Research Article

Network Pharmacology Analyses of the Pharmacological Targets and Therapeutic Mechanisms of Salvianolic Acid A in Myocardial Infarction

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Objective. Salvianolic acid A, a natural polyphenolic ingredient extracted from traditional Chinese medicine, possesses excellent pharmacological activity against cardiovascular diseases. Herein, therapeutic mechanisms of salvianolic acid A in myocardial infarction were explored through systematic and comprehensive network pharmacology analyses.

Methods. The chemical structure of salvianolic acid A was retrieved from PubChem database. Targets of salvianolic acid A were estimated through SwissTargetPrediction, HERB, and TargetNet databases. Additionally, by GeneCards, OMIM, DisGeNET, and TTD online tools, myocardial infarction-relevant targets were predicted. Following intersection, therapeutic targets were determined. The interaction of their products was evaluated with STRING database, and hub therapeutic targets were selected. GO and KEGG enrichment analyses of therapeutic targets were then implemented. H9C2 cells were exposed to oxygen-glucose deprivation/reoxygenation (OGD/R) to mimic myocardial infarction and administrated with salvianolic acid A. Cellular proliferation was assayed via CCK-8 assay, and hub therapeutic targets were verified with RT-qPCR.

Results. In total, 120 therapeutic targets of salvianolic acid A in myocardial infarction were identified. There were close interactions between their products. Ten hub therapeutic targets were determined, covering SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, ITGB1, MAPK8, and NFkB1. Therapeutic targets were significantly correlated to myocardial infarction-relevant pathways, especially PI3K-Akt signaling pathway. Salvianolic acid A administration remarkably ameliorated the viability of OGD/R-induced H9C2 cells, and altered the expression of hub therapeutic targets.

Conclusion. Our work uncovers therapeutic mechanisms of salvianolic acid A for the treatment of myocardial infarction, providing a new insight into further research on salvianolic acid A.

1. Introduction

Cardiovascular disease is a general term for diseases that damage the heart and blood vessels, characterized by rapid onset and high morbidity [1]. In accordance with the WHO, approximately 18 million individuals died from cardiovascular disease in 2019, which represented 32% of overall global deaths as the dominating cause of deaths in all diseases [2]. Myocardial infarction, often triggered by blood clot blocking artery or bypass graft, has the features of a sudden decrease in blood flow to myocardium, eventually resulting in heart failure even death [3]. Among cardiovascular diseases, myocardial infarction has become a primary international health issue [4]. Thrombolysis, percutaneous coronary intervention as well as coronary artery bypass graft remain the most commonly applied approaches in treating myocardial infarction. Nevertheless, patients often present complications such as bleeding, ischemia-reperfusion damage as well as coronary restenosis. Therefore, more effective therapeutic approaches to alleviate apoptosis of cardiomyocytes and facilitate local angiogenesis are urgently required for preventing the expansion of irreversible myocardial damage [5].
Salvianolic acids are extracted from *Salvia miltiorrhiza Bunge* (Danshen). Salvianolic acid A is the strongest anti-oxidant among salvianolic acids, which is an effective free radical scavenger because of the polyphenolic structure [6]. It has been suggested that salvianolic acid A exerts diverse pharmacological properties, especially for cardiovascular diseases [7]. Preclinical evidence has demonstrated the cardioprotective property of salvianolic acid A against myocardial infarction. Salvianolic acid A attenuates myocardial infarction-triggered apoptosis and inflammation through activation of thioredoxin [8]. Also, it exhibits the antiapoptotic and cardioprotective effects on rat cardiomyocytes under ischemia/reperfusion via DUSP-induced modulation of ERK1/2/JNK signaling [9]. Experimental evidence also demonstrates that salvianolic acid A possesses antioxidant activity and exerts a remarkable protective function against isoproterenol-triggered myocardial infarction [10]. Additionally, salvianolic acid A exhibits cardioprotective effects by facilitating angiogenesis [11] as well as lowering plasma uric acid levels [12] and plasma and tissue dimethylarginine levels [13] in acute myocardial infarction animal models. Despite this, there is a lack of systematic and comprehensive analysis of the therapeutic mechanisms of salvianolic acid A in myocardial infarction.

