Atorvastatin ameliorates chronic subdural hematomas by interrupting the 'chronic subdural hematoma cycle' via inhibition of the inflammatory response

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Abstract. Chronic subdural hematomas (CSDHs) are prevalent neurosurgical occurrences characterized by progressive hemorrhagic expansion, which is primarily mediated by persistent inflammation, angiogenesis and fibrinolytic dysregulation. Atorvastatin, a widely used lipid-lowering agent with known anti-inflammatory and angiogenesis-modulating properties, has shown therapeutic potential in CSDH management. In the present study, network pharmacology and experimental validation were combined to elucidate the underlying mechanisms of atorvastatin in CSDH treatment. Potential targets were identified through database mining and Venn analysis, followed by Gene Ontology/Kyoto Encyclopedia of Genes and Genomes enrichment, protein-protein interaction network construction and molecular docking. *In vitro* experiments were performed to evaluate the effects of atorvastatin on a tumor necrosis factor-α-induced endothelial inflammation model, including on inflammatory cytokine secretion, target gene expression, endothelial permeability and tube formation. A total of 19 candidate therapeutic targets were identified, which were predominantly involved in the inflammatory response, coagulation, fibrinolysis and angiogenesis pathways. Core targets, including matrix metalloproteinase (MMP)-2, MMP-9, interleukin (IL)-6, C-X-C motif chemokine ligand 8/IL-8 and serpin family E member 1, demonstrated strong binding affinities with atorvastatin via molecular docking analyses. Furthermore, functional experiments revealed that atorvastatin significantly suppressed the expression of pro-inflammatory cytokines and adhesion molecules, mitigated endothelial barrier dysfunction, reversed the inhibitory effect of inflammation on endothelial tube formation and downregulated key pathogenic genes. Collectively, these findings suggest that atorvastatin may disrupt the 'CSDH cycle' by modulating critical inflammatory, angiogenic and fibrinolytic mechanisms, providing a scientific rationale for its therapeutic application in CSDH management. Further *in vivo* studies are warranted to validate these preliminary observations and to explore clinical translation to the clinic.

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Introduction

Chronic subdural hematomas (CSDHs) are neurological events characterized by the gradual accumulation of blood and its breakdown products in the subdural space between the dura mater and the cerebral cortex. CSDHs are most commonly associated with minor head trauma, which can rupture the bridging veins spanning the subdural space that then drain into the dural venous sinuses (1). CSDHs primarily affect the elderly population and have become one of the most frequently encountered neurosurgical disorders in clinical settings. Recent epidemiological data indicate that the annual incidence of CSDH ranges from 1 to 13.5 per 100,000 individuals in the general population, with a marked increase observed with advancing age. In individuals aged 65 years and older, the incidence rises to ~58.1 per 100,000 individuals per year (2). The incidence of CSDHs has steadily increased over recent decades; for example, a temporal analysis conducted in Finland from 1990 to 2015 reported a doubling in annual incidence, from 8.2 to 17.6 per 100,000 individuals per year, likely driven by population aging and the widespread use of antithrombotic agents (3). These epidemiological trends highlight the growing clinical burden of CSDHs and the need for improved therapeutic strategies.

The pathophysiology of a CSDH is multifactorial, involving a complex interplay of biological processes, among which inflammation has a central role. Following the initial hemorrhagic event, a persistent inflammatory cascade is triggered, which facilitates immune cell infiltration, activation of the fibrinolytic system and the formation of a vascularized outer membrane encasing the hematoma. The neovasculature within this membrane is typically immature and highly permeable,

resulting in recurrent microbleeding and the extravasation of plasma components. This self-perpetuating cycle, often referred to as the 'CSDH cycle', drives progressive hematoma expansion over time (2,3).

Considering the key roles of inflammation and aberrant angiogenesis in CSDH progression, recent pharmacological efforts have increasingly focused on targeting these pathways using established anti-inflammatory and anti-angiogenic therapies (4,5). Atorvastatin, a widely prescribed 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor primarily used to treat hyperlipidemia and atherosclerosis, has demonstrated a variety of biological effects beyond lipid-lowering (6). Notably, the anti-inflammatory and angiogenesis-modulating properties of atorvastatin render it a promising candidate for the management of CSDHs (7,8). Preliminary clinical investigations have indicated that atorvastatin may facilitate hematoma resolution and improve neurological outcomes in patients with CSDHs (9-11). These observations are further supported by animal studies showing similar therapeutic benefits (12,13); however, the precise molecular mechanisms by which atorvastatin mediates its effects on CSDHs have not yet been fully elucidated.

Network pharmacology offers an integrative framework for investigating the complex pharmacodynamic profiles of therapeutic agents by identifying their interactions with a number of molecular targets (14). This approach, which integrates computational modeling with systems biology, enables the construction of comprehensive drug-target networks and facilitates the discovery of novel mechanisms of action as well as drug repurposing opportunities. In the present study, network pharmacology analysis was combined with experimental *in vitro* validation to investigate the potential molecular mechanisms by which atorvastatin exerts therapeutic effects in the treatment of CSDHs. Specifically, this study aimed to identify the key molecular targets and signaling pathways through which atorvastatin modulates inflammation in CSDHs, and to provide mechanistic insights supporting its potential as a therapeutic agent for this condition.

