g., TNF-α, IL-6, IL-17) and anti-inflammatory (e.g., IL-10) cytokines. TNF-α, secreted by macrophages, monocytes, neutrophils, CD4 + T cells, and NK cells, drives synovial inflammation in 50%–70% of RA patients by promoting fibroblast-like synovial cell (e.g., MH7A) proliferation and cytokine/matrix metalloproteinase expression, exacerbating disease progression via interactions with IL-1β and IL-6[3].

Current RA treatments, including nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying antirheumatic drugs (DMARDs), and biologics, alleviate symptoms but have limitations. For example, Methotrexate can cause hepatotoxicity and bone marrow suppression, TNF-α inhibitors are costly and increase infection risk, and long-term glucocorticoids lead to osteoporosis and metabolic disorders[4].

Angelicae Pubescentis Radix (Duhuo) is the dried root of Angelica pubescens Maxim. f. biserrata Shan et Yuan, a plant belonging to the Apiaceae family. It is one of the key medicinal herbs in traditional Chinese medicine for treating rheumatic pain[5]. Modern pharmacological studies have demonstrated that Duhuo possesses significant anti-inflammatory, analgesic, and immunomodulatory properties. In traditional Chinese medicine practice, it is frequently combined with other herbs such as Notopterygium incisum and Taxillus chinensis to form classical formulas (e.g., Duhuo Jisheng Decoction), which are widely used for rheumatoid arthritis and related conditions[6].

Modern analyses reveal Duhuo's active constituents, including coumarins (e.g., neocnidilide, O-acetylcolumbianetin), volatile oils (e.g., α-pinene, limonene), sterols (e.g., β-sitosterol), and polysaccharides[7]. underpin its anti-inflammatory, analgesic, and immunomodulatory effects. In collagen-induced arthritis models, Duhuo reduces joint swelling, arthritis index, and synovial hyperplasia by downregulating TNF-α, IL-6, and IL-17 while upregulating IL-10.

Network pharmacology, aligning with traditional Chinese medicine's holistic, multi-component approach, integrates omics data and bioinformatics to map drug-disease networks[8].Complementing this, molecular docking simulates compound-target interactions at the atomic level.

This study used network pharmacology to identify Duhuo's key constituents and RA targets, built a constituent-target-disease network, validated bindings via molecular docking, and confirmed effects in TNF-α-induced MH7A cells. These findings elucidate Duhuo's pharmacological mechanisms in RA.

2. Methods

2.1 Databases and softwares

Disease databases: GeneCards (https://www.genecards.org/), DrugBank (https://go.drugbank.com/); Traditional Chinese Medicine and Component Databases: TCMSP Database (https://tcmsp-e.com/), SwissTargetPrediction Database (http://swisstargetprediction.ch/); Protein Databases: PDB Database (Protein Data Bank, http://www.rcsb.org/); Bioinformatics Databases: DAVID Database (https://david.ncifcrf.gov/); Network Visualisation Databases and Software: Venny 2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/),WeiShengXin platform (https://www.bioinformatics.com.cn/login/), Cytoscape 3.10.3 software, AutoDock Tools 1.5.7 software, pymol 3.7.0 software.

2.2 Collection of RA Disease-Related Targets

RA targets were retrieved from GeneCards and DrugBank using 'rheumatoid arthritis' as the keyword. Duplicates were removed, and targets with a relevance score ≥ 3.5 were retained. Gene names were standardized via UniProt[8].

2.3 Identifying the active constituents and targets of Duhuo

Duhuo constituents were screened from TCMSP with oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18[8]. SMILES structures (from PubChem) were input into SwissTargetPrediction for target prediction (probability > 0).

2.4 Key component genes and disease genes

Venny 2.1.0 identified intersecting genes between Duhuo targets and RA genes.

2.5 Drug-Component-Gene Target Network Construction

Cytoscape 3.10.3 generated the network. Topology analysis ranked top constituents by degree value [8].

2.6 GO, KNEE enrichment analysis

Intersecting genes underwent GO and KEGG analysis in DAVID (Homo sapiens)[9], Top 20 pathways by -logP were visualized on WeiShengXin.Detailed enrichment results are presented in Supplementary Tables 7–10.

2.7 Verification of molecular docking results

Top three constituents (by degree) were docked with core targets using AutoDock Tools 1.5.7. Structures were from TCMSP and PDB. Results were evaluated by binding energy and visualized in PyMOL3.7.

2.8 Cell culture and treatment

MH7A cells (human rheumatoid arthritis synovial fibroblasts) were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China; Cat. No. CL-0747) and cultured in RPMI-1640 medium with 10% fetal bovine serum and 1% antibiotics at 37°C, 5% CO_2, and 70%–80% humidity [10].

2.9 Cell counting Kit-8 (CCK-8) assay

Cells (5×10³/well) in 96-well plates were treated with Duhuo extract (1,4 mg/mL) and 10 ng/mL TNF-α for 24–48h. Viability was measured at 450 nm after CCK-8 incubation[11].

2.10 Flow Cytometry Analysis

Seed cells at a density of 5 × 10³ cells per well in a 96-well plate and incubate in a cell culture incubator for 24 hours to allow attachment. Following cell attachment, treat with different concentrations (1, 4 mg/mL) of Duhuo extract and 10 ng/mL TNF-α. Continue culture for 48 hours, then centrifuge at 1000 × g for 5 minutes. Aspirate the cell culture medium and wash with PBS. Subsequently, add 195 μL Annexin V-FITC binding buffer, 5 μL Annexin V-FITC, and 10 μL propidium iodide (PI) staining solution. Gently mix and incubate at room temperature (20–25°C) in the dark for 10–20 minutes, then place on ice. Proceed with analysis using a flow cytometer.