Network pharmacology is an effective approach to establish a “compound-protein/gene-disease” network, which reveals the regulation principle of small molecule compounds with a high-throughput manner, thus providing a broader selection of pharmacologically relevant targets [14]. Herein, we applied network pharmacology analyses to dissect the therapeutic mechanisms of salvianolic acid A systematically and comprehensively in myocardial infarction. Additionally, the effects and therapeutic targets of salvianolic acid A in myocardial infarction were verified in oxygen-glucose deprivation/reoxygenation (OGD/R)-induced H9C2 cells that mimicked myocardial infarction.

## 2. Materials and Methods

### 2.1. Retrieval of the Chemical Structure of Salvianolic Acid A

PubChem (https://pubchem.ncbi.nlm.nih.gov) is an important chemical information resource, which comprises over 293 million depositor-provided substance descriptions, 111 million unique chemical structures as well as 271 million biological activity data points from 1.2 million bioassay experiments [15]. The chemical structure of salvianolic acid A was accessed from PubChem.

### 2.2. Analyses of Salvianolic Acid A Targets

The SwissTargetPrediction web tool (http://www.swisstargetprediction.ch) allows users to predict the most possible macromolecular targets of a specific small molecule compound on the basis of 2D and 3D similarity with a library of 370000 known actives on over 3000 proteins from three distinct species [16]. The canonical SMILES “C1=CC(=C(C=CClC(=O)O)OC(=O)C=C2C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=C(C(=O)O)O)O)” of salvianolic acid A was uploaded to SwissTargetPrediction, and potential molecular targets of salvianolic acid A were downloaded. HERB (http://herb.ac.cn/) is a high-throughput experiment and reference guide database of traditional Chinese medicine [17]. Salvianolic acid A ingredient was input into HERB database, and relevant gene targets were gathered from curated references. TargetNet (http://targetnet.scbdd.com) is a web service to estimate potential drug-target interactions through multitarget SAR models [18]. Potential targets of salvianolic acid A were screened on the basis of ECFP2 molecular fingerprint in accordance with the area under the receiver operating characteristic curve = 1. On the basis of SwissTargetPrediction, HERB, and TargetNet databases, potential molecular targets of salvianolic acid A were merged and deduplicated. Through the Uniprot database (https://www.uniprot.org/) [19], gene name of targets was corrected and matched.

### 2.3. Acquisition of Targets of Myocardial Infarction

GeneCards (http://www.genecards.org/) [20] and Online Mendelian Inheritance in Man (OMIM; http://omim.org) [21] are comprehensive, and authoritative research resources of annotative information of human genes. DisGeNET (http://www.disgenet.org/) is a knowledge management platform that integrates and standardizes data about disease-relevant genes and variants from a variety of sources, covering over 24,000 diseases and phenotypes, 17,000 genes as well as 117,000 genomic variants [22]. Therapeutic Target Database (TTD; http://db.idrblab.net/TTD/) is a popular information resource of the known therapeutic protein and nucleic acid targets, the targeted diseases, the pathway information as well as the matched drugs or ligands [23]. Potential targets of myocardial infarction were searched from above databases, followed by merging, deduplication, and correction through the Uniprot database.

### 2.4. Identification of Targets Shared by Salvianolic Acid A and Myocardial Infarction

Targets of salvianolic acid A and myocardial infarction were intersected, and imported into Venny 2.1 online tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The Venn diagram of targets shared by salvianolic acid A and myocardial infarction was drawn.

### 2.5. Protein-Protein Interaction (PPI) Analyses

The PPIs of myocardial infarction targets of salvianolic acid A were estimated with the STRING online tool (https://string-db.org/) that integrates all known and predicted PPIs comprising physical and functional interactions [24]. The criteria included organism, Homo sapiens; settings, highest confidence (0.900). The PPI network was drawn via Cytoscape 3.7.2 (https://cytoscape.org/) [25]. The degree of targets was computed with Count package. Through cytoHubba plugin, hub genes were selected.

