The Mechanism Study of Xiao-Xian-Xiong Decoction in the Treatment of Atherosclerosis with Network Pharmacology

Liucheng Xiao
Hunan University of Chinese Medicine
Zonghuan Li
Hunan University of Chinese Medicine
Chongyuan Fan
Hunan University of Chinese Medicine
Chenggong Zhu
Hunan University of Chinese Medicine
Xingyu Ma
Hunan University of Chinese Medicine
Rong Yu
Hunan University of Chinese Medicine
Xuejiao Xie (99511298@qq.com)
Hunan University of Chinese Medicine https://orcid.org/0000-0001-7420-9681

Research

Keywords: Xiao-Xian-Xiong decoction, Atherosclerosis, Mechanism, Inflammation

Posted Date: September 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-73640/v1

Abstract

Background: Xiao-Xian-Xiong decoction is a useful formula in the treatment of atherosclerosis in traditional Chinese medicine. In this study, we aimed to investigate the function of Xiao-Xian-Xiong decoction in the treatment of atherosclerosis.

Methods: In this study, we conducted the method of network pharmacology and molecular docking to discover the mechanism of Xiao-Xian-Xiong decoction against atherosclerosis. Then, we validated the function of Xiao-Xian-Xiong decoction in atherosclerosis *in vitro*. We investigated the function and mechanism of Xiao-Xian-Xiong decoction in RAW264.7 macrophage-derived foam cells.

Results: We identified 213 targets of Xiao-Xian-Xiong decoction and 331 targets of atherosclerosis. The PPI networks of Xiao-Xian-Xiong decoction and atherosclerosis were constructed. Furthermore, the two PPI networks were merged and the core PPI network was obtained. Then, functional enrichment analysis was conducted with GO and KEGG signaling pathway analysis. KEGG analysis indicated Xiao-Xian-Xiong decoction was correlated with ubiquitin mediated proteolysis pathway, PI3K-AKT pathway, MAPK pathway, Notch signaling pathway, and TGF-β signaling pathway. At last, we validated the function of Xiao-Xian-Xiong decoction with atherosclerosis *in vitro*. Xiao-Xian-Xiong decoction reduced lipid accumulation and promoted the outflow of cholesterol in RAW264.7-derived foam cells. Xiao-Xian-Xiong decoction increased the expression of ABCA1 and ABCG1 protein in foam cells. ABCA1 and ABCG1 were related with regulation of the inflammatory pathway and cell proliferation in atherosclerosis.

Conclusions: Combined the mechanism of available treatments of atherosclerosis, we inferred Xiao-Xian-Xiong decoction could alleviate atherosclerosis by inhibiting inflammatory response and cell proliferation.

1. Introduction

Atherosclerosis is a chronic vascular disease characterized with plaque formation in artery. It is the reason of various diseases including stroke, coronary artery disease (CAD), and vascular dementia (1). The risk factors of atherosclerosis contain high blood pressure, diabetes, obesity, smoking, and unhealthy diet (2). Atherosclerosis is regard as the chronic inflammatory response to lipid accumulation in the artery initially with intimal plaques for several years (3). The plaque in the layer of artery is consisted with cholesterol, calcium, fat, and lipoproteins. Complex series of cellular and molecular events are involved in the mechanism of plaque formation. In the early stage of atherosclerosis, monocytes in the blood circulation adhere to the endothelium and derived to macrophages (4). Activated by inflammatory response and oxidative stress, inflammatory cells infiltrate to artery wall and stimulate plaque formation (5). Later, smooth muscle cells form sub-endothelial cap structure to stabilize the plaque. The process of plaque formation may be silent for several years and without clinical manifestations. However, thrombotic occlusion might ensue if the surface of plaque is damaged. So, it is important to deal with atherosclerosis to prevent thrombotic occlusion. The medications of atherosclerosis include stains,

aspirin, and fibrates (6). Surgery such as vascular bypass surgery and angioplasty are considered as available treatments of atherosclerosis. However, previous treatments have some adverse effects including muscle toxicity and rhabdomyolysis (7). So, it is important to discover novel treatments in dealing with atherosclerosis.

Xiao-Xian-Xiong decoction is a famous traditional Chinese medicine formula which has been used by Chinese medicine practitioners for more than 2,000 years. There are 3 substances in this formula including Gualou (*Fructus Trichosanthis Kirlowii*), Huanglian (*Rhizoma Coptidis*), and Banxia (*Rhizoma Pinelliae Tematae*). This formula could relieve cough and expel phlegm under the principle of Chinese medicine. Previous study has been revealed Xiao-Xian-Xiong decoction could alleviate obesity by inactivating catalytic activity of human pancreatic lipase (8). However, the mechanism of Xiao-Xian-Xiong decoction in the treatment of atherosclerosis has not been discovered clearly.

Network pharmacology is a novel subject focused on systemic pharmacology to determine the relationship between diseases and drugs. The network of targets to drugs is helpful to understand mechanism of drug, while the network of targets to diseases is critical to conduct drug design and discovery. Owning to the cardiovascular diseases and cerebral-vascular diseases are characterized with multiple molecule mutations or signaling pathways dysfunction, it is necessary to conduct network pharmacology to determine the mechanism of drugs.

In this study, we investigated the functions of Xiao-Xian-Xiong decoction in the treatment of atherosclerosis with the principle of network pharmacology. Firstly, we determined the targets of atherosclerosis and Xiao-Xian-Xiong decoction, respectively. Two PPI networks were merged and core PPI network was obtained under network topological features. Gene Ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis were performed to reveal the related signaling pathways and biological processes. Molecular docking was conducted in our study, either. Then, we validated the function of Xiao-Xian-Xiong decoction in atherosclerosis *in vitro*. We investigated the function and mechanism of Xiao-Xian-Xiong decoction in RAW264.7 macrophage-derived foam cells. This research was hoped to provide novel insight and meaningful analysis of Xiao-Xian-Xiong decoction in treating atherosclerosis.

2. Methods

2.1 Targets determination for atherosclerosis

We used 4 related databases to determine the targets of atherosclerosis. The four databases were DrugBank database, Genetic Association Database (GAD), Therapeutic Target Database (TTD), Online Mendelian Inheritance in Man (OMIM). DrugBank database (https://www.drugbank.ca/) is a useful bioinformatics database to offer information on drugs and disease. GAD database (https://geneticassociationdb.nih.gov/) is a comprehensive database to investigate the association between genes and proteins in complex disorders and diseases. TTD (https://db.idrblab.org/ttd/) is a

comprehensive database to disclose data about therapeutic protein and nucleic acid targets, the targeted disease. OMIM (https://www.omim.org/) is a useful and comprehensive database on human genes and genetic diseases.

2.2 Targets determination for Xiao-Xian-Xiong decoction

We used 3 related databases to determine the targets of Xiao-Xian-Xiong decoction. The 3 databases were traditional Chinese medicine systems pharmacology (TCMSP) database, STITCH database, and Swiss Target Prediction database. TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php) is a useful pharmacological database especially on traditional Chinese medicines describing the correlations between targets, drugs, and diseases. STITCH database (http://stitch.embl.de/) is a comprehensive tool to investigate the relationship between chemicals and proteins. Swiss Target Prediction database is a useful web server to predict the bioactive molecule of ligands.

2.3. PPI network construction

Cytoscape plugin BisoGenet was used to obtain the protein-protein interaction (PPI) in this research. Cytoscape plugin BisoGenet had 6 PPI databases containing the Biomolecular Interaction Network Database (BIND), Human Protein Reference Database (HPRD), Database of Interacting Proteins (DIP), Biological General Repository for Interaction Datasets (BioGRID), IntAct Molecular Interaction Database (IntAct), Molecular INTeraction Database (MINT). Then, Cytoscape software (version 3.7.1, Boston, MA, USA) was used to construct and visualize targets of atherosclerosis and Xiao-Xian-Xiong decoction. Furthermore, we merged these two PPI networks and obtained core PPI network under network topological features.

2.4. Network topological features analysis

In this study, we used 6 parameters to analyze the topological features of nodes in the interaction network. The 6 parameters contained betweenness centrality (BC), degree centrality (DC), eigenvector centrality (EC), closeness centrality (CC), local average connectivity (LAC), and network centrality (NC). Cytoscape software plugin cytoNCA was used to conduct the analysis.

2.5 GO and KEGG analysis

In this study, we used the database for Annotation, Visualization and Integrated Discovery (DAVID) to perform functional enrichment analysis of GO and KEGG analysis. GO analysis is a powerful bioinformatics tool to discover biological process (BP), cellular components (CC), and molecular functions (MF) of genes. KEGG signaling pathways analysis is a combination of database about genomes, biological pathways, diseases, and drugs. At last, OmicShare platform (https://www.omicshare.com/) was performed to visualize the bubble chart.