Materials and methods

Prediction of the therapeutic targets of atorvastatin in CSDHs. Potential molecular targets of atorvastatin in CSDHs were systematically retrieved from the ChEMBL (https://www.ebi.ac.uk/chembl/), NCBI PubChem Compound (https://www.ncbi.nlm.nih.gov/pccompound) and SwissTargetPrediction (http://www.swisstargetprediction.ch/) databases using the keyword 'atorvastatin'. All identified targets were standardized to the official gene symbols via the UniProt database (https://www.uniprot.org/) and duplicate entries were removed to generate a non-redundant gene list. Concurrently, CSDH-related genes were obtained from the GeneCards database (https://www.genecards.org/) using the keyword 'CSDH' and from the DisGeNET database (https://www.disgenet.org/) using the term 'subdural hematoma' and duplicates were similarly excluded to ensure uniqueness. The intersection of the atorvastatin-associated and CSDH-associated genes was determined using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/), and the overlapping genes were defined as the putative therapeutic targets of atorvastatin in CSDHs.

Enrichment analysis. Enrichment analysis of the identified target genes was performed using R software (version 4.2.1; RStudio, Inc.). The gene identifiers were converted to Entrez IDs using the 'org.Hs.eg.db' package (15). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted using the 'clusterProfiler,' (16) 'enrichplot,' (17) 'org.Hs.eg.db' and 'pathview' (18) packages. $P<0.05$ and $Q<0.05$ was considered to indicate a statistically significant difference. Visualization of the enrichment results was performed using the 'ggplot2' (19) package.

Protein-protein interaction (PPI) network construction. To investigate the functional interactions among the candidate target proteins, a PPI network was constructed using the STRING database (https://string-db.org/). The resulting network was subsequently visualized using Cytoscape software (version 3.9.1; Cytoscape Consortium) (20).

Molecular docking analysis. The 2D chemical structure of atorvastatin was retrieved from the ChEMBL database (https://www.ebi.ac.uk/chembl/), whereas the 3D structures of the target proteins were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/; accession numbers: 8QY5, 6WZM, 8H78, 6ESM and 4G8O). Molecular docking simulations were conducted using AutoDockTools (version 1.5.7; Molecular Graphics Laboratory; The Scripps Research Institute). The ligand-receptor binding conformations and interactions were visualized using PyMOL software (version 2.5.4; Schrödinger, Inc.).

Cell culture. Human umbilical vein endothelial cells (HUVECs; cat. no. QS-H002; Keycell Biotechnology Co., Ltd.), authenticated via short tandem repeat profiling by the manufacturer, were purchased at passage 3 and cultured in endothelial cell-specific medium (cat. no. QS-H002A; Keycell Biotechnology Co., Ltd.) supplemented with 10% fetal bovine serum (HyClone™; Cytiva). The cells were maintained in a humidified incubator at 37˚C with 5% CO_2 and all experiments were performed using cells between passages 5 and 8.

Induction of inflammation and atorvastatin treatment. HUVECs were seeded into 6-well culture plates at a density of $5x10^5$ cells per well. The cells were treated with 10 μg/l tumor necrosis factor-α (TNF-α; cat. no. 300-01A-10UG; PeproTech Inc.; Thermo Fisher Scientific, Inc.) at 37˚C for 24 h to induce inflammation. To assess the effects of atorvastatin dose-dependently, cells were assigned to five groups: Control group (untreated), inflammation group (TNF-α treated only) and three intervention groups co-treated with TNF-α and 2.5, 10.0 or 20.0 μmol/l atorvastatin (cat. no. S5715: Selleck Chemicals) at 37˚C for 24 h; both agents were added to the culture medium at the same time to evaluate the direct protective effect of atorvastatin against TNF-α-induced inflammation. Based on the results of the dose-response experiments and previous studies (21,22), 10 μmol/l was selected to investigate the effect of atorvastatin on the expression of inflammation-related genes in endothelial cell models.

Table I. Primers used for reverse transcription-quantitative PCR.

Gene	Forward sequence (5' to 3')	Reverse sequence (5' to 3')	Size, bp
MMP-2	GTGTGAAGTATGGGAACGCC	CCTGGAAGCGGAATGGAAAC	231
MMP-9	GCTACCACCTCGAACTTTGAC	TCAGTGAAGCGGTACATAGGG	161
SERPINE-1	TGCCCTCACCAACATTCT	TGCCACTCTCGTTCACCT	54
GAPDH	TCAAGAAGGTGGTGAAGCAGG	TCAAAGGTGGAGGAGTGGGT	115

MMP, matrix metalloproteinase; SERPINE-1, serpin family E member 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Quantification of inflammatory cytokine levels by enzyme-linked immunosorbent assay (ELISA). After 24 h of treatment, cell culture supernatants were collected and centrifuged at 560 x g for 5 min at 4˚C. The concentrations of interleukin (IL)-6, C-X-C motif chemokine ligand 8 (CXCL-8)/IL-8, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) were measured using ELISA kits (IL-6, cat. no. EH0201; CXCL-8, cat. no. EH0205; ICAM-1, cat. no. EH0161; VCAM-1, cat. no. EH0326; Wuhan Fine Biotech Co., Ltd), in accordance with the manufacturers' instructions.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the HUVECs in each group after 24 h of treatment using TRIzol™ reagent (cat. no. 15596-026; Ambion; Thermo Fisher Scientific, Inc.), then RT was performed with the HiScript® II Q Select RT SuperMix kit (cat. no. R233; Vazyme Biotech Co., Ltd.) under the following conditions: 50˚C for 15 min, 85˚C for 5 sec and 4˚C for 10 min. qPCR was conducted using the AceQ qPCR SYBR Green Master Mix (cat. no. Q111; Vazyme Biotech Co., Ltd.) on a ViiA™ 7 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The qPCR cycling conditions were: 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 60 sec. A melting curve was obtained by 95˚C for 15 sec, 60˚C for 60 sec, and a gradual increase to 95˚C with continuous fluorescence acquisition to verify amplification specificity. Data were analyzed using QuantStudio™ Real-Time PCR software (v1.6.1; Thermo Fisher Scientific, Inc.). All primers were synthesized by Beijing Tsingke Biotech Co., Ltd. (Table I). Gene expression was normalized to GAPDH and the relative changes in gene expression were quantified using the $2^{-\Delta\Delta Cq}$ method (23).