### 2.6. Functional Enrichment Analyses

Utilizing clusterProfiler package [26], functional enrichment analyses of myocardial infarction targets of salvianolic acid A were implemented. Gene ontology (GO) analyses were utilized for describing biological functions of gene products, comprising
biological processes (BPs), cellular components (CCs), along with molecular functions (MFs). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were applied for probing out signaling pathways enriched by myocardial infarction targets of salvianolic acid A. A P value < 0.05 was regarded as significant enrichment. The map of KEGG pathways was drawn via pathview package [27].

2.7. Cell Culture, OGD/R Injury, and Administration. H9C2 cells (ATCC, USA) were maintained in DMEM (Gibco, USA) with 10% fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL) in a humidified incubator with 5% CO₂ at 37°C. The medium was exchanged every three days. H9C2 cells were pretreated with 50 μm salvianolic acid A with 98.15% purity (MedChemExpress, China) for 24 h, as previously described [8]. Following OGD, they were grown lasting 3 h in an incubator flushed with a gas mixture (1% O₂, 5% CO₂ as well as 94% N₂). Control cells were grown in DMEM with normoxia.

2.8. Cell Viability Assay. Cell viability was assayed with cell counting kit-8 (CCK-8) kit (MedChemExpress, China). H9C2 cells were grown in a 96-well plate (1 × 10⁴ cells/well). 10 μL CCK-8 reagent was added to each well with H9C2 cells. The plate was cultured at 37°C for 2 h away from light. The optical density was monitored with microplate reader at 450 nm.

2.9. Reverse Transcription-Quantitative PCR (RT-qPCR). Total RNA extraction from H9C2 cells was implemented via TRIzol reagent (Solarbio, China). The RNA content was measured with UV spectrophotometry. Extracted RNA was utilized for reverse transcription and cDNA was synthesized. The qPCR assays were implemented utilizing SYBR Premix Ex Taq II and RT-qPCR detection system. GAPDH was utilized for normalizing mRNA expressions. Relative expressions were computed with 2^−ΔΔCT. Sequences of primers (SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, ITGB1, MAPK8, and NFKB1) utilized for RT-qPCR are listed in Table 1.

2.10. Statistical Analyses. Data were analyzed through appropriate R packages and GraphPad Prism 8 software. ANOVA was applied for comparisons between groups, and the difference was statistically significant when P < 0.05.

3. Results

3.1. The Chemical Structure and Potential Targets of Salvianolic Acid A. The chemical structure of salvianolic acid A was retrieved from the PubChem database, as illustrated in Figure 1. To determine potential molecular targets of salvianolic acid A, we employed three web tools, comprising SwissTargetPrediction, HERB, and TargetNet databases. As a result, 100 (Table 2), 33 (Table 3), and 79 (Table 4) potential targets of salvianolic acid A were predicted on the basis of SwissTargetPrediction, HERB, and TargetNet databases, respectively. After merging and deduplication, we finally retrieved 180 potential targets of salvianolic acid A (Figure 2(a)). SwissTargetPrediction-, HERB-, and TargetNet-predicted targets of salvianolic acid A occupied 47%, 16%, and 37% of all targets, respectively. Through SwissTargetPrediction web tool, target classes of the top 15 potential targets of salvianolic acid A were analyzed. In Figure 2(b), 40.0% belonged to protease, with 26.7% for lyase, 13.3% for membrane receptor, and with 6.7% for secreted protein, enzyme, or kinase.

3.2. Estimation of Targets of Myocardial Infarction. To estimate the potential targets of myocardial infarction, this study integrated four comprehensive databases comprising