2.6 Molecular docking

We used CB-Dock (http://cao.labshare.cn/cb-dock/) to predict the binding activities of Xiao-Xian-Xiong decoction compounds to targets of core PPI network. The Vina score and cavity size were calculated,

either. The “cartoon” and “spacefill” were set as receptor and ligand, respectively. “Chain” and “element” were colored receptor and ligand.

2.7. Drug and chemicals

Fetal bovine serum (FBS) was purchased from Sera Pro (S601S-500), ox-LDL was purchased from Guangzhou Yiyuan (YB-002), ABCA1 antibody was purchased from Abcam (ab18180), ABCG1 antibody was purchased from Abcam (ab52617), β-actin antibody was purchased from proteintech (20536-1AP), NBD-cholesterol was purchased from Thermo (N1148).

2.8. The preparation of cell model and Xiao-Xian-Xiong decoction

RAW264.7 cells were cultured at 37 °C in a 5% CO2 cell incubator. The cells were replaced with RPMI1640 medium and supplemented with ox-LDL at a concentration of 25 μg/ml for 24 h to obtain the macrophage-derived foam cells.

Xiao-Xian-Xiong decoction contained 6 g Coptis chinensis, 12 g Pinellia chinensis, and 30 g Melon fruit. After soak and boiling, the final concentration of Xiao-Xian-Xiong decoction was 0.5 g/ml. Then, we sterilized Xiao-Xian-Xiong decoction by high temperature and high pressure, filtered with a 0.22 μm sterile filter, and stored in a refrigerator at -20 ℃.

2.9. CCK-8 measurement

96-well plates were used for cell plating detection. The drug stimulation was given according to the experimental design. We added 10 μl CCK-8 reagent to each well, and measure the absorbance at 450 nm with a microplate reader. The cell survival rate was calculated after administration of different concentrations. Then, the appropriate concentration was selected.

2.10. Oil red O staining

The cells were fixed with 4% paraformaldehyde and rinsed with 60% isopropanol solution. Oil red working solution was added and stained for 30 min. Then, we removed the oil red dye and counterstained the cell nucleus with hematoxylin. At last, the cells were observed under microscope.

2.11. Western blot analysis

We used SDS-PAGE to separate the proteins. Then, the proteins were transferred to nitrocellulose membranes. TBST buffer with 5% nonfat dry milk was used to block the membranes at room temperature for 2 h. The membranes were incubated with primary antibodies (β-actin, ABCA1, and ABCG1) overnight at 4 °C. In the next day, we washed the membrane with TBST 3 times. Secondary antibodies were conducted to cover the membrane about 2 h. At last, enhanced chemiluminescence (ECL) reagent was used to visualize the proteins.

2.12. Cell cholesterol efflux detection

RAW264.7 macrophage-derived foam cells were incubated in phenol red-free RPMI1640 medium containing 5 μmol/l NBD Cholesterol for 4 h, and then incubated with HDL (50 μg/ml) as lipid receptor. The culture medium and cell lysate were collected to detect the fluorescence intensity (FI), and the cells were lysed with RIPA in a 12-well plate. Using a fluorescence microplate reader to measure the FI of NBD-cholesterol in the culture medium and cell lysate, the excitation wavelength was 469 nm and the emission wavelength was 537 nm. The percentage of NBD-cholesterol efflux is calculated by dividing the FI in the medium by the sum of the FI in the medium and cell lysates.

2.13. Statistical analysis

SPSS 21.0 statistical software was used for statistical analysis of experimental data. The results were expressed as means ± standard error of the mean (SEM). Differences of the results values were analyzed by Student t test or one-way analysis of variance (ANOVA) for multiple comparisons. When $P < 0.05$, the difference was statistically significant.

3. Results

3.1. Determination of atherosclerosis targets

In this study, we determined 331 potential targets in atherosclerosis with four databases including DrugBank, TTD, GAD, and OMIM databases. The potential atherosclerosis targets contained ABCG2, APOA1, CDKN2A, MAPK9, etc. Figure 1A described the detailed data of potential targets in atherosclerosis.

3.2. Determination of compounds and targets of Xiao-Xian-Xiong decoction

A total of 38 compounds of Xiao-Xian-Xiong decoction were recognized by TCMSP database. 213 potential targets of Xiao-Xian-Xiong decoction were predicted with databases of TCMSP, Swiss Target Prediction, and STITCH. The potential Xiao-Xian-Xiong decoction targets contained ADH1C, BCL2, CDK2, HSF1, etc. Figure 1B showed the detailed data of compounds and potential targets of Xiao-Xian-Xiong decoction.

3.3. Construction of PPI networks

We used PPI networks to discover the relationships of proteins in atherosclerosis and Xiao-Xian-Xiong decoction. Firstly, we constructed PPI network of atherosclerosis targets. Figure 2A indicated PPI network of atherosclerosis targets possessed 5451 nodes and 130891 edges. Secondly, we constructed PPI network of Xiao-Xian-Xiong decoction targets. Figure 2B indicated PPI network of Xiao-Xian-Xiong decoction targets possessed 6170 nodes and 150379 edges. Thirdly, we constructed a new PPI network

with Cytoscape software by merging previous two networks to investigate the function of Xiao-Xian-Xiong decoction in treating atherosclerosis. Figure 2C indicated the merged PPI network possessed 3969 nodes and 108203 edges.

3.4. Topological features analysis of PPI network

We conducted network topological features analysis to construct core PPI network. Firstly, we performed the parameter DC≥68 to filter the data. The results indicated that the PPI network had 945 nodes and 42941 edges (Fig. 3). Secondly, six parameters in topological features analysis were used to acquire core PPI network. In the six parameters, BC≥0.00043, CC≥0.516, DC≥146, EC≥0.0212, LAC≥18.49, NC≥21.11. The core PPI network was showed in Fig. 3C.

The core PPI network possessed 113 nodes and 2764 edges. The nodes of core PPI network contained neurotrophic receptor tyrosine kinase 1 (NTRK1), cullin 3 (CUL3), tumor protein p53 (TP53), fibronectin 1 (FN1), estrogen receptor 1 (ESR1), cyclin-dependent kinase 2 (CDK2), etc.

3.5. GO and KEGG analysis

We performed GO and KEGG analysis to conduct functional enrichment analysis with DAVID dataset. GO analysis was used to investigate the biological process (BP) of Xiao-Xian-Xiong decoction in treating atherosclerosis. The results of GO analysis indicated the BP was related with translational initiation, epigenetic gene expression, cell-cell adhesion, cellular protein metabolic process, apoptotic process, etc (Fig. 4).

Furthermore, KEGG signaling pathway was conducted to discover the related signaling pathways of Xiao-Xian-Xiong decoction in treating atherosclerosis. The signaling pathway were showed as follows: cell cycle, ubiquitin mediated proteolysis, PI3K-AKT signaling pathway, DNA replication, protein processing in endoplasmic reticulum, HIF-1 signaling pathway, estrogen signaling pathway, MAPK signaling pathway, notch signaling pathway, TGF-β signaling pathway, etc (Fig. 5).

3.6. Compound-target docking

The top targets in core PPI network were docked with compounds in Xiao-Xian-Xiong decoction. The Vina score with lowest binding energy and highest cavity size were determined. Vina score showed binding activity about the compound and protein. Lower Vina score indicated more stable of target binding compound, while higher cavity size indicated the increased accuracy of docking trends. The cartoon chain and spacefill indicated the protein and ligand, respectively. The results of molecular docking indicated that Xiao-Xian-Xiong decoction compounds had efficient binding activities to AS targets (Table 1). The specific binding activities of compound to targets were showed in Fig. 6.