FITC-dextran permeability assay for detecting endothelial cell barrier function. HUVECs were seeded into the upper chambers of Transwell inserts (0.4 μm microporous PET membrane; BD Biosciences) at a density of $5x10^4$ cells per well. The cells were cultured for 3-5 days until confluency was achieved, and a continuous monolayer was formed. The same experimental grouping and treatments as aforementioned were applied. After 24 h of intervention, the medium in the upper chamber was removed and replaced with 200 μl serum-free medium (cat. no. QS-H002A; Keycell Biotechnology Co., Ltd.) containing 1 g/l FITC-dextran (10 kDa; cat. no. HY-128868; MedChemExpress). Simultaneously, 800 μl serum-free medium was added to the lower chamber. The plates were then incubated at 37˚C with 5% CO_2 for 3 h. Following incubation, 100 μl of the medium was collected from the lower chamber and transferred to a 96-well plate for fluorescence measurement. As a reference, 100 μl of the initial FITC-dextran solution was used to determine the fluorescence intensity of the upper chamber. The permeability rate (%) was calculated as follows: (Fluorescence intensity of the lower chamber/initial fluorescence intensity of the upper chamber) x100.

Matrigel tube formation assay. To evaluate the effect of atorvastatin on the angiogenesis of endothelial cell models, a Matrigel tube formation assay was performed. Experimental procedures were conducted as previously described (24). Briefly, HUVECs ($5x10^5$ cells/well) were seeded on Matrigel, which had been thawed overnight at 4˚C and used to precoat the culture plates at 37˚C for 1 h before cell seeding, and were treated as aforementioned. Tube formation was visualized and images were collected under an inverted light microscope (IX51; Olympus Corporation).

Statistical analysis. The data are presented as the mean ± standard deviation. Statistical analysis was conducted using SPSS 25.0 software (IBM Corp.). Data were first assessed for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. Then, one-way analysis of variance (ANOVA) was employed to compare the differences among groups, followed by the least significant difference or Tukey's honestly significant difference post hoc test for pairwise comparisons. The overall P-values from ANOVA are reported in the text, whereas the significance levels from pairwise comparisons are indicated in the figures. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Potential targets of atorvastatin in the treatment of CSDHs. An initial list of 324 potential molecular targets of atorvastatin was obtained without applying a specific confidence score threshold, retaining only those with annotated gene symbols and functional annotations. After standardization and deduplication, 271 unique targets were retained. To identify the genes associated with CSDHs, 125 genes were retrieved from databases, resulting in 121 non-redundant CSDH-related genes. Cross-referencing these genes with the atorvastatin targets identified 19 overlapping genes through strict symbol matching via Venny, which were defined as the candidate

Table II. Potential target genes of atorvastatin in the treatment of chronic subdural hematoma.

Gene symbol	Gene name
MMP-9	Matrix metalloproteinase 9
SERPINE-1	Serpin family e member 1
MMP-2	Matrix metalloproteinase 2
FGF-2	Fibroblast growth factor 2
CXCL-10	C-X-C motif chemokine 10
PLAT	Tissue-type plasminogen activator
AKT-1	AKT serine/threonine kinase 1
ANGPT-2	Angiopoietin 2
CCL-2	C-C motif chemokine ligand 2
CD-36	CD36 molecule
CRP	C-reactive protein
CXCL-8/IL-8	C-X-C motif chemokine ligand 8/ Interleukin 8
EDN-1	Endothelin 1
F2	Coagulation factor II
F5	Coagulation factor V
IL-6	Interleukin 6
INS	Insulin
VWF	Von Willebrand factor
F7	Coagulation factor VII

Table III. Binding energies of potential target gene proteins with atorvastatin and other known ligands.

Target	Binding energy with Atorvastatin, kcal/mol	Binding energy with other ligands, kcal/mol
IL-6	-8.1	-5.9-5.8
MMP-2	-6.0	-8.2-5.7
MMP-9	-8.0	-8.1-5.9
SERPINE-1	-6.7	-8.2-5.3
CXCL-8/IL-8	-6.1	-8.7-4.4

IL-6, Interleukin 6; MMP, matrix metalloproteinase; SERPINE-1, serpin family E member 1; CXCL-8/IL-8, C-X-C motif chemokine ligand 8/Interleukin 8.

therapeutic targets of atorvastatin in the context of CSDHs (Table II).