2.11 Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis

RNA from treated cells was extracted and analyzed using TOROBlue® OneHiFi PCR Mix (Supplementary Table 2).

3. Results

3.1 RA Disease-Related Targets

GeneCards and DrugBank yielded 6,705 genes associated with RA pathogenesis. 1,453 with scores > 3.5 were selected (Supplementary Table 3).Certain RA-associated genes include HLA-DRB1 (Uniport ID P01911, relevance score 109.9435425), TNF (P01375, 105.8718872), PTPN22 (Q9Y2R2, 105.4918976), IL10 (P22301, 90.30067444), IL6 (P05231, 90.11192322), PSTPIP1 (O43586, 79.11911011), PADI4 (Q9UM07, 78.68096924), CIITA (P33076, 75.19916534), STAT4 (Q14765, 68.16044617), and IL2RA (P01589, 65.44426727).

3.2 The active constituents and targets of Angelica pubescens

Nine potential active constituents were identified based on screening criteria of oral bioavailability (OB) ≥ 30% and drug similarity (DL) ≥ 0.18(Supplementary Table 4), predicting 268 targets (e.g., MAOA, JAK2, PIK3CG).The nine potential active constituents include beta-sitosterol (OB 36.91%, DL 0.75), nodakenin (OB 57.12%, DL 0.69), [(1R,2R)-2,3-dihydroxy-1-(7-methoxy-2-oxochromen-6-yl)-3-methylbutyl] 3-methylbutanoate (OB 45.19%, DL 0.34), Angelol D (OB 34.85%, DL 0.34), [(1R,2R)-2,3-dihydroxy-1-(7-methoxy-2-oxochromen-6-yl)-3-methylbutyl] (Z)-2-methylbut-2-enoate (OB 46.03%, DL 0.34), O-Acetylcolumbianetin (OB 60.04%, DL 0.26), Angelicone (OB 30.99%, DL 0.19), Ammidin (OB 34.55%, DL 0.22), and isoimperatorin (OB 45.46%, DL 0.23).To identify the target sites for the above compounds,

target prediction was conducted using the Swiss-PTA target prediction database. Following screening and deduplication, a total of 268 relevant target sites were ultimately identified, including MAOA, JAK2, PIK3CG, PIK3CD, PIK3CA, JAK1, EGFR.

3.3 Major component genes and disease genes

To further investigate the association between potential target sites of active components in Duhuo and core pathogenic genes of RA, an intersection analysis was conducted between the 268 target genes of Duhuo's principal components and 1,453 RA genes. This yielded 85 intersecting genes, including PIK3CD, PIK3CG, PIK3CA, JAK2, HDAC1, MTOR, and JAK1(Fig. 1).

A:venn diagram, 85 represents that Duhuo acts on 85 RA targets;B:PPI network analysis,Identified 9 core targets:PIK3CA、PIK3CG、JAK2、PARP1、FLT3、MAPK8、MAPKAPK2、PIK3CD、NOS2、MAPK14;C: drug-constituent-target network diagram.

3.4 Drug-Component-Target Pathway Network

Five key constituents emerged (e.g., O-Acetylcolumbianetin, Imperatorin, Angelicone; Supplementary Table 5, Fig. 1). Core targets (degree ≥ 3) included PIK3CA, PIK3CG, and JAK2(Supplementary Table 6).Target analysis indicates that targets with a degree ≥ 3 comprise phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha(PIK3CA), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma(PIK3CG), Janus kinase 2 (JAK2), poly(ADP-ribose) polymerase 1(PARP1), FMS-like tyrosine kinase 3 (FLT3), Mitogen-Activated Protein Kinase 8 (MAPK8), MAPK activated protein kinase 2 (MAPKAPK2), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD), nitric oxide synthase (NOS2), and mitogen-activated protein kinase 14 (MAPK14). Consequently, PIK3CA, PIK3CG, and JAK2 are predicted to be core therapeutic targets for Duhuo in treating RA.

3.5 GO, KEGG enrichment analysis

Analyses of the 85 intersecting genes yielded 429 biological processes (BP), 56 cellular components (CC), 127 molecular functions (MF), and 148 KEGG pathways. The top 20 pathways were selected based on -logP values.

In GO_BP, the most significantly enriched terms were protein phosphorylation, chromatin remodelling, and inflammatory response. These processes are central to synovial fibroblast activation in RA. Detailed gene lists and counts are provided in Supplementary Table 8. GO_CC analysis showed that the enriched genes were primarily located in cell membranes, cytosol, receptor complexes, and cytoplasm (Supplementary Table 9). GO_MF highlighted protein tyrosine kinase activity, protein kinase activity, and ATP binding as the dominant functions (Supplementary Table 10).

KEGG pathway enrichment revealed strong involvement in cancer pathways, Kaposi’s sarcoma-associated herpesvirus infection, endocrine resistance, and hepatitis B virus infection pathways. The cancer pathway exhibited the highest number of enriched genes, followed by pathways related to PI3K/Akt signalling, EGFR signalling, and apoptosis regulation. Full pathway lists with gene counts and

P-values are shown in Supplementary Table 7. These findings indicate that Duhuo exerts its effects through multiple overlapping signalling networks that regulate inflammation and cell survival in RA.