| Table 1: Sequences of primers utilized for RT-qPCR. |
|----------------|----------------|
| Target | Primer sequence (5′-3′) |
| SRC | F: GAGCCGGCTCCAGATTGTCAA R: CTGGGAGATGTCGCTGTTCTG |
| CTNNB1 | F: AAAAGGGCTGTGAAGCTACTGG R: CGATCTTCTGATCCATG |
| PIK3CA | F: CCACGACCACATCATGAGTGAA R: CTCATCAAGAGCTTAACTG |
| AKT1 | F: ATGGTTGAGATCATGGAGACAGC R: CCTGTGCTCTGATCCATT |
| RELA | F: AGGGAGATCAAGAATGTCACC R: AGGAGTGGTGAAAGATG |
| EGFR | F: ATGGAGAACATTCAGCAGCAGC R: CCTACTCTGAGCATGGATAG |
| FYN | F: ATGGGGCTTGTGGACGAAATG |
| ITGB1 | F: CCTTTGCTACGGTTGATCATT R: CTTGTTGGAATCAAGCAGCCTT |
| MAPK8 | F: AGGGGTCTCATCAAAACTGCTTC |
| NFKB1 | F: AACAGAGAGTTTCTCCTT |
| GAPDH | F: CTGGGCTACATGAGGAC |
| R: AATGGAGGTCGGTTCGCC |

Figure 1: The chemical structure of salvianolic acid A.
GeneCards, OMIM, DisGeNET and TTD. As a result, 4633, 39, 1800, and 36 myocardial infarction-relevant targets were separately retrieved from GeneCards, OMIM, DisGeNET and TTD databases (Figure 3). After merging and deduplication, 5172 disease targets of myocardial infarction were finally obtained.

3.3. Identification of Targets Shared by Salvianolic Acid A and Myocardial Infarction. To determine myocardial infarction targets of salvianolic acid A, we took the intersection between targets of salvianolic acid A and myocardial infarction. As illustrated in Figure 4, 120 targets shared by salvianolic acid A and myocardial infarction were eventually obtained.

3.4. Interactions between Products of Myocardial Infarction Targets of Salvianolic Acid A. Through the STRING online tool, the interactions between myocardial infarction targets and salvianolic acid A-related targets were identified. The results showed that there were significant interactions among these targets, indicating potential therapeutic targets for myocardial infarction.

Table 2: Potential molecular targets of salvianolic acid A by the SwissTargetPrediction web tool.

| Target    | Target Class | Target    | Target Class |
|-----------|--------------|-----------|--------------|
| CA12      | Lyase        | AURKA     | Kinase       |
| CA4       | Lyase        | AKR1B10   | Enzyme       |
| CA7       | Lyase        | DHFR      | Oxidoreductase|
| CA2       | Lyase        | F3 F7     | Protease     |
| MMP1      | Protease     | CASP1     | Protease     |
| AKR1B1    | Enzyme       | AGTR1     | Family A G protein-coupled receptor |
| FYN       | Kinase       | HMGCR     | Oxidoreductase|
| TTR       | Secreted protein | THRBR   | Nuclear receptor |
| MMP9      | Protease     | ITGB5 ITGA V | Membrane receptor |
| MMP12     | Protease     | IMPDH1    | Oxidoreductase|
| MME       | Protease     | ERBB2     | Kinase       |
| SLC28A2   | Electrochemical transporter | LCK | Kinase |
| ADAMTS4   | Protease     | HSP90AB1  | Other cytosolic protein |
| ADORA3    | Family A G protein-coupled receptor | LDHA | Enzyme |