Table 1
The binding activities of Xiao-Xian-Xiong decoction compounds to targets

Compound	Target	Vina Score	Cavity Size	Center			Size		
				x	y	z	x	y	z
Baicalein	HSP90AB1	-8	474	36	25	73	20	20	20
Baicalein	TP53	-6.1	93	10	20	2	20	20	20
Berberine	ESR1	-6.9	430	2	-2	11	22	22	22
Berberine	HSP90AB1	-8.1	489	13	41	74	22	22	22
Berlambine	HSP90AB1	-8.4	474	36	25	73	22	22	22
Beta-sitosterol	HSP90AB1	-8.6	504	32	30	29	25	25	25
Cavidine	HSP90AB1	-7.9	504	32	30	29	22	22	22
Coniferin	ESR1	-7	816	17	-15	14	22	22	22
Coptisine	ESR1	-9.6	1254	-11	2	18	22	22	22
Diosmetin	HSP90AB1	-7.7	491	45	73	64	22	22	22
Epiberberine	ESR1	-7.2	430	2	-2	11	22	22	22
Hydroxygenkwanin	HSP90AB1	-7.6	489	13	41	74	21	21	21
Moupinamide	EGFR	-8	1009	119	71	42	23	23	23
Palmatine	ESR1	-6.7	1069	7	7	13	22	35	22
Palmatine	HSP90AB1	-7.6	545	14	41	74	22	22	22
Quercetin	EGFR	-7.7	970	47	43	57	27	21	21
Quercetin	HSP90AB1	-7.9	652	29	61	69	21	21	21
Quercetin	MYC	-5.6	154	46	45	8	21	21	21
Quercetin	PARP1	-9.7	914	9	3	31	21	31	21
Quercetin	TP53	-5.7	93	10	20	2	21	21	21
Quercetin	VCAM1	-7.3	157	11	12	72	21	21	21
Worenine	ESR1	-8.3	1288	-11	2	18	22	22	28

3.6. Effect of Xiao-Xian-Xiong decoction on the proliferation of RAW264.7-derived foam cells

The extracts of Xiao-Xian-Xiong decoction with different concentrations were co-cultured with RAW264.7 foam cells for 24 h. The cell survival rate was determined by CCK-8 measurement. The results showed that Xiao-Xian-Xiong decoction had obviously inhibitory effects on cells compared with normal group when the concentration was ≥ 5 mg/ml. On the contrary, Xiao-Xian-Xiong decoction did not have obviously inhibitory effects on cells compared with normal group when the concentrations were 0.625 mg/ml, 1.25 mg/ml, and 2.5 mg/ml (Fig. 7). So, the concentrations about 0.625 mg/ml, 1.25 mg/ml, and 2.5 mg/ml were selected as low concentration group, medium concentration group, and high concentration group in the follow-up experiments.

3.7. Xiao-Xian-Xiong decoction reduced lipid accumulation in RAW264.7-derived foam cells

After incubation with 25 μg/ml ox-LDL, a large number of lipid droplets were accumulated in the foam cells. Oil red O staining was used to evaluate the lipid accumulation in foam cells. As shown in Fig. 8, medium concentration and high concentration groups had significantly inhibitory effects on lipid accumulation in foam cells ($p < 0.001$). However, low concentration group did not have significantly inhibitory effects on lipid accumulation in foam cells ($p > 0.05$).

3.8. Xiao-Xian-Xiong decoction promoted the outflow of cholesterol in foam cells

The foam cell cholesterol efflux was detected by fluorescence with NBD cholesterol. The results showed that Xiao-Xian-Xiong decoction significantly promoted the outflow of intracellular cholesterol compared with model group. In addition, there was no obviously different between Xiao-Xian-Xiong decoction group and negative normal group (Fig. 9). This indicated that Xiao-Xian-Xiong decoction could induce the outflow of cholesterol in foam cells.

3.9. Xiao-Xian-Xiong decoction increased the expression of ABCA1 and ABCG1 protein in foam cells

In this study, we measured the expression of ABCA1 and ABCG1 protein in foam cells. The results showed the expression of ABCA1 and ABCG1 protein were significantly reduced in model group compared with negative normal group (Fig. 10). This result indicated ox-LDL inhibited the expression of ABCA1 and ABCG1 in foam cells. As for ABCA1, Xiao-Xian-Xiong decoction could up-regulate the expression of ABCA1 in a dose manner. As for ABCG1, Xiao-Xian-Xiong decoction also significantly up-regulated the expression of ABCG1 compared with the model group.

4. Discussion

Atherosclerosis is responsible for the cause of many diseases such as myocardial infarction, ischemic stroke, and vascular dementia (9). Chronic inflammatory response to the lipid accumulation in artery wall is the feature of atherosclerosis (10). The preventive strategies include healthy diet, exercise, and no

smoking. The treatments of atherosclerosis aim to control risk factors and maintain perfusion of affected arteries. Stains reduce atherosclerotic lesions and restrain the progression (11, 12). Aspirin inhibits platelet aggregation to prevent coronary heart disease (13). Stains and aspirin have multiple functions in the treatment of atherosclerosis. Apart from medications, surgery such as angioplasty and vascular bypass surgery could re-establish flow and re-open narrowed arteries. However, the previous methods of atherosclerosis have some side effects. It is important to investigate novel strategy of atherosclerosis.

Network pharmacology may provide important reference in safety evaluation and efficacy in traditional Chinese medicine (14). The network based analysis accelerates the comprehension of traditional medicine to promote drug discovery. In the opinion of traditional Chinese medicine, Xiao-Xian-Xiong decoction alleviates viscous sputum, cough, and constipation. Xiao-Xian-Xiong decoction is widely used in the treatment of atherosclerosis in clinical practice. In this study, we used the method of network pharmacology and molecular docking to discover the mechanism of Xiao-Xian-Xiong decoction against atherosclerosis. We also demonstrated the function of Xiao-Xian-Xiong decoction in atherosclerosis *in vitro*.

In this study, KEGG analysis indicated Xiao-Xian-Xiong decoction was correlated with ubiquitin mediated proteolysis pathway, PI3K-AKT pathway, MAPK pathway, Notch signaling pathway, and TGF-β signaling pathway. These signaling pathways were inflammatory pathways involved in inflammatory response to different extracellular and intracellular stimuli. Molecular docking also indicated that the compounds in Xiao-Xian-Xiong decoction bound to the inflammatory proteins such as EGRF, ESR1, HSP90AB1, etc. Atherosclerosis is characterized with a chronic inflammatory disorder that chronic inflammatory play key factor in the prognosis of atherosclerosis (15). Inflammatory cells including macrophages infiltrate into vascular endothelium in the early stage. These inflammatory cells produce and secret inflammatory cytokines to exacerbate atherosclerosis (16). Previous studies have been demonstrated the available treatments such as statins and aspirin could alleviate atherosclerosis by inhibiting inflammation. Statins inhibit the inflammatory response of macrophages to reduce the expression of IL-6, TNF-α, and IFN-γ (17). In addition, statins suppress the infiltration of inflammatory cells by inhibiting chemokines including monocyte chemoattractant protein-1 (MCP-1). As for the other available treatment, aspirin reduces vascular inflammation to alleviate atherosclerosis (18). Aspirin down-regulates the activities of inflammatory mediators such as NF-κB, MCP-1, and TNF-α. Furthermore, aspirin could protect the endothelial function by inhibiting inflammatory response (19). In our study, we revealed Xiao-Xian-Xiong decoction was correlated with inflammatory pathways including ubiquitin mediated proteolysis pathway, PI3K-AKT pathway, MAPK pathway, Notch signaling pathway, and TGF-β signaling pathway. These inflammatory signaling pathways play important role in the pathogenesis of atherosclerosis. The ubiquitin proteasome system regulates process of oxidative stress and inflammation that dysfunction of ubiquitin proteasome system accelerates plaque progression and rupture of atherosclerosis (20). PI3K-AKT signaling pathway regulates inflammation in atherosclerosis such as fibrous cap formation. Down-regulated activity of PI3K-AKT signaling pathway increases the plaque stability of atherosclerosis (21). MAPK pathway facilitates the formation of foam cells in atherosclerosis (22). The components in MAPK pathways including c-Jun N-terminal kinases 2 (JNK2) and p38α MAPK are important in foam cell

formation. Notch signaling pathway regulates atherosclerosis by polarizing macrophages into M1 or M2 subtypes (23). TGF-β signaling pathway orchestrates fibro-proliferative response to tissue damage in atherosclerosis (24). Similar to the anti-inflammatory response of available treatments, we assumed that Xiao-Xian-Xiong decoction could alleviate atherosclerosis by regulating inflammatory signaling pathways.