This yielded 429 biological processes (BP), 56 cellular components (CC), 127 molecular functions (MF), and 148 KEGG pathways. The top 20 pathways were selected based on -logP values and visualised on the WeiShengXin platform (larger bubbles in the enrichment plot indicate more genes enriched in that pathway; bubble colour denotes significance, with redder hues corresponding to smaller P-values and greater statistical significance). Results indicate that Duhuo primarily influences biological processes in RA treatment, including protein phosphorylation, chromatin remodelling, and inflammatory responses. Key cellular components involved comprise cell membranes, cytosol, receptor complexes, and cytoplasm. Principal molecular functions encompass protein tyrosine kinase activity, protein kinase activity, and ATP binding. The pathways predominantly involved include cancer pathways, Kaposi's sarcoma-associated herpesvirus (KSHV) infection pathways, endocrine resistance pathways, and hepatitis B virus (HBV) infection and pathogenesis pathways. The cancer pathway exhibited the highest enrichment of target genes (Supplementray Tables 7–10 and Figs. 2).

A:GO_BP(biological processes),The signalling pathway exhibits the highest enrichment of genes, indicating that its protein phosphorylation pathway represents the most significant enrichment of genes;B:GO_CC(cell components),The cell membranes harbour the greatest number of enriched genes and exhibit the most pronounced representation.C:The ATP-binding pathway exhibits the highest enrichment of genes, indicating that its protein tyrosine kinase activity pathway represents the most significant enrichment of genes.D:The cancer pathway exhibits the highest number of enriched genes and the most pronounced representativeness.

3.6 Molecular docking

Imperatorin bound PIK3CA (-6.5 kcal/mol), Angelicone bound JAK2 (-3.67 kcal/mol), and O-Acetylcolumbianetin bound PIK3CG (-8.4 kcal/mol; Fig. 3).

A: Molecular docking of Imperatorin with the PIK3CA protein;B:Molecular docking of Angelicone with the JAK2 protein;C:Molecular docking of O-Acetylcolumbianetin with the PIK3CG protein.

3.7 Duhuo extract inhibits the proliferative activity of MH7A cells.

Duhuo extract reduced viability by 34.5%–39.3% (24 h) and 23.6%–48.9% (48 h) in a dose-dependent manner (Supplementary Tables 11–12, Fig. 4).Duhuo extract reduced cell viability in a dose- and time-dependent manner. At 24 h, viability decreased by 34.5% at 1 mg/mL and 39.3% at 4 mg/mL compared with the TNF-α group. At 48 h, the reductions were 23.6% and 48.9%, respectively (Supplementary Tables 11 and 12 for raw OD values and statistical details). These results confirm that Duhuo directly suppresses the proliferative capacity of RA synovial fibroblasts.

A: Histogram of cell viability following 24-hour treatment of MH7A cells with Duhuo extract.

Control denotes the PBS control group; TNF-α concentration is 10 ng/mL; '**' indicates P < 0.01; 24 h denotes treatment of MH7A cells with AE for 24 hours.B:Cell survival rate after treatment of MH7A cells with Duhuo extract for 48 hours Control denotes the PBS control group; TNF-α concentration is 10 ng/mL; '**' indicates P < 0.01; 48 h denotes MH7A cells treated with Angelica pubescens extract for 48 hours.C:Apoptosis effects following treatment of MH7A cells with Duhuo extract for 48 hours The four quadrants represent distinct cellular states of activity: Q1 quadrant (Annexin V^-/PI^+): Necrotic cells or late apoptotic cells Q2 quadrant (Annexin V^+/PI^+): Late apoptotic cells Q3 quadrant (Annexin V^+/PI^-): Early apoptotic cells Q4 quadrant (Annexin V^-/PI^-): Viable cells;D:Apoptosis rate of MH7A cells after treatment with Duhuo for 48 hours Control denotes the PBS control group; TNF-α concentration is 10 ng/mL; '**' indicates P < 0.01; 48 h denotes MH7A cells treated with Angelica pubescens extract for 48 hours.E:Relative expression levels of target genes were assessed by RT-qPCR following treatment of MH7A cells with Duhuo extract for 24 hours.TNF-α concentration is 10 ng/mL; '**' indicates P < 0.01;

3.8 Duhuo extract induces apoptosis in MH7A cells

Apoptosis rates increased 285.8% (1 mg/mL) and 416.8% (4 mg/mL; Supplementary Table 13, Fig. 4). Flow cytometry showed that early and late apoptosis rates increased markedly after 48 h treatment. The total apoptosis rate rose by 285.8% at 1 mg/mL and 416.8% at 4 mg/mL compared with the control (Supplementary Table 13 for quadrant percentages and statistical analysis)

3.9 Duhuo extract inhibits the expression of MH7A target genes.

RT-qPCR showed dose-dependent reductions in JAK2 (27.1%–45.5%), PIK3CG (13.1%–30.3%), and PIK3CA (36.6%–46.8%; Fig. 4).

4. Discussion

Network pharmacology identified nine Duhuo constituents (Supplementary Table 4), with Imperatorin, Angelicone, and O-Acetylcolumbianetin as key players (Supplementary Table 5). Core targets included PIK3CA, PIK3CG, and JAK2, with docking confirming bindings (Fig. 3). To further elucidate the mechanism of action of Duhuo in treating RA, the 85 intersecting genes were imported into the DAVID bioinformatics database for functional enrichment analysis. GO annotation revealed the biological processes and molecular functions in which these genes participate, while KEGG pathway analysis identified core regulatory pathways and key hub targets involving these genes. This yielded 429 biological processes (BP), 56 cellular components (CC), 127 molecular functions (MF), and 148 KEGG pathways. The top 20 pathways were selected based on -logP values and visualised on the WeiShengXin platform (larger bubbles in the enrichment plot indicate more genes enriched in that pathway; bubble colour denotes significance, with redder hues corresponding to smaller P-values and greater statistical significance). Results indicate that Duhuo primarily influences biological processes in RA treatment, including protein phosphorylation, chromatin remodelling, and inflammatory responses. Key cellular components involved comprise cell membranes, cytosol, receptor complexes, and cytoplasm. Principal

molecular functions encompass protein tyrosine kinase activity, protein kinase activity, and ATP binding. The pathways predominantly involved include cancer pathways, Kaposi's sarcoma-associated herpesvirus (KSHV) infection pathways, endocrine resistance pathways, and hepatitis B virus (HBV) infection and pathogenesis pathways. The cancer pathway exhibited the highest enrichment of target genes.