| ACE       | Protease     | THRA      | Nuclear receptor |
| SLC5A1    | Electrochemical transporter | MMP8 | Protease |
| GRM2      | Family C G protein-coupled receptor | ECE1 | Protease |
| ITGB7 ITGA4 | Membrane receptor | PTGDR2 | Family A G protein-coupled receptor |
| F7        | Protease     | CA14      | Lyase       |
| ALB       | Secreted protein | DHOHDH | Oxidoreductase|
| MMP2      | Protease     | TDP1      | Enzyme       |
| PIM1      | Kinase       | GALK1     | Enzyme       |
| SELP      | Adhesion     | ABL1      | Kinase       |
| MMP13     | Protease     | PAD1I     | Enzyme       |
| SELF      | Adhesion     | MAP3K9    | Kinase       |
| IGFBP3    | Secreted protein | F3     | Surface antigen |
| KDM4C     | Eraser       | MMP10     | Protease     |
| SLC5A2    | Electrochemical transporter | PAD14 | Enzyme |
| MMP3      | Protease     | AMPD3     | Enzyme       |
| SELE      | Adhesion     | ITGA2B ITGB3 | Membrane receptor |
| ITGB1 ITGA4 | Membrane receptor | C3AR1 | Family A G protein-coupled receptor |
| HCAR2     | Family A G protein-coupled receptor | TYR | Oxidoreductase |
| CA1       | Lyase        | ADK       | Enzyme       |
| TYMS      | Transferase  | AMPD2     | Enzyme       |
| SLC6A2    | Electrochemical transporter | EGFR | Kinase       |
| AKR1C2    | Enzyme       | YARS      | Enzyme       |
| APP       | Membrane receptor | ITGAV ITGB3 | Membrane receptor |
| ITGAV ITGB1 | Membrane receptor | SLC5A4 | Electrochemical transporter |
| MAPK8     | Kinase       | SRD5A1    | Oxidoreductase|
| ROCK2     | Kinase       | PAD12     | Enzyme       |
| GART      | Ligase       | PAD13     | Enzyme       |
| PRKCA     | Kinase       | EPHA2     | Kinase       |
| CASP3     | Protease     | FGFR1     | Kinase       |
| F10       | Protease     | LDHB      | Enzyme       |
| CA9       | Lyase        | BTK       | Kinase       |
| SRC       | Kinase       | SLC28A3   | Electrochemical transporter |
| KDR       | Kinase       | CA5A      | Lyase       |
| SLC29A1   | Electrochemical transporter | CA6 | Lyase |
| ESR1      | Nuclear receptor | CA5B | Lyase |
| ACE2      | Protease     | CA13      | Lyase       |
tool, we evaluated the interactions between products of myocardial infarction targets of salvianolic acid A. Figure 5 illustrates their interactions. We computed the degree of each target. Figure 6(a) visualizes the top twenty myocardial infarction targets of salvianolic acid A in accordance with the degree, comprising SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, ITGB1, MAPK8, NFKB1, ESR1, PLG, MAPK14, ERBB2, IL6, ITGB3, ITGAV, KDR, MORT, and APP. With cytoHubba plugin, ten hub myocardial infarction targets of salvianolic acid A were determined, covering SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, ITGB1, MAPK8, and NFKB1 (Figure 6(b)).