Then, we used RAW264.7-derived foam cells to validate the function of Xiao-Xian-Xiong decoction. Xiao-Xian-Xiong decoction reduced lipid accumulation and promoted the outflow of cholesterol in foam cells. We also found Xiao-Xian-Xiong decoction increased the expression of ABCA1 and ABCG1 protein in foam cells. ABCA1 is a protein related with cholesterol efflux regulatory function (25). It could regulate the lipid transport between cell membrane and Golgi. ABCA1 activation was correlated with multiple inflammatory signaling pathways such as MAPK and TGF-β (26). ABCA1 deficiency would induce decreased cholesterol efflux about the macrophages. In cell and animal models, ABCA1 deficiency macrophages were correlated with enhanced inflammatory mediators and gene expression such as IL-6, TNF-α, IL-1β, etc (27). In addition, ABCA1 was act as anti-inflammatory mediator that ABCA1 knockout mice might increase the infiltration of inflammatory cells in vessel wall, tissue, and blood. In ABCA1 over-expressed macrophages, the inflammatory cytokines including IL-1β, IL-6, and TNF-α were reduced. Apart from inflammation, ABCA1 could also regulate apoptosis in cell model (28). ABCA1 was correlated with high-density lipoprotein (HDL) in plasma. ABCG1 was other ABC family member similar to ABCA1. ABCG1 also play critical role in efflux of cholesterol with macrophage derived foam cells. ABCA1 and ABCG1 had similar function on cholesterol efflux (29). ABCG1 also exerted anti-inflammatory effects on inflammatory macrophage foam cells (30). ABCG1 knockout mice might increase the infiltration of inflammatory cells in atherosclerosis. ABCG1 also correlated with apoptosis of macrophages (31). The results of network pharmacology showed that Xiao-Xian-Xiong decoction could alleviate atherosclerosis by inhibiting inflammatory response.

Thus, we considered that Xiao-Xian-Xiong decoction alleviates atherosclerosis by inhibiting inflammatory response and cell proliferation. The inflammatory signaling pathways included ubiquitin mediated proteolysis pathway, PI3K-AKT pathway, MAPK pathway, Notch signaling pathway, and TGF-β signaling pathway. Abnormal cell proliferation might be inhibited by Xiao-Xian-Xiong decoction, either. The cell model of RAW264.7 macrophage derived foam cells indicated that Xiao-Xian-Xiong decoction reduced lipid accumulation and promoted the outflow of cholesterol in foam cells. ABCA1 and ABCG1 might play important in the cholesterol efflux by regulating inflammation and apoptosis.

Declarations

Conflicts of Interest

All the authors declare that they have no conflicts of interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding Statement

This research was supported by the National Natural Science Foundation of China [Grant no.81603600], Hunan Provincial Department of Education Innovation Platform Open Fund Project (Grant no. 19K069).

Authors' contributions

X.L., L.Z. performed statistical analysis, and drafted the manuscript. F.C., Z.C., M.X., Y.R. contributed to database building. X.X., conceived the design of the study and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Availability of data and materials

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

References

1. Libby P, Bornfeldt KE, Tall AR. Atherosclerosis: Successes, Surprises, and Future Challenges. Circ Res. 2016;118(4):531-4.
2. Avgerinos NA, Neofytou P. Mathematical Modelling and Simulation of Atherosclerosis Formation and Progress: A Review. Ann Biomed Eng. 2019;47(8):1764-1785.
3. Gistera A, Hansson GK. The immunology of atherosclerosis. Nat Rev Nephrol. 2017;13(6):368-380.
4. Businaro R, Tagliani A, Buttari B, et al. Cellular and molecular players in the atherosclerotic plaque progression. Ann N Y Acad Sci. 2012;1262:134-41.
5. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol. 2009;78(6):539-52.
6. Bruikman CS, Stoekenbroek RM, Hovingh GK, et al. New Drugs for Atherosclerosis. Can J Cardiol. 2017,33(3):350-357.
7. Natarajan P, Young R, Stitziel NO, et al. Polygenic Risk Score Identifies Subgroup With Higher Burden of Atherosclerosis and Greater Relative Benefit From Statin Therapy in the Primary Prevention Setting. Circulation. 2017;135(22):2091-2101.

8. Xiao S, Yu R, Ai N, et al. Rapid screening natural-origin lipase inhibitors from hypolipidemic decoctions by ultrafiltration combined with liquid chromatography-mass spectrometry. J Pharm Biomed Anal. 2015;104:67-74.
9. Singh RB, Mengi SA, Xu YJ, et al. Pathogenesis of atherosclerosis: A multifactorial process. Exp Clin Cardiol. 2002;7(1):40-53.
10. Bentzon JF, Otsuka F, Virmani R, et al. Mechanisms of plaque formation and rupture. Circ Res. 2014;114(12):1852-1866.
11. Storino Farina M, Rojano Rada J, Molina Garrido A, et al. Statins and atherosclerosis: the role of epigenetics. Medwave. 2015;15(10):e6324.
12. Okuyama H, Langsjoen PH, Hamazaki T, et al. Statins stimulate atherosclerosis and heart failure: pharmacological mechanisms. Expert Rev Clin Pharmacol. 2015;8(2):189-199.
13. Cyrus T, Sung S, Zhao L, et al. Effect of low-dose aspirin on vascular inflammation, plaque stability, and atherogenesis in low-density lipoprotein receptor-deficient mice. Circulation. 2002;106(10):1282-1287.
14. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008;4(11):682-90.
15. Taleb S. Inflammation in atherosclerosis. Arch Cardiovasc Dis. 2016;109(12):708-715.
16. Wu MY, Li CJ, Hou MF, et al. New Insights into the Role of Inflammation in the Pathogenesis of Atherosclerosis. Int J Mol Sci. 2017;18(10). pii: E2034.
17. Antonopoulos AS, Margaritis M, Lee R, et al. Statins as anti-inflammatory agents in atherogenesis: molecular mechanisms and lessons from the recent clinical trials. Curr Pharm Des. 2012;18(11):1519-1530.
18. Tousoulis D, Oikonomou E, Economou EK, et al. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. Eur Heart J. 2016;37(22):1723-1732.
19. Cyrus T, Sung S, Zhao L, et al. Effect of low-dose aspirin on vascular inflammation, plaque stability, and atherogenesis in low-density lipoprotein receptor-deficient mice. Circulation. 2002;106(10):1282-1287.
20. Wang F, Lerman A, Herrmann J. Dysfunction of the ubiquitin-proteasome system in atherosclerotic cardiovascular disease. Am J Cardiovasc Dis. 2015;5(1):83-100.
21. Eisenreich A, Rauch U. PI3K inhibitors in cardiovascular disease. Cardiovasc Ther. 2011;29(1):29-36.
22. Reustle A, Torzewski M. Role of p38 MAPK in Atherosclerosis and Aortic Valve Sclerosis. Int J Mol Sci. 2018;19(12). pii: E3761.
23. Vieceli Dalla Sega F, Fortini F, Aquila G, et al. Notch Signaling Regulates Immune Responses in Atherosclerosis. Front Immunol. 2019;10:1130.
24. Goumans MJ, Ten Dijke P. TGF-β Signaling in Control of Cardiovascular Function. Cold Spring Harb Perspect Biol. 2018;10(2). pii: a022210.

25. Talbot CPJ, Plat J, Ritsch A, et al. Determinants of cholesterol efflux capacity in humans. Prog Lipid Res. 2018;69:21-32.
26. Bi X, Vitali C, Cuchel M. ABCA1 and Inflammation: From Animal Models to Humans. Arterioscler Thromb Vasc Biol. 2015;35(7):1551-3.
27. McPeek M, Malur A, Tokarz DA, et al. Alveolar Macrophage ABCG1 Deficiency Promotes Pulmonary Granulomatous Inflammation. Am J Respir Cell Mol Biol. 2019;61(3):332-340.
28. Wang M, Li C, Zhang Y, et al. LncRNA MEG3-derived miR-361-5p regulate vascular smooth muscle cells proliferation and apoptosis by targeting ABCA1. Am J Transl Res. 2019;11(6):3600-3609.
29. Sag D, Cekic C, Wu R, et al. The cholesterol transporter ABCG1 links cholesterol homeostasis and tumour immunity. Nat Commun. 2015;6:6354.
30. Ren K, Li H, Zhou HF, et al. Mangiferin promotes macrophage cholesterol efflux and protects against atherosclerosis by augmenting the expression of ABCA1 and ABCG1. Aging (Albany NY). 2019;11(23):10992-11009.
31. Yvan-Charvet L, Pagler TA, Seimon TA, et al. ABCA1 and ABCG1 protect against oxidative stress-induced macrophage apoptosis during efferocytosis. Circ Res. 2010;106(12):1861-9.