Target gene enrichment analysis in the treatment of CSDHs with atorvastatin. GO enrichment analysis of the 19 candidate genes yielded 948 enriched terms, comprising 897 biological processes (BPs), 15 cellular components (CCs) and 36 molecular functions (MFs). The top 20 terms in each category are presented in Fig. 1A-C. These results suggest that atorvastatin may exert therapeutic effects in CSDH management by modulating BPs such as 'Wound healing', 'Coagulation', 'Chemotaxis', 'Hemostasis' and 'Leukocyte migration'. The enriched MFs included 'Cytokine receptor binding', 'Cytokine activity' and 'Serine hydrolase activity'. The associated CCs included 'Collagen-containing extracellular matrix', 'Peptidase inhibitor complex', 'Secretory granule lumen' and 'cytoplasmic vesicle lumen'.

KEGG pathway enrichment analysis revealed 62 pathways associated with the putative targets of atorvastatin in treating CSDHs, with the top 20 pathways shown in Fig. 1D and E. These pathways are primarily involved in the regulation of inflammation, coagulation and angiogenesis, including the 'HIF-1 signaling pathway', 'TNF signaling pathway', 'Complement and coagulation cascades', 'Toll-like receptor signaling pathway', 'PI3K-Akt signaling pathway' and 'IL-17 signaling pathway'.

PPI network analysis. A PPI network was constructed using the STRING database (Fig. 1F). Node degree centrality was assessed to identify the highly connected hub genes (Fig. 2A), with darker colors representing nodes of higher degree (greater connectivity). Based on both the topological importance and functional relevance, five core targets [matrix metalloproteinase (MMP)-2, MMP-9, IL-6, CXCL-8/IL-8 and serpin family E member 1 (SERPINE-1)] were selected for further validation through molecular docking and *in vitro* experiments. Other network metrics such as betweenness and closeness centrality were also calculated, although they were not used as primary selection criteria. These parameters reflect the extent to which a node acts as a bridge or is close to other nodes in the network, respectively, and were included to provide a more comprehensive topological characterization. However, node degree centrality was prioritized since it more directly represents the number of connections a protein has, which is commonly regarded as a reliable indicator of functional importance in biological networks.

Molecular docking. Results of the molecular docking simulations indicated that atorvastatin had strong binding affinities with all five core target proteins, with binding energies ≤-5.0 kcal/mol. This threshold value indicates strong ligand-receptor interactions (25), with binding energies <-7.0 kcal/mol suggesting a particularly high affinity (26). Furthermore, compared with the binding energies of these target proteins with other known ligands (27-31), atorvastatin also exhibited a moderate to strong binding affinity (Table III). Visualization of the molecular docking results (Fig. 2B) revealed stable binding conformations characterized by key hydrogen bonds and hydrophobic interactions (Table SI). These results provide mechanistic insights into the potential modulation of these targets by atorvastatin.

Atorvastatin reduces the inflammatory response in an endothelial cell inflammation model. As shown in Fig. 2C, stimulation with TNF-α significantly increased the secretion of the inflammatory biomarkers, ICAM-1 and VCAM-1, in the HUVEC culture supernatants compared with the control group. Treatment with atorvastatin at 2.5, 10.0 and 20.0 μmol/l significantly reduced the ICAM-1 ($F=60.544$; $P<0.001$) and VCAM-1 ($F=41.848$; $P<0.001$)

