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Revealing the Regulatory Mechanism of Astragaloside IV in Treating Chronic Heart Failure through Network Pharmacology and Animal Experiments

Revelando o mecanismo regulatório do Astragalosídeo IV no tratamento da insuficiência cardíaca crónica por meio de farmacologia de rede e experimentação em animais

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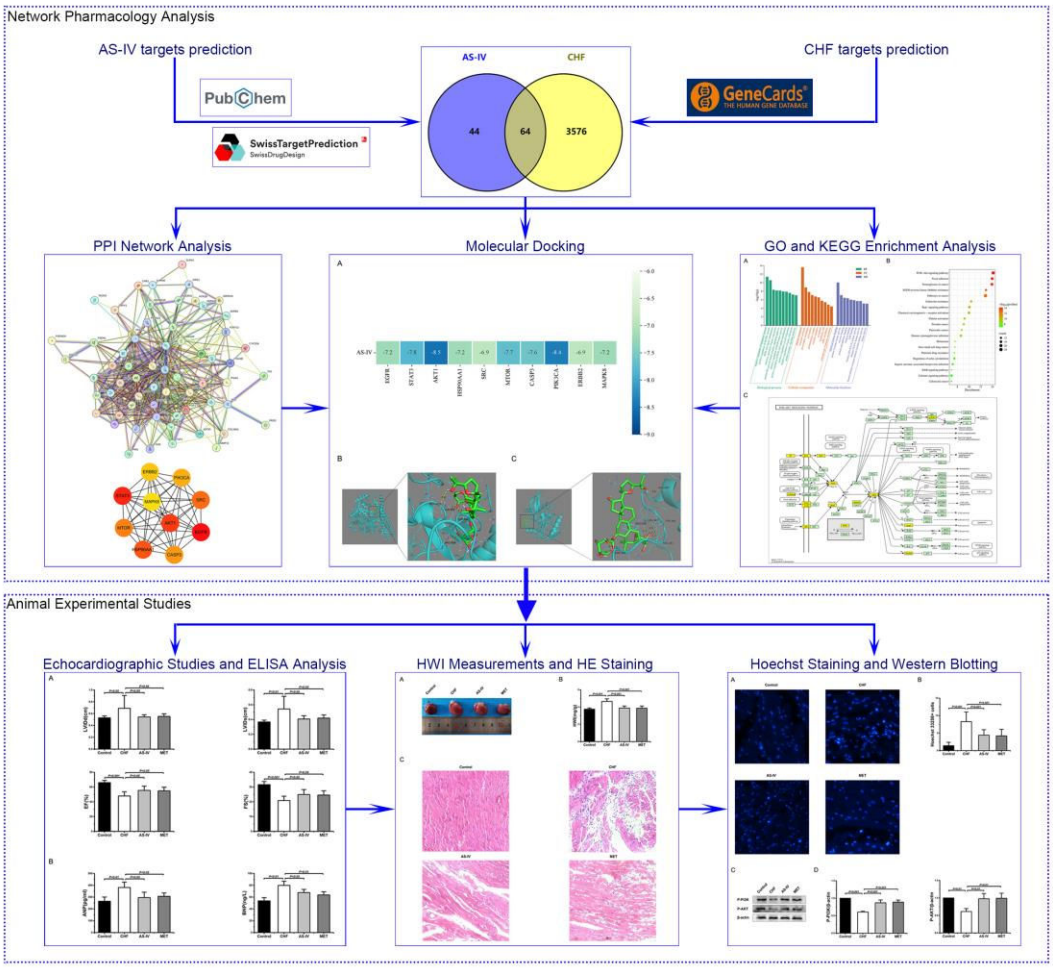
Graphical abstract



Resumo:

Introdução e objetivos: O astragalosídeo IV (AS-IV) é um composto ativo que demonstrou um potencial terapêutico significativo no tratamento da insuficiência cardíaca crónica (ICC), mas o seu mecanismo permanece obscuro. Este estudo teve como objetivo explorar os mecanismos regulatórios da AS-IV no tratamento da ICC.