Figures

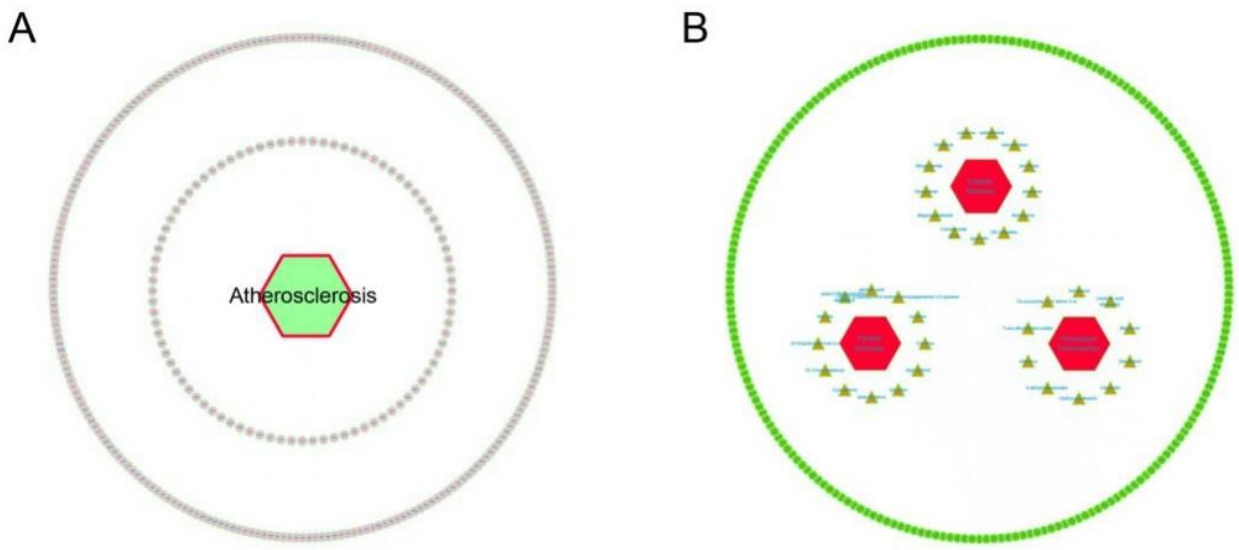

Figure 1

A. Targets of atherosclerosis. Green node indicated atherosclerosis. Pink nodes indicated the targets of atherosclerosis. B. Targets of Xiao-Xian-Xiong decoction. Red nodes indicated the 3 substances of Xiao-Xian-Xiong decoction including Gualou (Fructus Trichosanthis Kirlowii), Huanglian (Rhizoma Coptidis), and Banxia (Rhizoma Pinelliae Tematae). Yellow nodes indicated the compounds of Xiao-Xian-Xiong decoction. Green nodes indicated the targets of Xiao-Xian-Xiong decoction.

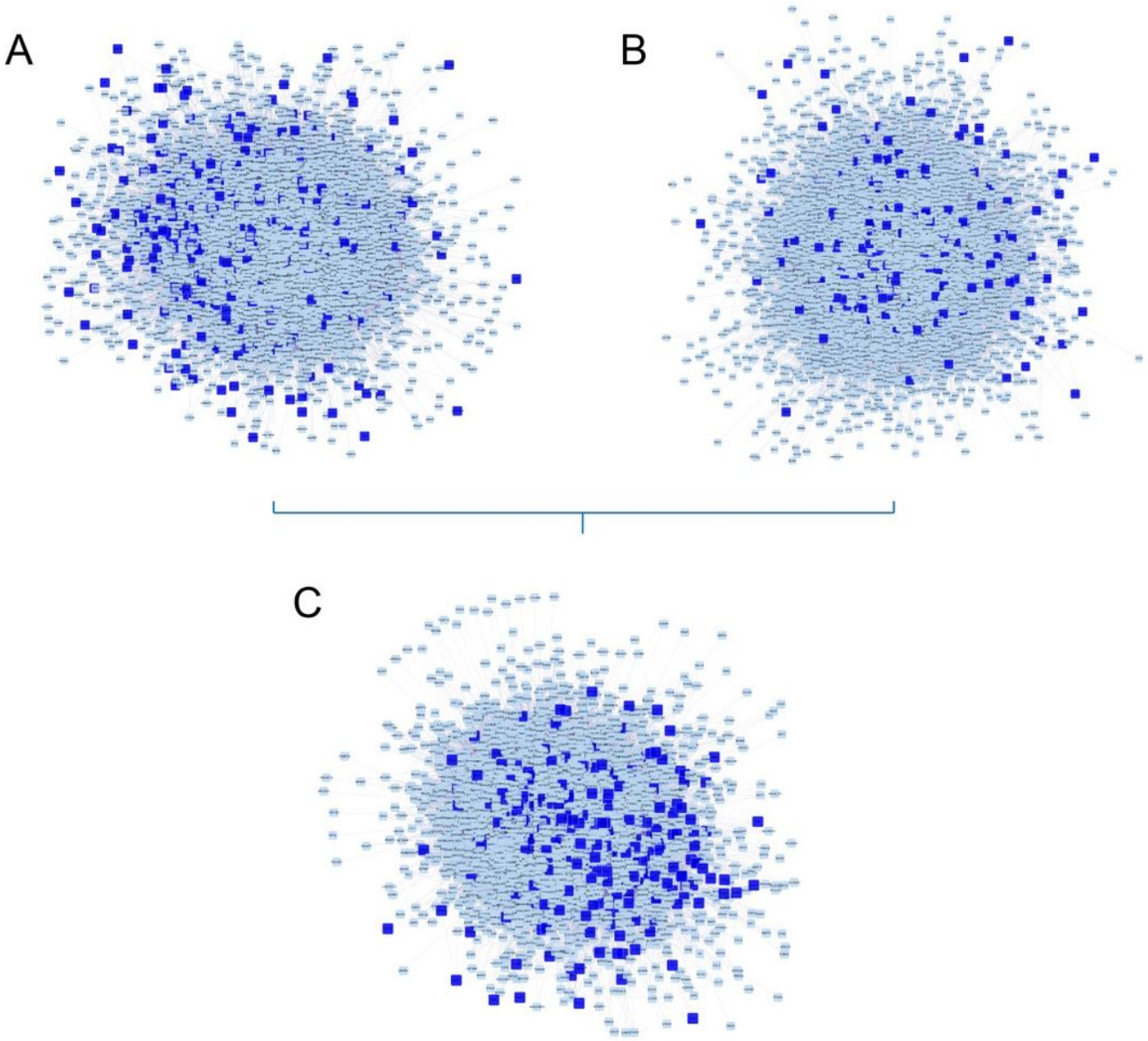

Figure 2

A. PPI network of atherosclerosis targets. Dark blue square nodes indicated atherosclerosis targets. Light blue square nodes indicated the proteins interacted with the targets of atherosclerosis. The edges indicated the relationship between proteins. B. Xiao-Xian-Xiong decoction targets PPI network. Dark blue square nodes indicated the Xiao-Xian-Xiong decoction targets. Light blue square nodes indicated the proteins interacted with the Xiao-Xian-Xiong decoction targets. The edges indicated the relationship between proteins. C. Merged PPI network of Xiao-Xian-Xiong decoction targets PPI network and atherosclerosis targets PPI network. Dark blue square nodes indicated the Xiao-Xian-Xiong decoction and atherosclerosis targets. Light blue square nodes indicated the proteins interacted with the Xiao-Xian-Xiong decoction and atherosclerosis targets. The edges represented the relationship between them.

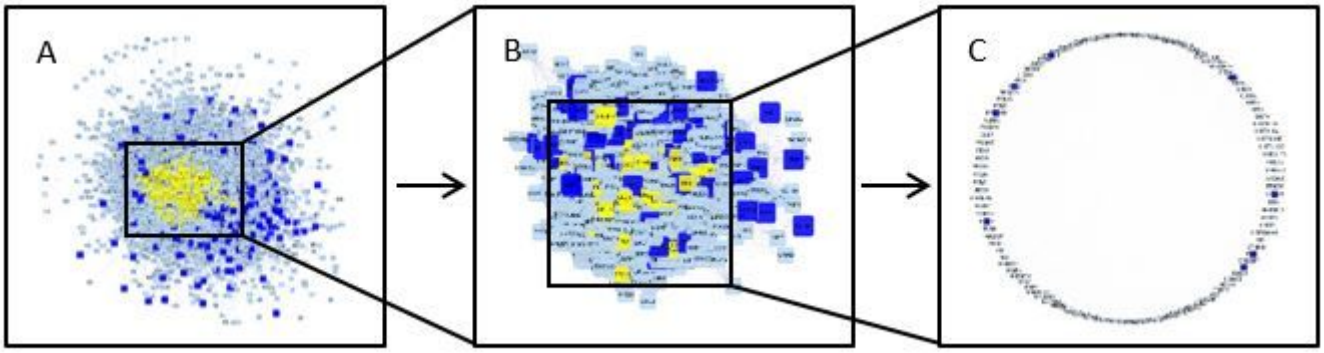

Figure 3

Core PPI network with network topological features analysis. (A) Merged network of Xiao-Xian-Xiong decoction targets PPI network and atherosclerosis targets PPI network. (B) Network built by first topological features analysis of merged PPI network. (C) Core PPI network. Six parameters in topological features were used to acquire core PPI network. The parameters were listed as follows: BC≥0.00043, CC≥0.516, DC≥146, EC≥0.0212, LAC≥18.49, NC≥21.11.