3.5. Biological Functions and Pathways of Myocardial Infarction Targets of Salvianolic Acid A. GO enrichment analyses were implemented for probing out biological functions of 120 myocardial infarction targets of salvianolic acid A. In Figure 7(a), the myocardial infarction targets of salvianolic acid A were remarkably linked to biological processes of response to molecule of bacterial origin, response to lipopolysaccharide, cellular response to chemical

| Paper ID | Target ID | Target name | PubMed ID |
|----------|-----------|-------------|-----------|
| HBREF001977 | HBTAR005574 | BCL2L11 | 31193821 |
| HBREF001977 | HBTAR001405 | FO XO3 | 31193821 |
| HBREF001977 | HBTAR003003 | PIK3CA | 31193821 |
| HBREF001977 | HBTAR003004 | PIK3CB | 31193821 |
| HBREF001977 | HBTAR003006 | PIK3CD | 31193821 |
| HBREF001977 | HBTAR003007 | PIK3CG | 31193821 |
| HBREF001977 | HBTAR001030 | AKT1 | 31193821 |
| HBREF001977 | HBTAR002705 | NFKB1 | 28303221 |
| HBREF001980 | HBTAR000636 | CDK5 | 27609227 |
| HBREF001980 | HBTAR001009 | DCX | 27609227 |
| HBREF001980 | HBTAR002705 | NFKB1 | 27609227 |
| HBREF001980 | HBTAR003003 | PIK3CA | 27609227 |
| HBREF001980 | HBTAR003004 | PIK3CB | 27609227 |
| HBREF001980 | HBTAR003006 | PIK3CD | 27609227 |
| HBREF001980 | HBTAR003007 | PIK3CG | 27609227 |
| HBREF001980 | HBTAR000374 | BDNF | 27609227 |
| HBREF001980 | HBTAR000360 | BCL2 | 27609227 |
| HBREF001981 | HBTAR000130 | AKT1 | 24486344 |
| HBREF001981 | HBTAR001437 | MTOR | 24486344 |
| HBREF001981 | HBTAR001851 | HMOX1 | 24486344 |
| HBREF001981 | HBTAR001851 | HMBOX1 | 24486344 |
| HBREF001982 | HBTAR002700 | NFE2L2 | 24486344 |
| HBREF001982 | HBTAR000151 | ALOX5 | 24486344 |
| HBREF001982 | HBTAR001851 | HMOX1 | 24303467 |
| HBREF001982 | HBTAR003295 | PTGS2 | 24303467 |
| HBREF001982 | HBTAR002055 | IL6 | 24303467 |
| HBREF001982 | HBTAR003006 | PIK3CD | 24303467 |
| HBREF001982 | HBTAR002705 | NFKB1 | 24303467 |
| HBREF001982 | HBTAR002737 | NOS2 | 24303467 |
| HBREF001982 | HBTAR0004140 | TNF | 24303467 |
stress, reactive oxygen species metabolic process, response to oxidative stress, regulation of reactive oxygen species metabolic process, response to reactive oxygen species, peptidyl-serine phosphorylation, cellular response to biotic stimulus, and peptidyl-serine modification. Additionally, cellular components of membrane raft, membrane microdomain, membrane region, vesicle lumen, secretory granule lumen, cytoplasmic vesicle lumens, integrin complex, platelet alpha granule, protein complex involved in cell adhesion, and cell projection membrane were significantly enriched by the myocardial infarction targets of salvianolic acid A. We also found the significant enrichment of molecular functions of protease binding, endopeptidase activity, metalloproteinase activity, metalloendopeptidase activity, serine-type peptidase activity, serine hydrolase activity, serine-type endopeptidase activity, protein tyrosine kinase activity, 1-dipeptidyl peptidase activity, serine hydrolase activity, serine-type endopeptidase activity, metalloendopeptidase activity, serine-type peptidase binding, endopeptidase activity, metallopeptidase activity.  

Table 4: Continued.

| Uniprot ID | Protein |
|------------|---------|
| P19099     | Cytochrome P450 11B2, mitochondrial |
| Q96B1      | Gamma-secretase subunit APH-1A |
| Q87DV5     | Glucose-dependent insulinotropic receptor |
| O77636     | Disintegrin and metalloproteinase domain-containing protein 17 |
| P40238     | Thrombopoietin receptor |
| P13945     | Beta-3 adrenergic receptor |
| P49430     | Thromboxane-A synthase |
| Q77MR0     | Lysozomal Pro-X carboxypeptidase |
| P16184     | Dihydrofolate reductase |
| O95822     | Malonyl-CoA decarboxylase, mitochondrial |
| Q05469     | Hormone-sensitive lipase |
| P34976     | Type-1 angiotensin II receptor |
| P05093     | Steroid 17-alpha-hydroxylase/17,20 lyase |
| O70536     | Sterol O-acyltransferase 1 |
| Q95232     | Carbonic anhydrase 4 |
| P49682     | C-X-C chemokine receptor type 3 |
| P14740     | Dipeptidyl peptidase 4 |
| Q16602     | Calcitonin gene-related peptide type 1 receptor |
| P55263     | Adenosine kinase |
| P31648     | Sodium- and chloride-dependent GABA transporter |
| P09958     | Furin |
| P08842     | Steryl-sulfatase |
| P15538     | Cytochrome P450 11B1, mitochondrial |
| P30557     | Prostaglandin E2 receptor EP3 subtype |
| P13516     | Acyl-CoA desaturase 1 |
| Q62053     | Prostaglandin E2 receptor EP2 subtype |

KEGG pathway enrichment analyses were conducted for unveiling the pathways involved in the myocardial infarction targets of salvianolic acid A. As illustrated in Figure 7(b), myocardial infarction-relevant pathways (PI3K-Akt signaling pathway, lipid and atherosclerosis, fluid shear stress and atherosclerosis, focal adhesion, AGE-RAGE signaling pathway in diabetic complications, sphingolipid signaling pathway, HIF-1 signaling pathway, TNF signaling pathway, IL-17 signaling pathway, etc.) were significantly correlated to the myocardial infarction targets of salvianolic acid A. Especially, the details of PI3K-Akt signaling pathway were visualized, as illustrated in Figure 7(c).