Métodos: Os alvos terapêuticos e as vias de sinalização do AS-IV na ICC foram identificados utilizando a farmacologia de rede. Posteriormente, foram utilizadas técnicas de *docking* molecular para validar essas previsões. Além disso, foi realizada experimentação *in vivo* para validação adicional, incluindo estudos ecocardiográficos, análises de ELISA, medições de HWI, coloração com HE, coloração com Hoechst e análise de *western blotting*.

Resultados: A análise farmacológica da rede mostrou que a apoptose celular e a via de sinalização PI3K-Akt podem ser os mecanismos primários envolvidos no tratamento da ICC pelo AS-IV. O *docking* molecular mostrou que o AS-IV tem uma afinidade de ligação relativamente alta pelos principais alvos. A experimentação *in vivo* mostrou que o AS-IV melhora a disfunção cardíaca causada pela ICC, reduz os níveis de biomarcadores, alivia a hipertrofia cardíaca, atenua as alterações patológicas do miocárdio, inibe a apoptose do miocárdio e regula positivamente os níveis de expressão das proteínas P-PI3K e P-AKT.

Conclusão: Este estudo demonstra que o AS-IV retarda a progressão da ICC, o que pode ser alcançado pela inibição da apoptose miocárdica por meio da ativação da via de sinalização PI3K-Akt. Esses achados lançaram as bases para a pesquisa e aplicação do AS-IV no tratamento da ICC.

Abstract:

1. Introduction and objectives: Astragaloside IV (AS-IV) is an active compound that has demonstrated significant therapeutic potential for chronic heart failure (CHF), but its mechanism remains unclear. This study aimed to explore the regulatory mechanisms of AS-IV in the treatment of CHF.

2. Methods: The therapeutic targets and signaling pathways of AS-IV on CHF were predicted using network pharmacology. Molecular docking techniques were then used to validate these predicted results. Additionally, in vivo experiments were carried out for further validation, including echocardiographic studies, ELISA analysis, HWI measurements, HE staining, Hoechst staining, and western blotting analysis.

3. Results: Network pharmacology analysis showed that cell apoptosis and the PI3K-Akt signaling pathway may be the primary mechanisms for AS-IV in the treatment of CHF. Molecular docking has shown that AS-IV has a relatively high binding affinity to core targets. In vivo experiments have shown that AS-IV improves cardiac dysfunction caused by CHF, reduces biomarker levels, alleviates cardiac hypertrophy, attenuates myocardial pathological changes, inhibits myocardial apoptosis, and up-regulates the expression levels of P-PI3K and P-AKT proteins.

4. Conclusion: This study demonstrates that AS-IV delays the progression of CHF, which may be achieved by inhibiting myocardial apoptosis via activating the PI3K-AKT signaling pathway. These

findings have laid the foundation for research and application of AS-IV in CHF treatment.

Palavra-chave: Farmacologia de rede; Astragalosídeo IV; Insuficiência cardíaca crônica; Via de sinalização PI3K-Akt; Apoptose celular.

Keywords: Network pharmacology; Astragaloside IV; Chronic Heart Failure; PI3K-AKT signaling pathway; Cell apoptosis.

1. Introduction

Chronic heart failure (CHF) refers to persistent ventricular dysfunction caused by abnormal cardiac structure or function. [1,2] Individuals with CHF typically experience symptoms such as breathlessness, [3] ankle swelling, [4] and fatigue, [5] which significantly impair their quality of life. [6] CHF remains one of the leading causes of human morbidity and mortality globally. [7] It is estimated that CHF affects approximately 26 million individuals worldwide, representing a major global health challenge. [8] The overall five-year survival rate for patients with CHF remains as low as 46% to date. [9] Unfortunately, despite advances in conventional CHF treatments, the overall morbidity and mortality rates remain high. With the growth of the aging population, the absolute number of hospital admissions for CHF is expected to further increase worldwide. [10,11]

Currently, the conventional methods for treating CHF in clinical practice mainly focus on neuroendocrine antagonists. [12,13] These drugs can alleviate some symptoms in CHF patients but fail to fully address the underlying cause of CHF and its associated complications. [14] Moreover, the prolonged use of these chemical drugs may cause adverse reactions, such as electrolyte depletion, fluid depletion, and hypotension. [15,16] Therefore, exploring more effective and safer therapeutic approaches for managing CHF has become a critical focus.