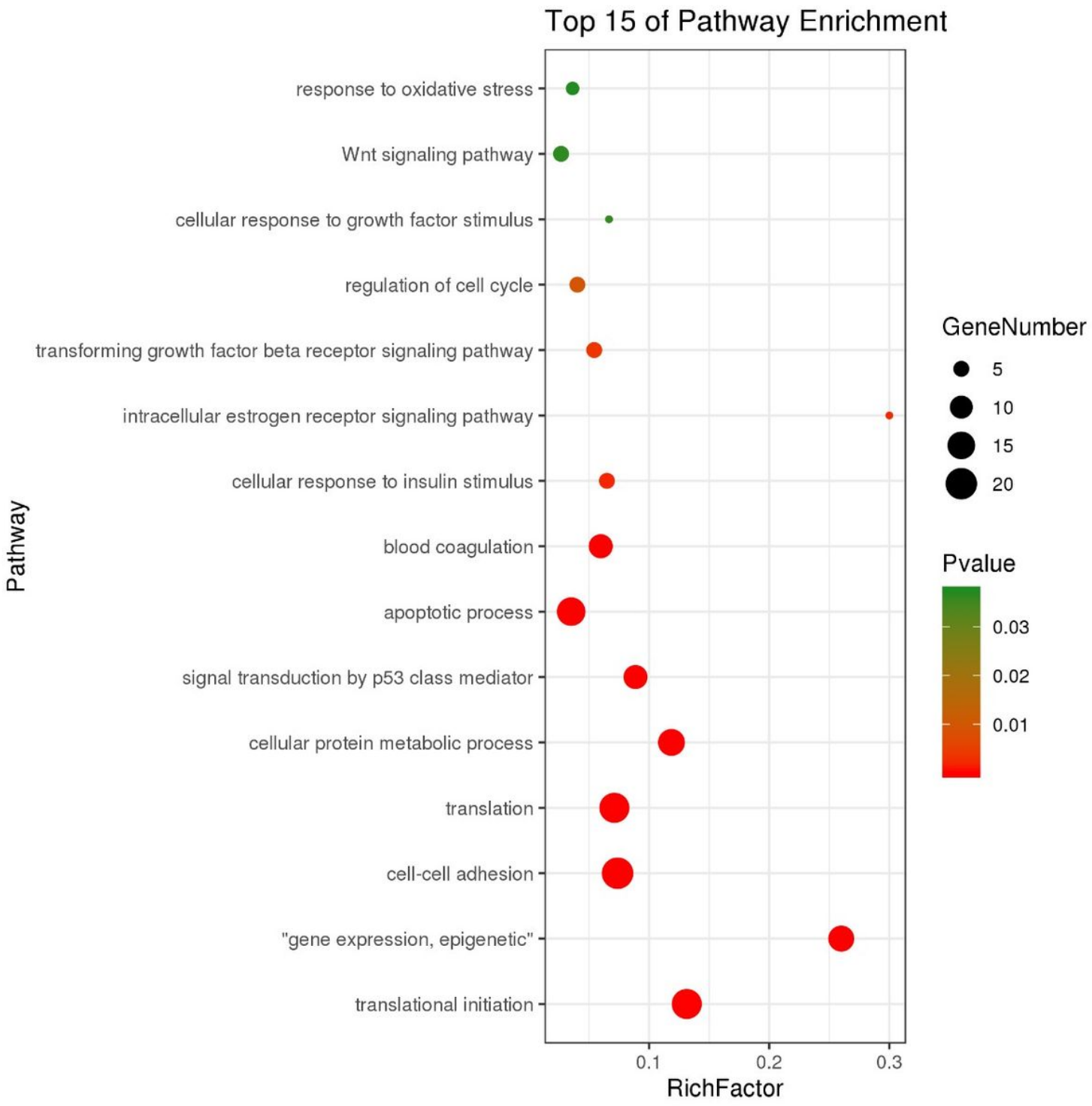

Figure 4

GO analysis of targets about Xiao-Xian-Xiong decoction in treating atherosclerosis. The X-axis indicated the enrichment scores of these terms, and the Y-axis indicated significantly enriched GO categories of the target genes. The size of the dots indicated the counts of gene. The color of the dots indicated the P-value.

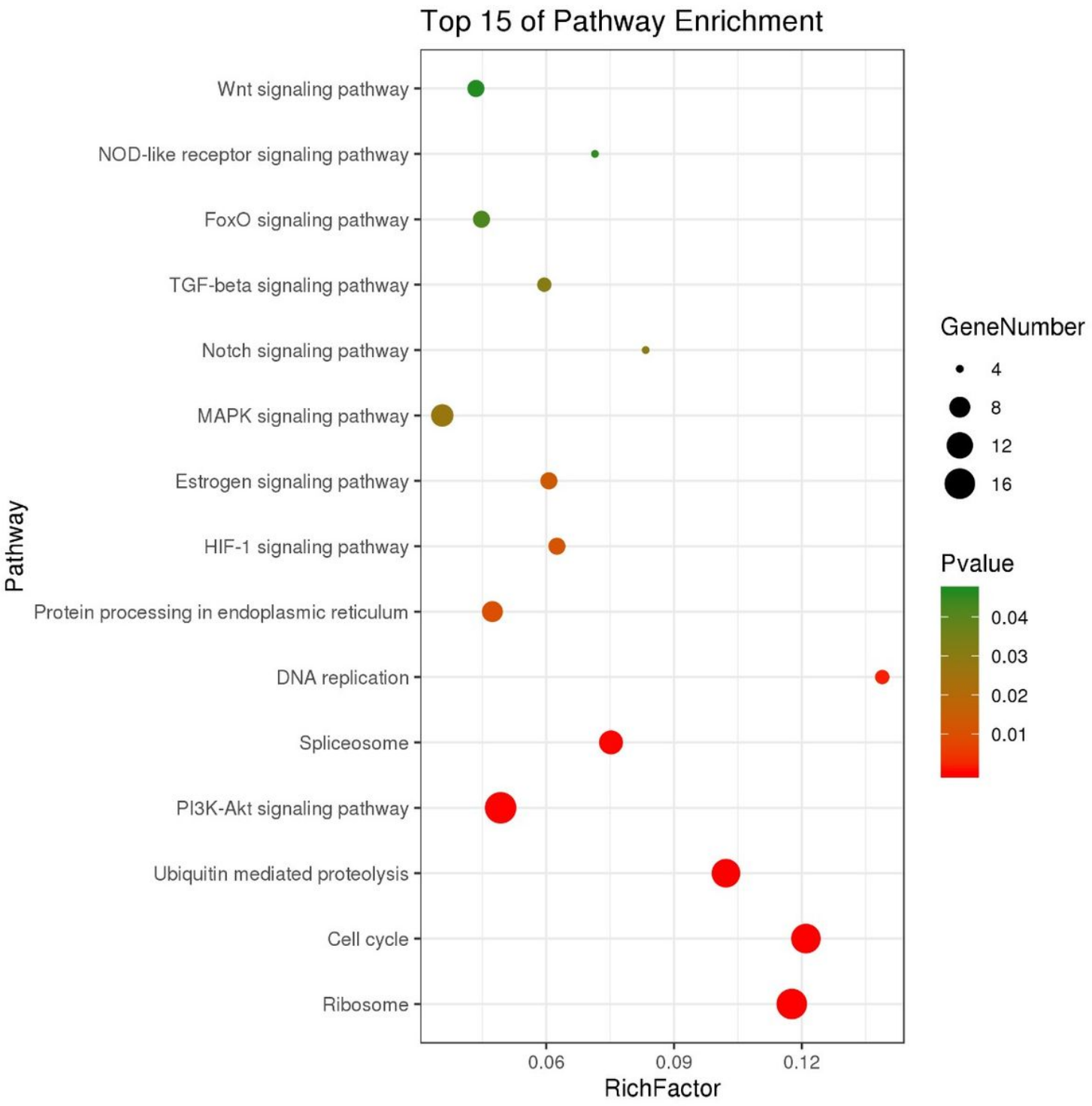

Figure 5

KEGG pathway analysis of candidate targets for Xiao-Xian-Xiong decoction in treating atherosclerosis. The X-axis indicated the enrichment scores of these terms, and the Y-axis indicated significantly enriched KEGG categories of the target genes. The size of the dots indicated the counts of gene. The color of the dots indicated the P-value.