JAK2 (Janus Kinase 2) is a non-receptor tyrosine protein kinase and a key member of the Janus kinase family. Its abnormal activation directly regulates the expression of inflammatory cytokines TNF-α and IL-6, thereby establishing a pro-inflammatory microenvironment[12]. GO enrichment analysis revealed that JAK2 is primarily localised to the cell membrane and cytoplasm of MH7A cells (Fig. 1, supplementary Table 9), participating in biological processes such as protein phosphorylation and chromatin remodelling (Fig. 1, supplementary Table 8), and molecular functions including protein tyrosine kinase activity and protein kinase activity (Fig. 1, supplementary Table 10). Research indicates that protein phosphorylation constitutes a pivotal process in cellular signal transduction, implicated in diverse physiological and pathological mechanisms including cell proliferation, differentiation, and Metabolism[13]. Proteins possessing protein tyrosine kinase activity participate in cellular signal transduction and regulate processes such as cell growth, differentiation, and survival[14]. Therefore, it may be concluded that during the treatment of RA by Duhuo, the active component Angelicone acts upon cellular membranes and cytoplasm. By inhibiting the abnormal expression of target gene JAK2 in biological processes such as protein phosphorylation and chromatin remodelling, as well as molecular functions including protein tyrosine kinase activity and protein kinase activity, it exerts an inhibitory effect on the proliferation of synovial fibroblasts in rheumatoid arthritis.protein phosphorylation constitutes a pivotal process in cellular signal transduction, implicated in diverse physiological and pathological mechanisms including cell proliferation, differentiation, and Metabolism. Proteins possessing protein tyrosine kinase activity participate in cellular signal transduction and regulate processes such as cell growth, differentiation, and survival. Therefore, it may be concluded that during the treatment of RA by Duhuo, the active component Angelicone acts upon cellular membranes and cytoplasm.

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α) encodes the p110α catalytic subunit of Phosphatidylinositol 3-kinase (PI3K), belonging to the Class I PI3K family. It regulates core cellular processes including survival, proliferation, and metabolism via the PI3K/AKT signalling pathway[15]. Under normal conditions, PIK3CA remains in a non-activated state, being activated only upon cellular stimulation by growth factors and similar agents to promote cell cycle progression and inhibit apoptosis[16]. However, when somatic mutations occur in the PIK3CA gene, the PI3K kinase activity it encodes becomes abnormally enhanced. This leads to sustained activation of the AKT signalling pathway, subsequently disrupting the normal regulation of apoptosis and affecting the expression of inflammatory mediators and the activity of inflammatory cells[17]. GO enrichment analysis revealed that PIK3CA primarily localises to the cell membrane and cytoplasm (Fig. 2, Supplementary Table 9), participating in biological processes such as epidermal growth factor receptor signalling and

PI3K/Akt signalling (Fig. 2, Supplementary Table 8), alongside molecular functions including protein serine/threonine kinase activity and protein serine kinase activity (Fig. 1,Supplementary Table 10). Relevant studies demonstrate that epidermal growth factor receptor (EGFR) signalling constitutes a pivotal intracellular pathway. It shortens the cell cycle and promotes DNA synthesis by activating the Ras/Raf/MAPK pathway, thereby increasing cell division rate and number. Additionally, it activates members of the MAPK family to regulate cell proliferation and differentiation[18]. Concurrently, it inhibits apoptosis and promotes cell survival by activating the PI3K/Akt pathway. PI3K/Akt signalling modulates diverse cellular functions. Fully activated Akt transmits signals intracellularly by phosphorylating multiple downstream target proteins, thereby regulating growth, proliferation, survival, and metabolism[19]. Both protein serine/threonine kinases and protein serine kinases transmit signals by phosphorylating specific substrate proteins[20], subsequently governing cellular behaviour. KEGG analysis revealed that PIK3CA is enriched in pathways including EGFR-TKI resistance, PI3K/Akt signalling, and apoptosis (Fig. 2, Supplementary Table 7). Research indicates that abnormal mutations in the PIK3CA gene within the EGFR-TKI resistance pathway led to dysregulated activation of the PI3K/AKT signalling pathway. This circumvents the EGFR signalling pathway, thereby sustaining the proliferation and survival of diseased cells[21]. Within the PI3K/Akt signalling pathway, aberrant mutations in the PIK3CA gene may enhance phospholipid kinase activity, amplifying downstream Akt signalling pathway and leading to uncontrolled cell proliferation[22], potentially conferring drug resistance. Along the apoptotic pathway,gene mutation may trigger abnormal activation of pro-apoptotic genes or excessive expression of anti-apoptotic genes, resulting in disrupted or inhibited cell death. Modern pharmacological studies indicate that Imperatorin can mitigate inflammatory responses by inhibiting the release of inflammatory mediators and the activation of inflammatory cells through blocking the PI3K/Akt signalling pathway[22, 23]. In summary, it can be concluded that when treating RA, the active component imperatorin in Angelica pubescens exerts its effects by acting on the cell membrane and cytoplasm of MH7A cells. It inhibits PIK3CA in biological processes such as epidermal growth factor receptor (EGFR) signalling and PI3K/Akt signalling, as well as molecular functions including protein threonine kinase activity and protein serine kinase activity. Furthermore, it suppresses molecular abnormalities in pathways such as EGFR-TKI resistance, PI3K/Akt signalling, and apoptosis pathways. This inhibits proliferation and induces apoptosis in synovial fibroblasts of osteoarthritis.KEGG analysis revealed that PIK3CA is enriched in pathways including EGFR-TKI resistance, PI3K/Akt signalling, and apoptosis. Research indicates that abnormal mutations in the PIK3CA gene within the EGFR-TKI resistance pathway led to dysregulated activation of the PI3K/AKT signalling pathway. This circumvents the EGFR signalling pathway, thereby sustaining the proliferation and survival of diseased cells. Within the PI3K/Akt signalling pathway, aberrant mutations in the PIK3CA gene may enhance phospholipid kinase activity, amplifying downstream Akt signalling pathway and leading to uncontrolled cell proliferation, potentially conferring drug resistance. Along the apoptotic pathway, gene mutation may trigger abnormal activation of pro-apoptotic genes or excessive expression of anti-apoptotic genes, resulting in disrupted or inhibited cell death.