Traditional Chinese medicine (TCM) has a long history of application in the treatment of CHF and has shown significant advantages, such as better efficacy, lower cost, and fewer side effects. [17,18] Astragaloside IV (AS-IV) is an active compound extracted from *Astragalus membranaceus* and has various pharmacological effects, including anti-inflammatory, anti-oxidative stress, anti-apoptotic, anti-fibrotic, and cardiac protection. [19-24] Several studies have demonstrated that AS-IV possesses significant therapeutic potential for CHF both in vivo and in vitro. [25-27] Moreover, AS-IV has good safety because it does not cause obvious toxicity or significant adverse reactions. [28-30] However, the therapeutic targets and regulatory mechanisms of AS-IV on CHF remain to be further clarified.

Network pharmacology, initially proposed by Hopkins, [31] has become a widely adopted approach in TCM research. [32] Research has shown that network pharmacology provides a distinct advantage in systematically revealing the effects of TCM on complex diseases and their molecular mechanisms. [33-35]

2. Objectives

This study first predicted the therapeutic targets and signaling pathways of AS-IV for CHF using the network pharmacology method, and then further verified these results through animal experiments.

3. Methods

3.1 Network pharmacology

3.1.1 Therapeutic targets screening

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [36] was used to obtain the molecular structure and SMILES information of AS-IV. The targets related to AS-IV were identified by the SwissTargetPrediction database (http://www.swisstargetprediction.ch/). [37] The keyword "chronic heart failure" was entered into the GeneCards database (https://www.genecards.org/), [38] and then the targets related to CHF were identified based on the screening criterion of a relevance score greater than 10. The intersection of AS-IV targets and CHF targets was analyzed using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/).

3.1.2 Protein-protein interaction network analysis

The overlapping genes were uploaded into the STRING database (https://cn.string-db.org/) [39] to construct a Protein-Protein Interaction (PPI) network with medium confidence (0.400). The tsv format file was downloaded and imported into Cytoscape 3.7.1 software. [40] Using the Maximal Clique Centrality (MCC) algorithm in the CytoHubba plugin, [41] the top 10 hub genes were identified according to their scores.

3.1.3 GO and KEGG analysis

The DAVID website (https://davidbioinformatics.nih.gov/) [42] was used to perform GO function and KEGG pathway enrichment analysis with $P\text{-}values < 0.05$. A bioinformatics online platform (https://www.bioinformatics.com.cn/) [43] was used for enrichment data visualization and graphing. The therapeutic target locations in key signaling pathways were marked using the KEGG mapper online platform (https://www.genome.jp/kegg/mapper/). [44]

3.1.4 Molecular docking

AS-IV was subjected to molecular docking with 10 core target proteins in the PPI network. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [45] was used to acquire the structural data format (sdf) file of AS-IV, and the RCSB PDB database (https://www.rcsb.org/) [46] was used to retrieve the pdb format files of the 10 target proteins. Autodock Vina was utilized for molecular docking and the evaluation of the binding affinity. [47] Subsequently, the best conformations were selected based on their binding affinities, and PyMOL 2.6.0 was used for conformation visualization. [48]

3.2 Animal experiments

3.2.1 Animals and grouping

In vivo experiments were authorized by the Medical Ethics Committee of Hunan University of Medicine (Approval No. 2021093043). SPF-grade male SD rats, weighing 160–180 g (Tianqin Biotechnology, Changsha, China), were randomly divided into four experimental groups ($n = 6$ per group): Control, CHF, AS-IV, and Metoprolol (MET, positive control). As previously reported, [49] a

5 mg/kg isoproterenol solution (Solarbio, Beijing, China) was intraperitoneally administered for 10 days to establish a CHF animal model. After the model was established, the rats in the AS-IV group were given 40 mg/kg/day AS-IV (Macklin, Shanghai, China) by gavage for 4 weeks, [50] and the MET groups were administered 10 mg/kg/day MET (Rhawn, Shanghai, China). [51] Meanwhile, the Control and CHF groups were only administered an equivalent amount of 0.9% NaCl solution.