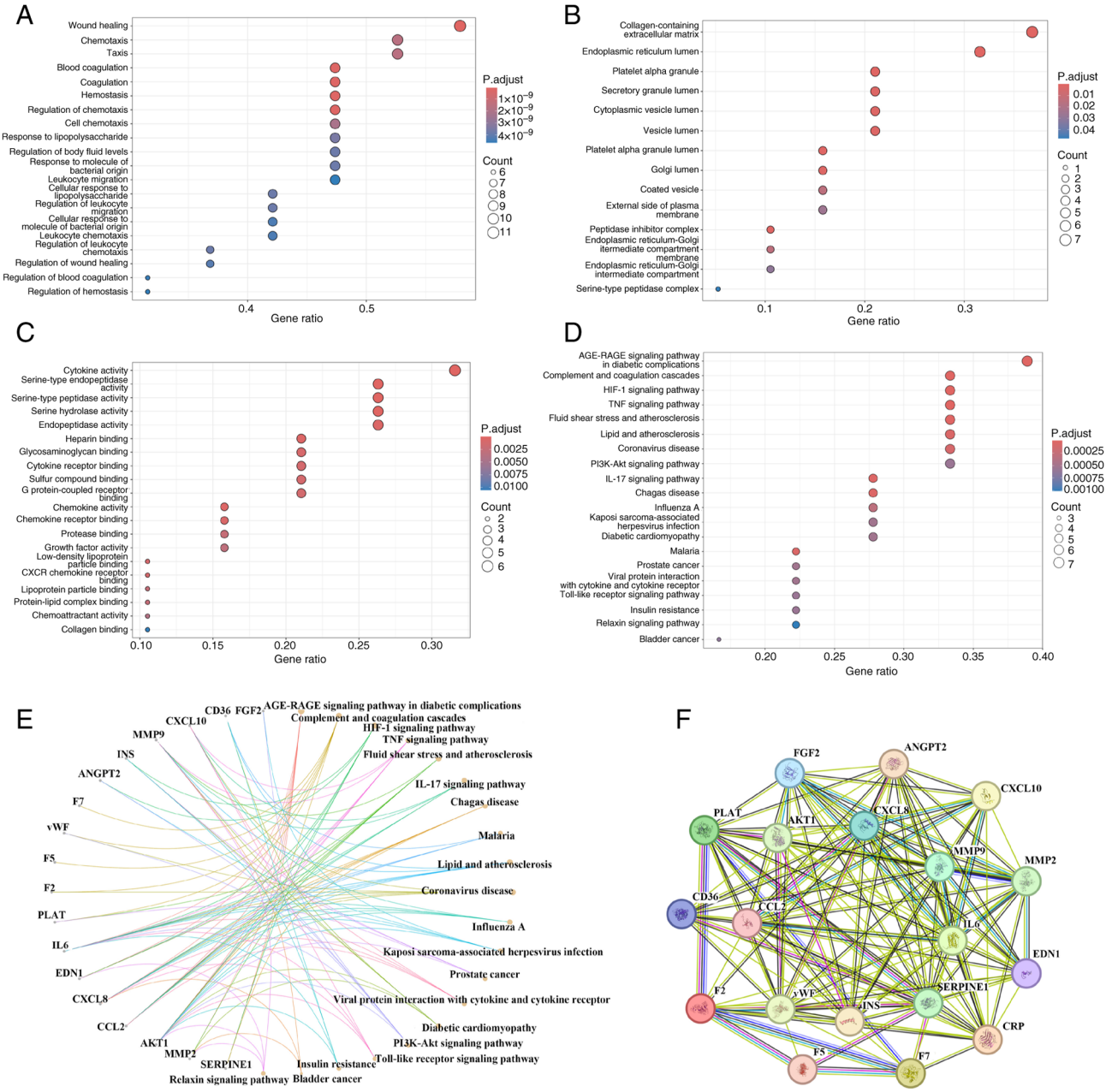

Figure 1. Enrichment analysis of the potential therapeutic targets of atorvastatin in treating chronic subdural hematoma. The top 20 enriched Gene Ontology terms included (A) biological processes, (B) cellular component and (C) molecular function. (D) The top 20 enriched pathways from the Kyoto Encyclopedia of Genes and Genomes analysis. (E) Network diagram of the target genes and related pathways. (F) Protein-protein interaction network of potential the target genes.

levels in a dose-dependent manner, compared with the inflammation group. However, no statistically significant difference was observed between the 10 and 20 μmol/l atorvastatin treatment groups (P>0.05).

Inhibitory effects of atorvastatin on TNF-α-induced inflammatory cytokine secretion and gene expression. As shown in Fig. 2D and E, TNF-α significantly increased the secretion of IL-6 and CXCL-8 in the HUVEC culture supernatants and significantly upregulated the mRNA expression of MMP-2, MMP-9 and SERPINE-1 in HUVECs. Treatment with 10 μmol/l atorvastatin significantly reduced the TNF-α-induced IL-6 (F=64.526; P<0.001) and CXCL-8 (F=37.779; P<0.001) secretion levels and significantly downregulated the mRNA expression of MMP-2 (F=264.413; P<0.001), MMP-9 (F=86.675; P<0.001) and SERPINE-1 (F=71.180; P<0.001).

Atorvastatin attenuates inflammation-induced endothelial barrier dysfunction. As shown in Fig. 2F, TNF-α stimulation markedly impaired the integrity of the endothelial barrier, resulting in a significant increase in FITC-dextran permeability from 1.14±0.08% in the control group to 11.68±0.13% in the inflammation group. Atorvastatin treatment effectively restored barrier function in a dose-dependent manner, reducing permeability to 7.83±0.17, 5.44±0.20 and 3.71±0.06% at 2.5, 10.0 and 20.0 μmol/l, respectively (F=2490.788; P<0.001). These results further support the hypothesis that atorvastatin