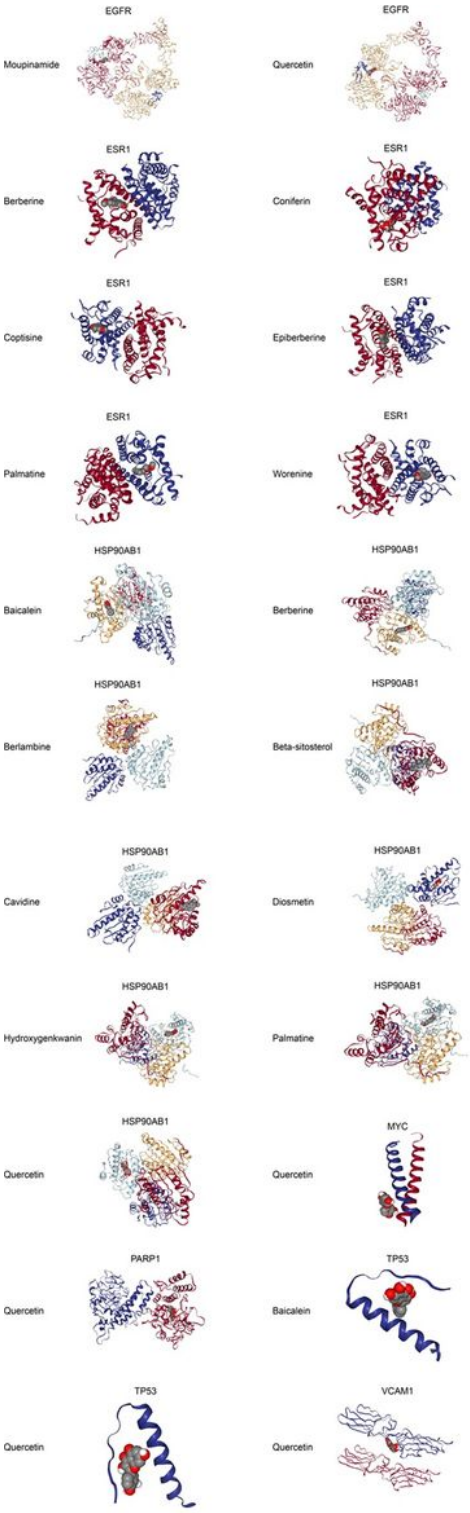

Figure 6

Molecular docking between Xiao-Xian-Xiong decoction compounds and targets.

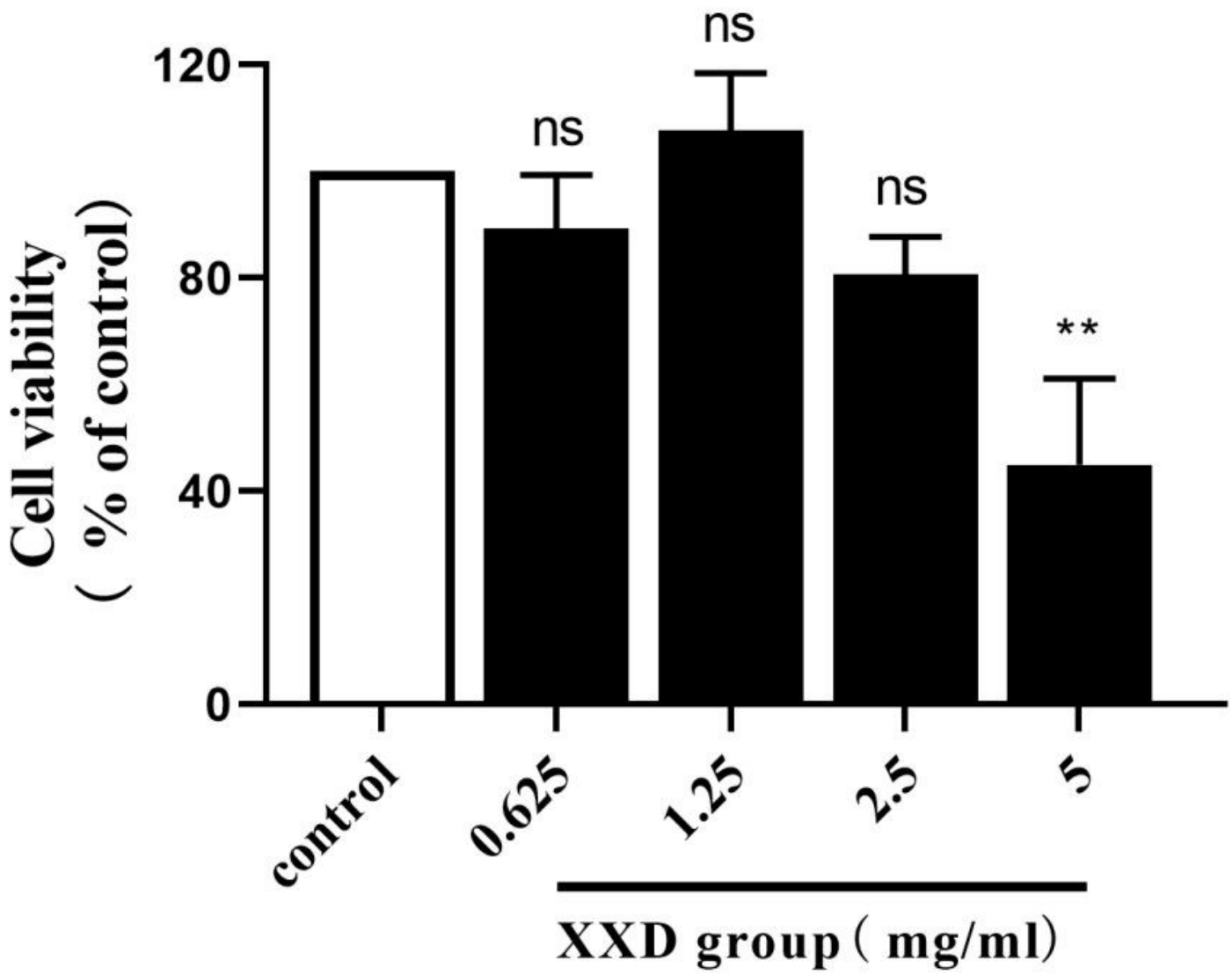

Figure 7

The effect of different Xiao-Xian-Xiong decoction concentration on the proliferation of RAW264.7 foam cells. ns mean P > 0.05 of XXX group compared with control group. ** mean P < 0.01 of 5 mg/ml Xiao-Xian-Xiong decoction group compared with control group.