3.2.2 Echocardiographic studies

After treatment, all animals in each group were anesthetized by intraperitoneal injection with 2% pentobarbital sodium. [52] Cardiac structural and functional parameters, including Left Ventricular Internal Diameter in diastole (LVIDd), Left Ventricular Internal Diameter in systole (LVIDs), Ejection Fraction (EF), and Fractional Shortening (FS), were then assessed by echocardiography using a color Doppler ultrasound system. [53]

3.2.3 ELISA analysis

After undergoing echocardiography, the rats were kept under anesthesia. Blood samples were collected from the abdominal aorta, centrifuged to obtain serum, which was then stored at -80°C for subsequent ELISA analysis. The content levels of Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) in the serum were quantitatively analyzed using the corresponding ELISA kits (Hengyuan, Shanghai, China). [54]

3.2.4 HWI measurements

After measuring body weight (BW), the rats in each group were euthanized. Subsequently, the hearts were immediately excised and washed with a 0.9% NaCl solution. After measuring heart weight (HW), the cardiac tissues were stored for subsequent experiments. The ratio of HW to BW was calculated to obtain the heart weight index (HWI), and the formula is HWI = HW/BW. [55]

3.2.5 HE staining

The cardiac tissues were immersed in a 4% paraformaldehyde solution for fixation, and then underwent alcohol dehydration, paraffin embedding, sectioning, and hematoxylin and eosin (H&E) staining. [56] Subsequently, the histopathological changes in the myocardial tissue were observed under the light microscope.

3.2.6 Hoechst staining

The cardiac tissues were immersed in a 4% paraformaldehyde solution for fixation, and then underwent alcohol dehydration, paraffin embedding, and sectioning. The sections were stained with the Hoechst 33258 staining kit (Beyotime, Shanghai, China) after conventional dewaxing and rehydration. A fluorescence microscope was employed to observe the Hoechst staining images, and the number of positive cells was evaluated by quantitative analysis.

3.2.7 Western blotting

The expressions of P-PI3K and P-AKT were quantitatively analyzed using Western blotting. Briefly, total proteins were extracted from the cardiac tissues, and their concentrations were determined. Following separation by SDS-PAGE and transfer to a membrane, the protein samples were blocked and then incubated with primary and secondary antibodies sequentially. The immunoreactive bands were visualized using an ECL developer, and the protein intensities were quantified using ImageJ

software. All the primary antibodies related to this study were purchased from ZEN-BIOSCIENCE (Chengdu, China).

3.2.8 Statistical analysis

The experimental data were statistically analyzed using SPSS Statistics 25.0. One-way ANOVA was used to compare the differences among groups, and a *P-value* less than 0.05 indicated statistical significance.

4. Results

4.1 Predictive outcomes of network pharmacology

4.1.1 Therapeutic target prediction and PPI network analysis

The molecular structure image of AS-IV is shown in Figure 1A. A total of 108 genes from the SwissTargetPrediction database may be associated with AS-IV. 3640 genes from the GeneCards database may be associated with CHF. Following intersection, 64 genes were eventually identified as potential therapeutic targets for AS-IV against CHF (Figure 1B). The 64 overlapping genes were input into the PPI network, resulting in 63 nodes with interactions and 470 edges. One node (SLC37A4) had no connections with other nodes in the PPI network, indicating that this target may not interact with other proteins (Figure 1C). After calculation using the MCC algorithm, 10 hub genes were obtained from the PPI network, specifically *EGFR, STAT3, AKT1, HSP90AA1*, *SRC, MTOR, CASP3, PIK3CA, ERBB2*, and MAPK8 (Figure 1D). These 10 hub genes might serve as the core therapeutic targets of AS-IV in CHF.