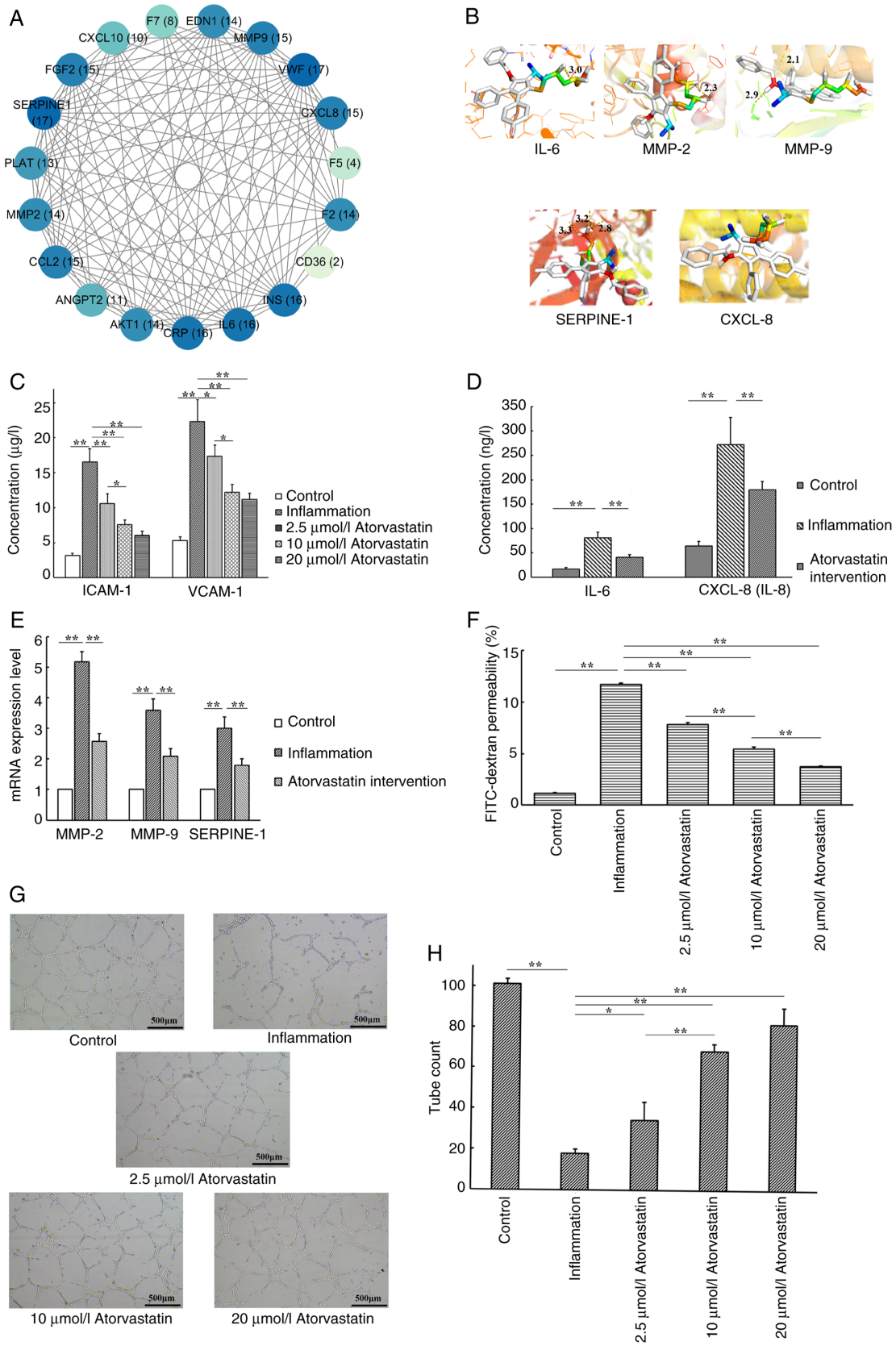

Figure 2. Molecular docking and functional validation of potential therapeutic mechanisms of atorvastatin in treating chronic subdural hematoma. (A) Degree values of the protein nodes that correspond to target genes in the protein-protein interaction network (values in parentheses indicate node degrees). (B) Molecular docking results of atorvastatin with proteins expressed by the core target genes. The anti-inflammatory effects of atorvastatin in the endothelial cell inflammation model were demonstrated. (C) Atorvastatin inhibited the TNF-α-induced inflammatory response in HUVECs, as evidenced by decreased ICAM-1 and VCAM-1 levels in a dose-dependent manner. (D) Atorvastatin at a concentration of 10 μmol/l reduced the secretion of inflammatory cytokines; (E) downregulated the expression of inflammation-related genes; and (F) protected against inflammation-induced endothelial barrier dysfunction. Atorvastatin also reversed the inhibitory effect of inflammation on endothelial tube formation. (G) Results of tube formation assay for each group. Scale bars 500 μm. (H) The tube counts in each group are shown. *P<0.05; **P<0.01. ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; MMP, matrix metalloproteinase; IL, interleukin; CXCL-8, C-X-C motif chemokine ligand 8; SERPINE-1, serpin family E member 1.

has a protective effect on endothelial barrier integrity under inflammatory conditions.

Atorvastatin protects endothelial angiogenesis from inflammation-induced inhibition. As shown in Fig. 2G and H, TNF-α stimulation significantly impaired tube formation in HUVECs compared with the control group. Co-treatment with atorvastatin at 2.5, 10.0 and 20.0 μmol/l significantly preserved angiogenic capacity in a dose-dependent manner, attenuating the inflammation-induced inhibition ($F=99.859$; $P<0.001$). However, no statistically significant difference was observed between the 10 and 20 μmol/l atorvastatin treatment groups ($P>0.05$).

Discussion

The pathophysiological mechanisms underlying the onset and progression of CSDHs have been increasingly clarified in recent decades. It is now accepted that the initial hemorrhage originates from ruptured bridging veins, often triggered by minor head trauma (particularly in elderly individuals with cerebral atrophy) or by low intracranial pressure and neurosurgical interventions (2,32). In the early phase following hemorrhage, inflammatory cells (including neutrophils, lymphocytes, macrophages and eosinophils) are recruited to the subdural space to initiate tissue repair (2,3). However, these immune cells also release inflammatory mediators and pro-angiogenic factors that contribute to the formation of the hematoma capsule and to the development of the neovascularization within it. The resulting vasculature is typically immature and highly permeable, allowing plasma leakage into the hematoma cavity and sustaining a cycle of inflammation and hematoma enlargement, a process often referred to as the 'CSDH cycle' (2,3). In addition to inflammation and angiogenesis, the dysregulation of coagulation and fibrinolysis is also involved in the pathogenesis of CSDHs. Enhanced fibrinolytic activity may lead to persistent microhemorrhage and progressive hematoma enlargement (33,34). Accordingly, fibrinolytic biomarkers such as tissue-type plasminogen activator (tPA) have been proposed as potential predictors of CSDH recurrence (35).