PIK3CG (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma, also known as PI3Kγ or p110γ) is one of the classes I catalytic subunits within the Phosphatidylinositol 3-kinase (PI3K)

family[24]. It exhibits high expression levels in immune cells, particularly in neutrophils, macrophages, and T cells. Its abnormal activation may lead to excessive inflammatory responses and is associated with the onset and progression of autoimmune diseases such as RA and systemic lupus erythematosus[25]. GO enrichment analysis revealed that PIK3CG is primarily localised to the cell membrane and cytoplasm of MH7A cells (Fig. 2, Supplementary Table 9). It participates in biological processes including inflammatory response and PI3K/Akt signalling (Fig. 2, Supplementary Table 8), and molecular functions such as protein kinase activity and histone H2AXY142 kinase activity (Fig. 2, Supplementary Table 10). Relevant studies indicate that mutations or dysregulation of PIK3CG may lead to excessive activation of immune cells and abnormal release of inflammatory mediators, thereby exacerbating tissue damage and inflammatory responses[24]. Concurrently, abnormal expression of PIK3CG can activate the PI3K/Akt signalling pathway, subsequently promoting proliferation in diseased cells[26]. Akt activation suppresses the expression of apoptosis-related proteins while promoting cyclin expression, thereby driving cell cycle progression and proliferation[27]. The dephosphorylated state of histone H2AX Y142 is closely linked to apoptosis regulation[28]: when Y142 remains phosphorylated, it may recruit pro-apoptotic proteins to promote cell death[29]; whereas its dephosphorylated state may attract repair proteins to maintain cell survival. Consequently, abnormal expression of Y142 dephosphorylation may disrupt apoptotic regulation, promoting cell survival and proliferation[30]. KEGG pathway analysis revealed PIK3CG enrichment in the PI3K/Akt signalling pathway (Fig. 2, Supplementary Table 7). Research indicates that within the PI3K/Akt pathway, abnormal expression of PIK3CG can bind with the p85 regulatory subunit to form an active PI3K complex. This complex catalyses the conversion of PIP2 into PIP3, thereby activating downstream Akt signalling molecules and influencing cellular processes such as proliferation and apoptosis[31]. In summary, it can be concluded that during the treatment of RA with Duhuo, its active component O-Acetylcolumbianetin acts upon cellular membranes and cytoplasm. By influencing PIK3CG's role in inflammatory responses, PI3K/Akt signalling, protein kinase activity, histone H2AXY142 kinase activity, and abnormal expression in the PI3K/Akt pathway. This achieves the suppression of proliferative activity in synovial fibroblasts and induces apoptosis.

In this study, in vitro cellular validation (Figs. 4) demonstrated that the active constituents in Duhuo achieve suppression of synovial fibroblast proliferation and induction of apoptosis by inhibiting the expression of target genes JAK2, PIK3CA, and PIK3CG in RA-induced cells.

5. Conclusion

Based on the multifaceted investigations into the treatment of RA with Duhuo in this study, the following conclusions may be drawn: The active constituents in Duhuo—Imperatorin binds directly to PIK3CA, Angelicone to JAK2, and O-Acetylcolumbianetin to PIK3CG—inhibiting the expression of target genes. This mechanism suppresses proliferation in RA synovial fibroblasts and induces apoptosis, thereby exerting therapeutic effects against RA.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Author Contribution

These authors contributed equally to this work.

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Data Availability

the data that support the findings of this study are available on request from the corresponding author

Further disclosure

The authors declare that there are no further disclosures. This work has not been presented at any conference or posted on a preprint server.