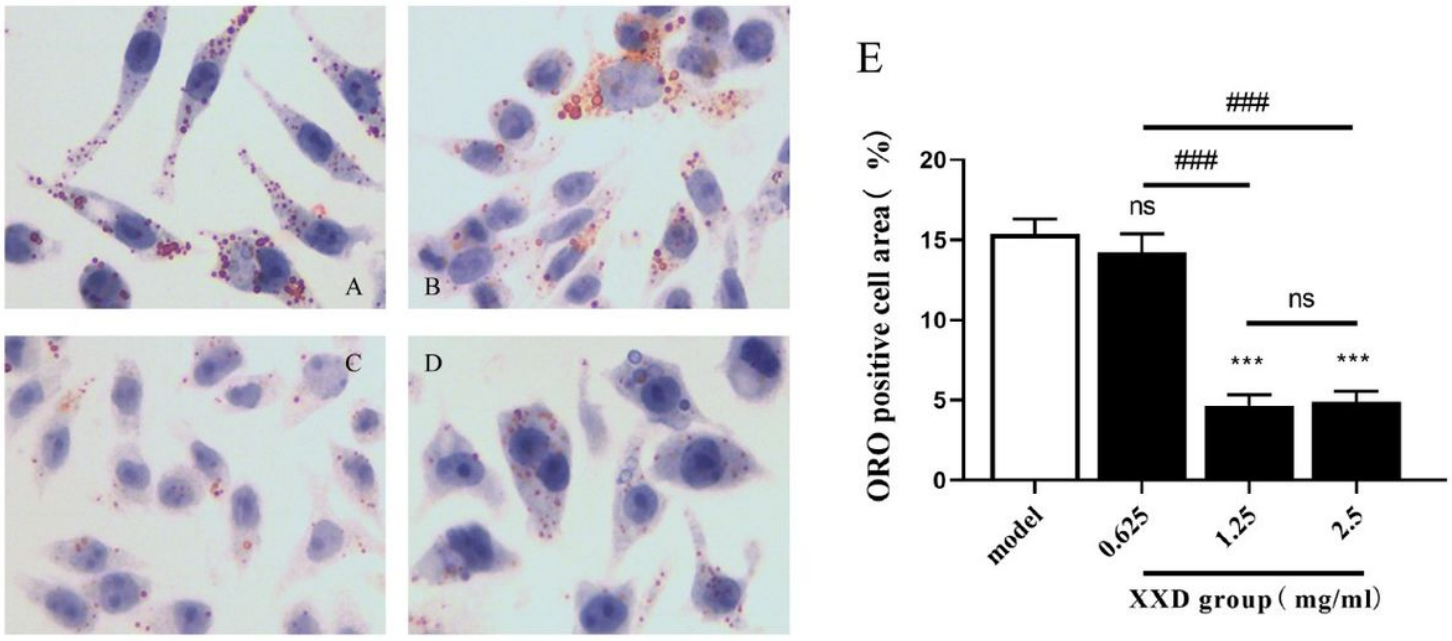

Figure 8

The effect of Xiao-Xian-Xiong decoction on lipid accumulation in macrophages. Oil red O staining morphology (×400): A. model group without Xiao-Xian-Xiong decoction, B. Xiao-Xian-Xiong decoction low concentration group, C. Xiao-Xian-Xiong decoction medium concentration group, D. Xiao-Xian-Xiong decoction high concentration group Concentration group. E. ORO positive cell area. ns mean P>0.05; *** mean P<0.001; ### mean P<0.001.

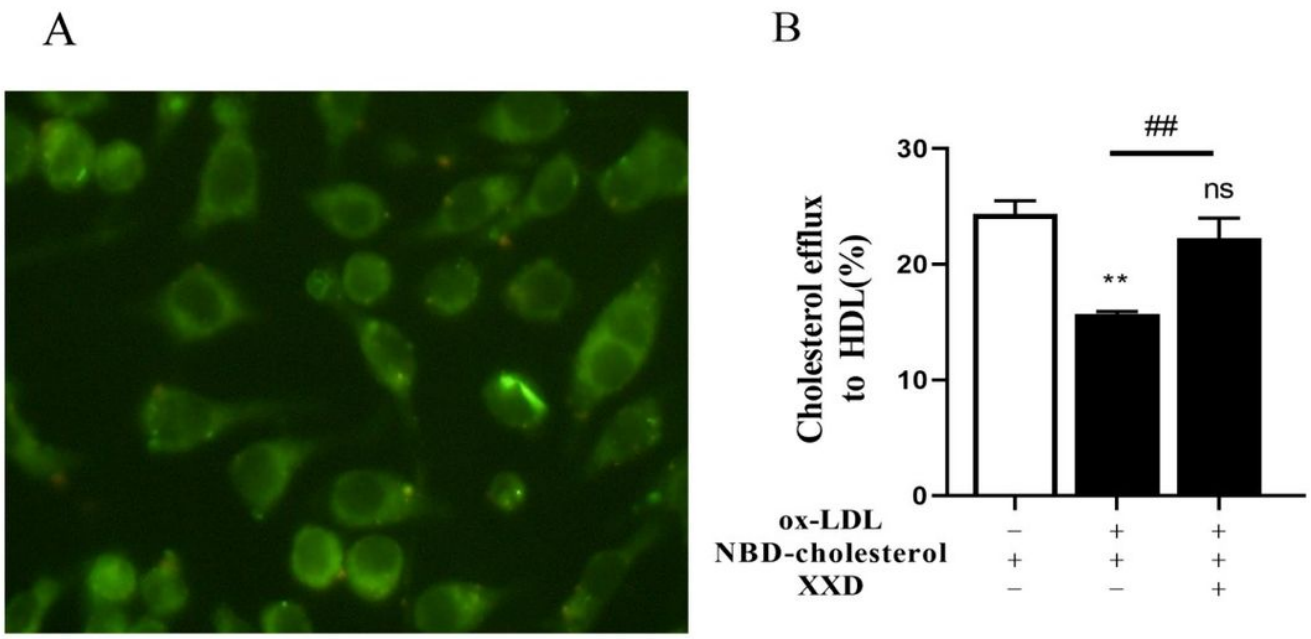

Figure 9

The effect of Xiao-Xian-Xiong decoction on cell cholesterol outflow. A. Cell morphology map of fluorescent cholesterol (×400), B. ns means P>0.05, ** mean P<0.01; ## mean P□0.01.

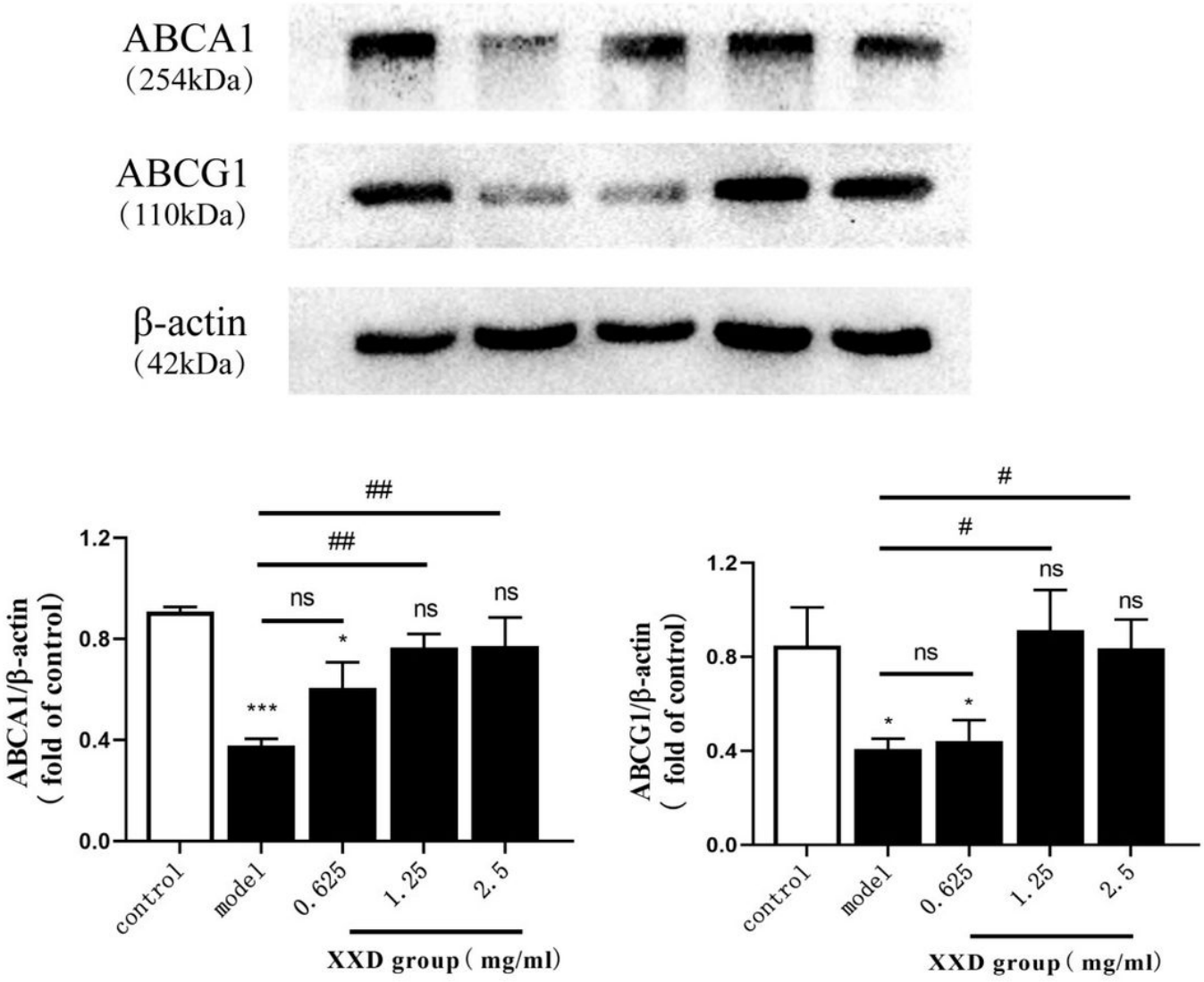

Figure 10

The effect of Xiao-Xian-Xiong decoction on ABCA1 and ABCG1 protein expression

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- actinwb.tif
- ABCG1wb.tif
- ABCA1wb.tif