In the present study, the overlapping genes identified in the network pharmacology analysis of the treatment of CSDHs with atorvastatin were notably enriched in BPs related to inflammation such as 'Hemostasis' and 'Leukocyte migration', as well as key pathways such as the 'TNF signaling pathway', 'IL-17 signaling pathway' and 'HIF-1 signaling pathway'. These results provide support for the hypothesis that atorvastatin may interrupt multiple pathogenic associations in the CSDH cycle. In the present study, GO enrichment analysis revealed that the atorvastatin-associated genes were significantly enriched in biological processes related to these key mechanisms. Among the top 10 enriched processes, 'Wound healing' reflected an association to angiogenesis and inflammation (36), 'Blood coagulation' and 'Hemostasis' were associated with coagulation-fibrinolysis balance (37) and terms such as 'Leukocyte chemotaxis,' 'Leukocyte migration' and 'Cell chemotaxis' were indicative of inflammatory regulation (38). Based on the subsequent PPI network analysis performed in the present study and supporting literature (1-3), five key genes (IL-6, CXCL-8, MMP-2, MMP-9 and SERPINE-1) were identified as central regulators of inflammation, angiogenesis and coagulation/fibrinolysis in the treatment of CSDHs with atorvastatin. These genes were selected for further mechanistic investigation to elucidate the molecular basis of the therapeutic effects of atorvastatin. IL-6 and CXCL-8 (also known as IL-8) are pro-inflammatory cytokines predominantly secreted by fibroblasts, endothelial cells and various immune cells such as monocytes, macrophages, neutrophils and T lymphocytes (39). IL-6 mediates acute-phase inflammatory responses, immune cell differentiation, thrombopoiesis and leukocyte recruitment (40), whereas CXCL-8 has a key role in immune cell chemotaxis and promotes angiogenesis by stimulating endothelial cell proliferation and tube formation (41). Elevated levels of both cytokines have been detected in the hematoma fluid from patients with CSDHs, particularly those with recurrent hematomas (42). MMP-2 and MMP-9 are involved in the degradation of extracellular matrix (ECM) components and are functionally linked to both inflammation and angiogenesis, and aberrant upregulation of these enzymes contributes to neovascular instability and increases the risk of recurrent hemorrhage in CSDHs (43). Additionally, MMP-2 and MMP-9 participate in inflammatory signaling cascades by modulating the activity of cytokines and their receptors (44). Elevated levels of both enzymes have been observed in the hematoma fluid from patients with CSDHs (45). SERPINE-1, also known as plasminogen activator inhibitor-1, is a serine protease inhibitor predominantly expressed by endothelial cells; it regulates fibrinolysis by inhibiting tissue-type and urokinase-type plasminogen activators and has been implicated in multiple pathological processes, including inflammation, oxidative stress, fibrosis and macrophage migration (46).

In the present study, molecular docking simulations demonstrated that atorvastatin stably bound to the protein structures encoded by the five identified target genes. Consistent with these predictions, *in vitro* experiments revealed that atorvastatin treatment significantly reduced the secretion of ICAM-1 and VCAM-1 in TNF-α-stimulated HUVECs. These adhesion molecules are well-established biomarkers of endothelial inflammation (47), and their downregulation indicates effective suppression of TNF-α-mediated inflammatory responses. Additionally, atorvastatin significantly decreased the secretion of IL-6 and CXCL-8 and the mRNA expression of MMP-2 and MMP-9, supporting the mechanistic insights derived from both the network pharmacology and docking analyses. Therefore, the reduction in IL-6/CXCL-8 secretion and MMP-2/MMP-9 expression in HUVECs, coupled with the improvement in endothelial barrier integrity, may help limit microvascular leakage and inflammatory cell infiltration in hematoma membranes. These effects could, in turn, contribute to stabilization of neovessels and reduced re-bleeding, ultimately facilitating hematoma resolution *in vivo*; these mechanisms will be validated in animal models of CSDH in future studies. It is noteworthy that fibrinolysis may exert bidirectional effects in the pathogenesis and progression of CSDHs; in the pathophysiological setting of CSDHs, excessive fibrinolysis may accelerate ECM breakdown, potentially triggering pro-MMP activation and compromising vascular stability, thereby increasing the risk of microvascular leakage or re-bleeding (33,34). Paradoxically, hyperfibrinolysis could

also promote hematoma resolution by facilitating clot degradation and reducing postoperative hematoma recurrence (48). Therefore, as an important regulator of fibrinolysis, it is necessary to avoid overly simplistic conclusions regarding the regulation of SERPINE-1 in CSDHs. Future work should employ time-course analyses of fibrinolytic markers (such as tPA, plasmin activity and D-dimer), ECM degradation products, MMP/tissue inhibitors of metalloproteinase activity profiles and fibrosis markers (such as collagen I/III, α-smooth muscle actin and fibronectin) in both *in vitro* co-culture systems and *in vivo* CSDH models. Such integrative approaches will enable a more precise understanding of whether the effects of atorvastatin on SERPINE-1 confer a benefit or risk in the context of CSDH management.

Notably, in the present study, atorvastatin was demonstrated to significantly improve endothelial barrier integrity under inflammatory conditions as evidenced by the reduced FITC-dextran permeability, a key pathological feature implicated in CSDH progression (2,3). This improvement was accompanied by a concentration-dependent decrease in both cytokine secretion and permeability, underscoring the therapeutic window and potency of atorvastatin in mitigating endothelial dysfunction. Moreover, the present study demonstrated that TNF-α suppressed tube formation in HUVECs, supporting the notion that inflammatory cytokines impair normal angiogenesis and contribute to the formation of fragile, leaky vessels in CSDH (2,3). Atorvastatin alleviated this inhibitory effect, which may be attributed to its anti-inflammatory and endothelial-protective properties. A previous study has demonstrated that atorvastatin reduces CSDH in animal models by promoting angiogenesis (49). Collectively, these *in vitro* findings support the hypothesis that atorvastatin may exert therapeutic benefits in CSDH not only through inflammation suppression but also by improving the quality of angiogenesis within the hematoma capsule.