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Figures

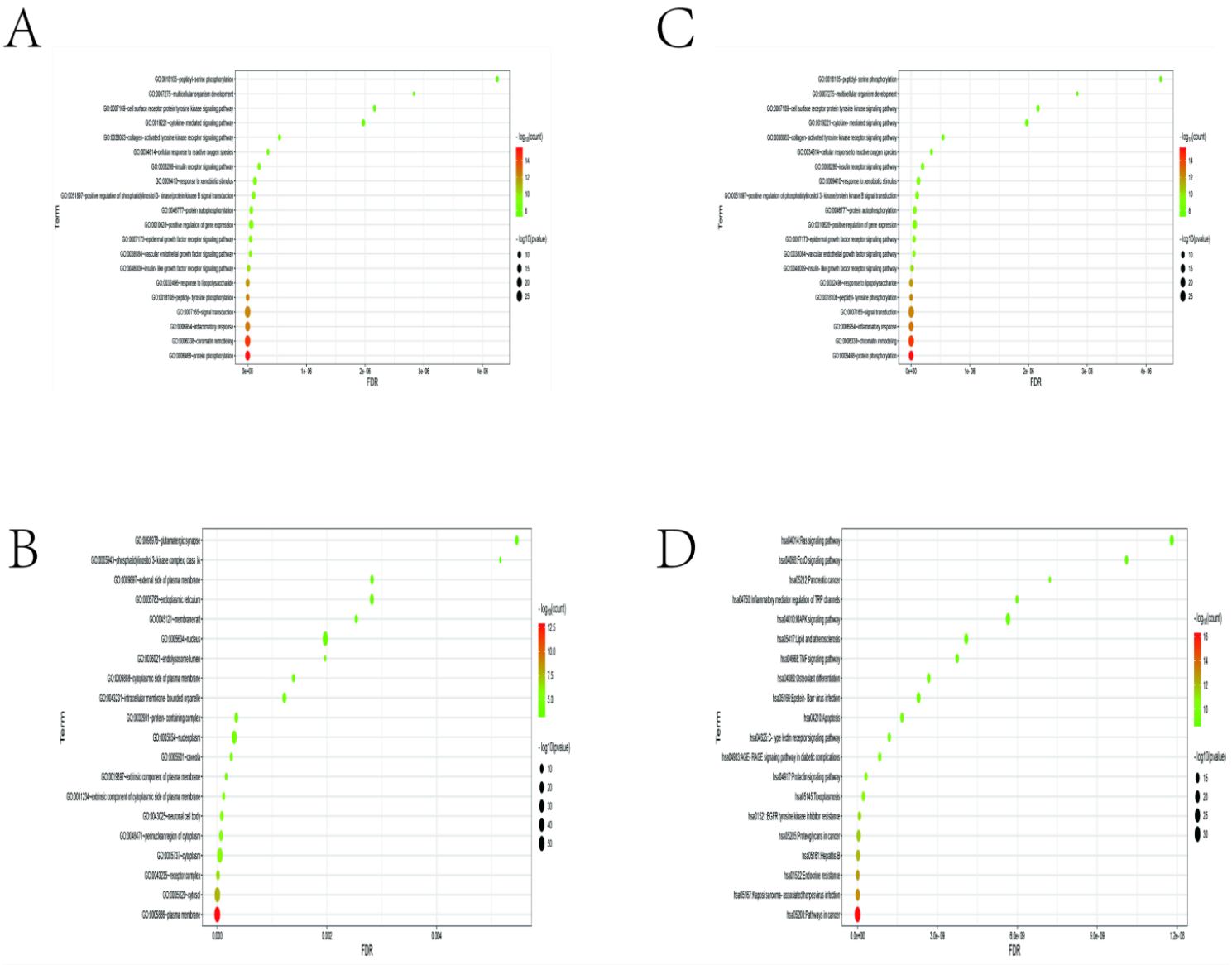


Figure 1

GO and KEGG enrichment analysis scatter diagram.

A:GO_BP(biological processes),The signalling pathway exhibits the highest enrichment of genes, indicating that its protein phosphorylation pathway represents the most significant enrichment of genes;B:GO_CC(cell components),The cell membranes harbour the greatest number of enriched genes

and exhibit the most pronounced representation.C:The ATP-binding pathway exhibits the highest enrichment of genes, indicating that its protein tyrosine kinase activity pathway represents the most significant enrichment of genes.D:The cancer pathway exhibits the highest number of enriched genes and the most pronounced representativeness.