3.6. Verification of the Effects and Therapeutic Targets of Salvianolic Acid A in Myocardial Infarction. H9C2 cells were exposed to OGD/R to mimic myocardial infarction. In comparison to normoxia, OGD/R-exposed H9C2 cells presented the reduced proliferation (Figure 8(a)). Pretreatment of 50 μM salvianolic acid A remarkably improved the proliferation of OGD/R-exposed H9C2 cells. Hub myocardial infarction targets of salvianolic acid A were further verified. Compared with normoxia, SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, MAPK8, and NFKB1 expressions were upregulated, and ITGB1 expression was downregulated in OGD/R-exposed H9C2 cells (Figures 8(b)–8(k)). Pretreatment of salvianolic acid A reduced SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, MAPK8, and NFKB1 expressions as well as elevated ITGB1 expression in OGD/R-exposed H9C2 cells.

4. Discussion

Salvianolic acid A is extracted from traditional Chinese medicine *Salvia miltiorrhiza*, which is a major water-soluble as well as a biologically active ingredient [28]. The present study employed network pharmacology analyses to uncover the pharmacological targets and therapeutic mechanisms of salvianolic acid A in myocardial infarction. Further, H9C2 cells were administrated with OGD/R to mimic myocardial infarction, and the effects and hub therapeutic targets were confirmed. Thus, our findings unveiled the possible functional mechanisms and pharmacological targets of salvianolic acid A as an antimyocardial infarction therapy.

Through intersecting the targets of salvianolic acid A and myocardial infarction, 120 pharmacological targets of salvianolic acid A in myocardial infarction were determined. Among them, ten hub therapeutic targets were identified, covering SRC, CTNNB1, PIK3CA, AKT1, RELA, EGFR, FYN, ITGB1, MAPK8, and NFKB1. Evidence has demonstrated the functions of the hub therapeutic targets in myocardial infarction. Blockage of SRC can stabilize Flk/cadherin complexing, reduce edema as well as tissue damage after myocardial infarction [29]. Additionally, SRC suppression reverses Cx43 remodeling and improves heart function following myocardial infarction [30]. CTNNB1 protein product β-catenin is a key integral part of the canonical Wnt/β-catenin pathway. Wnt/β-catenin damage response enables to activate the epicardium as well as cardiac fibroblasts for promoting cardiac repair [31]. The blockage of the Wnt/β-catenin pathway improves the cardiac function of myocardial infarction [32]. Activated RELA/p65 results in myocardial infarction [33], and activation of EGFR-dependent pathway strengthens cardiac fibrosis and exacerbates cardiac dysfunction in myocardial infarction [34]. The basic fibroblast growth factor activates Nrf2-triggered
antioxidant defenses through Akt/GSK3β/Fyn signaling in myocardial infarction [35]. Endothelial ITGB1 (β1 integrin) is essential for the heart to adapt cardiac ischemia and protects from myocardial infarction [36]. ITGB1 upregulation is capable of increasing cardiac function and clinical outcome after myocardial infarction [37, 38]. NFKB1 gene rs28362491 ins/del variation correlates to increased susceptibility to myocardial infarction among Chinese Han patients [39].

Therapeutic targets were significantly linked to myocardial infarction-relevant pathways, such as PI3K-Akt signaling pathway. Evidence demonstrates that salvianolic acid A is regarded as a potential PI3K/Akt inhibitor. For instance, salvianolic acid A enables to attenuate CCl4-triggered liver fibrosis through inactivation of the PI3K-Akt pathway [40]. Moreover, it hinders vasculogenic mimicry formation in human non-small cell lung carcinoma through the PI3K-Akt pathway [41]. Additionally, through suppressing PI3K-Akt pathway, salvianolic acid A triggers cellular apoptosis as well as blocks tumor growth in acute myeloid leukemia [42]. The PI3K-Akt pathway participates in myocardial ischemia/reperfusion damage in diabetic murine models, which is blocked by salvianolic acid A [43]. Salvianolic acid A hinders malignant development of glioma as well as strengthens temozolomide sensitivity through weakening PI3K-Akt pathway [44]. Because the PI3K-Akt pathway plays a key role in administering the process of myocardial infarction, targeting this aberrant signaling pathway as well as improving the pathological manifestation of myocardial infarction remains indispensable [5].