Taken together, the findings of the present study suggest that atorvastatin may interrupt the self-sustaining CSDH cycle by inhibiting inflammatory signaling, modulating fibrinolytic and angiogenic processes, suppressing the formation of immature leaky vessels, limiting immune cell infiltration and preserving endothelial function. These combined effects may facilitate hematoma liquefaction and enhance spontaneous resorption. Despite these promising results, it is important to recognize that the pathogenesis of CSDHs is multifaceted and that the regulatory effects of atorvastatin likely extend beyond the five core targets identified in the present study. Therefore, the proposed mechanism should be viewed as a partial representation of the therapeutic actions of atorvastatin. In the present study, KEGG pathway enrichment analysis further indicated that multiple classical inflammatory and angiogenic signaling pathways may be implicated in the mode of action of atorvastatin, including the 'HIF-1 signaling pathway', 'TNF signaling pathway', 'IL-17 signaling pathway', 'Toll-like receptor signaling pathway' and the 'PI3K-Akt signaling pathway'. Furthermore, clinical evidence has identified diabetes mellitus and atherosclerosis as notable risk factors for both the onset and recurrence of CSDH (50,51). Consistently, the KEGG analysis in the present study revealed the enrichment of pathways related to these metabolic and vascular disorders, such as 'AGE-RAGE signaling pathway in diabetic complications', 'Fluid shear stress and atherosclerosis' and 'Diabetic cardiomyopathy', providing potential avenues for exploring the broader mechanistic effects of atorvastatin in CSDHs. Additionally, several pathways associated with infectious diseases and malignancies were also enriched. Prior studies have reported associations between CSDH recurrence and underlying malignancy (52) or chronic infection (53). These findings suggest that the systemic health status of patients may influence disease progression and that statins may offer a benefit in patient subgroups with these comorbidities. The roles of these comorbidities in CSDH pathophysiology, as well as their potential modulation by atorvastatin, warrant further investigation. Although atorvastatin is primarily used to regulate lipid metabolism (6), the results of the enrichment analysis in the present study did not clearly indicate a potential role of lipid metabolism regulation in its treatment of CSDH. Therefore, the *in vitro* endothelial inflammation model established in the present study does not involve lipid metabolism, and the observed molecular effects are more likely attributable to the pleiotropic effects of atorvastatin rather than its lipid-lowering properties. Nonetheless, it must be acknowledged that *in vivo*, lipid regulation may synergistically contribute to endothelial protection (54). In future studies, control compounds with lipid-lowering effects but minimal pleiotropic activity will be used to help delineate the relative contributions of each mechanism.

Despite the promising findings of the present study, several limitations should be acknowledged. First, the study relied solely on *in vitro* experiments using HUVECs as a model for endothelial inflammation; although this approach provides mechanistic insights, it may not fully replicate the cerebrovascular endothelial environment of CSDHs, nor simulate interactions with macrophages and fibroblasts. Second, the key inflammatory regulatory signaling pathways of statins, such as the NF-κB pathway, have not been directly evaluated. Third, the network pharmacology analysis was based on existing public databases, which may not cover all relevant molecular interactions or reflect tissue-specific gene expression profiles. In terms of target gene selection, while the method employed in the present study provides clear traceability, it may underestimate the extent of functional overlap. The selection of five core target genes, while supported by the established PPI network and functional relevance, may overlook other key targets involved in CSDH progression. Moreover, the present study did not examine the long-term treatment outcomes of atorvastatin. Finally, the lack of *in vivo* validation, such as animal model experiments, limits the translational applicability of the current findings. Future studies should incorporate *in vivo* CSDH models for validation of the core molecular targets, integrate proteomic and transcriptomic analyses to delineate the downstream signaling networks and assess the dose-response relationships and pharmacokinetic profiles of atorvastatin in animal models. In addition, broader target selection and verification such as scoring-based or relevance-weighted intersections, along with the incorporation of clinical data, will be essential to more comprehensively elucidate the therapeutic mechanisms of atorvastatin on CSDHs. The next phase of research will also focus on p65 nuclear translocation assays and phosphorylated p65 expression by western blotting to investigate whether the anti-inflammatory

effects of atorvastatin are mediated, at least in part, through NF-κB inhibition. Meanwhile, pathway-specific verifications, including HIF-1α and IL-17 protein levels, will also be prioritized in future mechanistic studies. Furthermore, future studies will incorporate direct assessments of ECM remodeling, such as collagen degradation assays, gelatin zymography and immunofluorescent staining for ECM components, to confirm whether the downregulation of MMP-2 and MMP-9 contributes to the preservation of ECM integrity.

In conclusion, the present study identified key molecular targets and signaling pathways potentially involved in the therapeutic effects of atorvastatin on CSDHs. The results from the integrated network pharmacology analysis and *in vitro* experimental validation suggest that atorvastatin may exert its effects by modulating inflammation, angiogenesis and fibrinolysis. These findings provide a mechanistic foundation and valuable reference for future preclinical and clinical studies aimed at elucidating the therapeutic potential of atorvastatin in managing CSDHs.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CL conceived and designed the study. JY performed the *in vitro* experiments. XW conducted the bioinformatics analyses. CL and JY contributed primarily to manuscript drafting and confirm the authenticity of all the raw data. All authors reviewed and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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