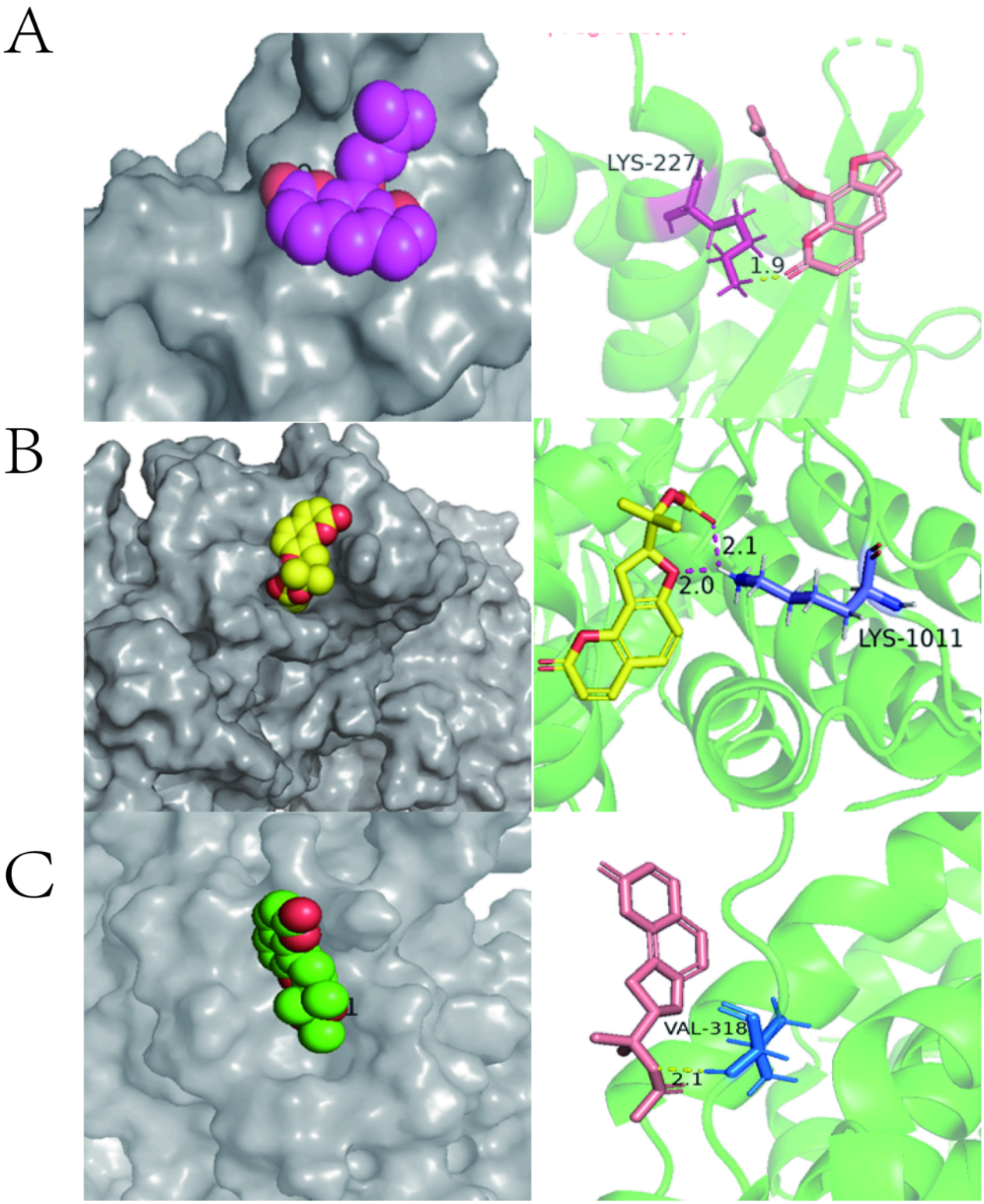


Figure 2

Molecular docking pattern results.

A: Molecular docking of Imperatorin with the PIK3CA protein;B:Molecular docking of Angelicone with the JAK2 protein;C:Molecular docking of O-Acetylcolumbianetin with the PIK3CG protein.

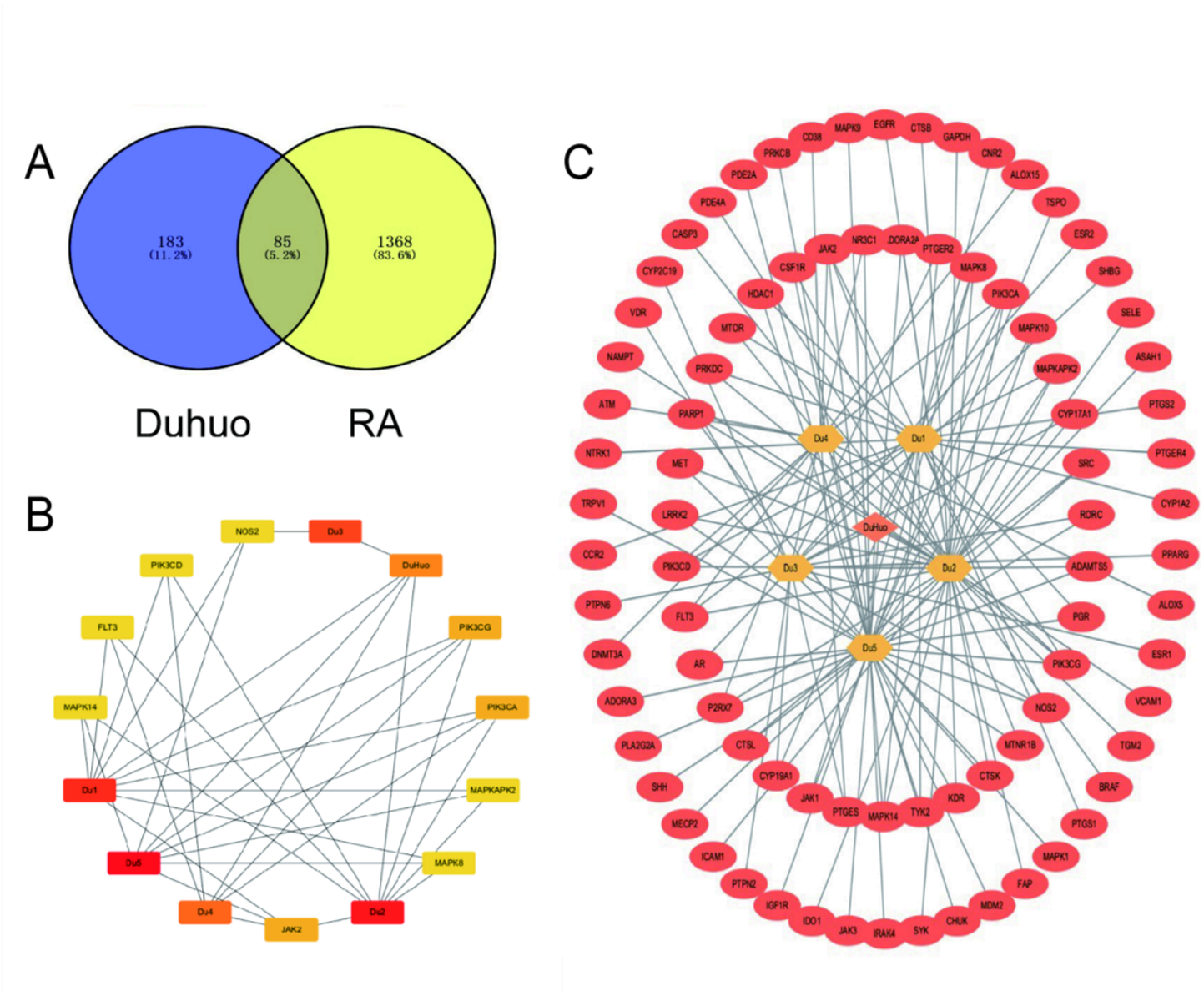


Figure 3

network pharmacology analysis of the core target of Duhuo in the treatment of RA.