We further verified the effects and therapeutic targets of salvianolic acid A in myocardial infarction in OGD/R-

Figure 2: Prediction of potential targets of salvianolic acid A on the basis of SwissTargetPrediction, HERB, and TargetNet web tools. (a) The distribution of SwissTargetPrediction-, HERB-, and TargetNet-predicted targets of salvianolic acid A. (b) Target classes of the top 15 potential targets of salvianolic acid A predicted by the SwissTargetPrediction web tool.
Figure 5: The protein-protein interaction (PPI) network of products of myocardial infarction targets of salvianolic acid A.

Figure 6: Hub myocardial infarction targets of salvianolic acid A. (a) The top twenty myocardial infarction targets of salvianolic acid A in accordance with the degree. (b) The interaction network of hub myocardial infarction targets of salvianolic acid A.
GO Results of Three Ontologies

- response to molecule of bacterial origin
- response to lipopolysaccharide
- cellular response to chemical stress
- reactive oxygen species metabolic process
- response to oxidative stress
- regulation of reactive oxygen species metabolic process
- response to reactive oxygen species
- peptidyl-serine phosphorylation
- cellular response to biotic stimulus
- peptidyl-serine modification
- membrane raft
- membrane microdomain
- membrane region
- vesicle lumen
- secretory granule lumen
- cytoplasmic vesicle lumen
- integrin complex
- platelet alpha granule
- protein complex involved in cell adhesion
- cell projection membrane
- protease binding
- endopeptidase activity
- metalloendopeptidase activity
- serine-type peptidase activity
- serine hydrolase activity
- serine type endopeptidase activity
- protein tyrosine kinase activity
- 1-phosphatidylinositol 3-kinase activity
- integrin binding

Enrichment Score

BP
CC
MF

(a) Figure 7: Continued.
Figure 7: Biological functions and pathways of myocardial infarction targets of salvianolic acid A. (a) The first ten biological processes (BPs), cellular components (CCs), and molecular functions (MFs) of myocardial infarction targets of salvianolic acid A BPs, CCs, and MFs are marked by unique colors. The length of the column is proportional to the enrichment score. (b) The first twenty Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of myocardial infarction targets of salvianolic acid A. (c) The closer the color is to red, the smaller the adjusted p-value. (c) The map of the PI3K-Akt signaling pathway.
induced H9C2 cells. As expected, salvianolic acid A administration enabled to ameliorate the viability of OGD/R-induced H9C2 cells as well as alter the expression of the hub therapeutic targets. Nevertheless, several limitations should be pointed out. First, the hub therapeutic targets of salvianolic acid A should be verified in myocardial infarction animal models. In our future research, we will further investigate the therapeutic effects as well as pharmacologically relevant targets of salvianolic acid A in myocardial infarction animal models. Second, further clinical trials are urgently needed to verify the therapeutic effects of salvianolic acid A against myocardial infarction.

5. Conclusion

The present study unveiled the pharmacological targets and therapeutic mechanisms of salvianolic acid A in myocardial infarction utilizing network pharmacology analyses and in vitro OGD/R H9C2 cellular models of myocardial infarction, which paved the way for further clinical trials.

### Abbreviations:

- **OGD/R**: Oxygen-glucose deprivation/reoxygenation
- **OMIM**: Online Mendelian Inheritance in Man
- **TTD**: Therapeutic Target Database
- **PPI**: Protein-protein interaction
- **GO**: Gene ontology
- **BPs**: Biological processes
- **CCs**: Cellular components
- **MFs**: Molecular functions
- **KEGG**: Kyoto Encyclopedia of Genes and Genomes
- **CCK-8**: Cell counting kit-8
- **RT-qPCR**: Reverse transcription-quantitative PCR

### Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.
Conflicts of Interest
The authors declare that they have no conflicts of interest.

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