A:venn diagram, 85 represents that Duhuo acts on 85 RA targets;B:PPI network analysis,Identified 9 core targets:PIK3CA、PIK3CG、JAK2、PARP1、FLT3、MAPK8、MAPKAPK2、PIK3CD、NOS2、MAPK14;C: drug-constituent-target network diagram.

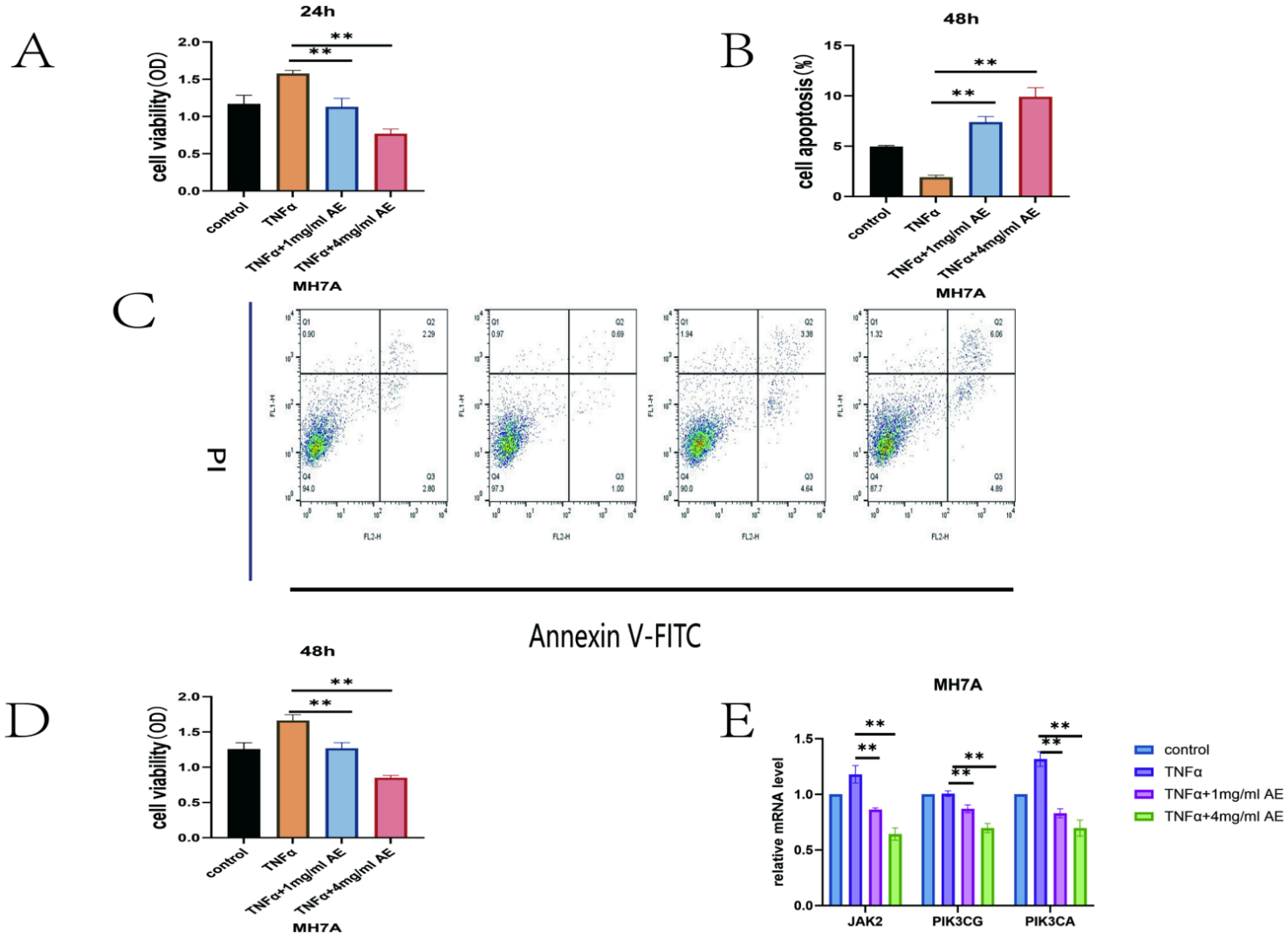


Figure 4

In vitro cell experiments.

A: Histogram of cell viability following 24-hour treatment of MH7A cells with Duhuo extract.

Control denotes the PBS control group; TNF-α concentration is 10 ng/mL; '**' indicates $P < 0.01$; 24 h denotes treatment of MH7A cells with AE for 24 hours.B:Cell survival rate after treatment of MH7A cells with Duhuo extract for 48 hours Control denotes the PBS control group; TNF-α concentration is 10 ng/mL; '**' indicates $P < 0.01$; 48 h denotes MH7A cells treated with Angelica pubescens extract for 48 hours.C:Apoptosis effects following treatment of MH7A cells with Duhuo extract for 48 hours The four quadrants represent distinct cellular states of activity: Q1 quadrant (Annexin V^-/PI^+): Necrotic cells or late apoptotic cells Q2 quadrant (Annexin V^+/PI^+): Late apoptotic cells Q3 quadrant (Annexin V^+/PI^-): Early apoptotic cells Q4 quadrant (Annexin V^-/PI^-): Viable cells;D:Apoptosis rate of MH7A cells after treatment with Duhuo for 48 hours Control denotes the PBS control group; TNF-α concentration is 10 ng/mL; '**' indicates $P < 0.01$; 48 h denotes MH7A cells treated with Angelica pubescens extract for 48

hours.E:Relative expression levels of target genes were assessed by RT-qPCR following treatment of MH7A cells with Duhuo extract for 24 hours.TNF-α concentration is 10 ng/mL; '**' indicates P < 0.01;