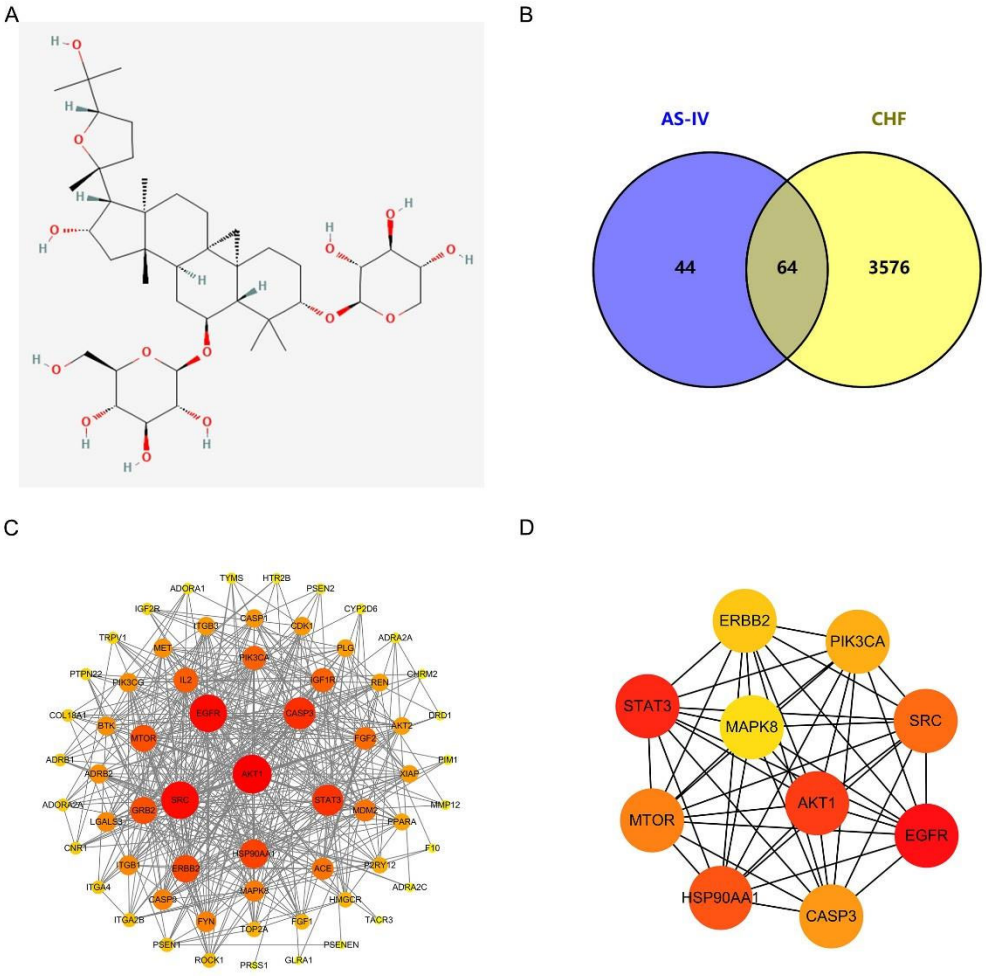


Figure 1. Therapeutic targets prediction and PPI network construction. (A) Molecular structure of AS-IV. (B) The intersection of AS-IV targets and CHF targets. (C) PPI network of 64 overlapping genes. (D) Interaction network of the 10 hub genes based on the screening of MCC scores.

4.1.2 GO and KEGG enrichment analysis

Biological process mainly includes negative regulation of apoptotic process, positive regulation of MAPK cascade, phosphatidylinositol 3-kinase/protein kinase B (PI3K-AKT) signal transduction, positive regulation of gene expression, insulin-like growth factor receptor signaling pathway, positive regulation of MAP kinase activity, positive regulation of PI3K-AKT signal transduction, positive regulation of cell migration, negative regulation of gene expression and insulin receptor signaling pathway (Figure 2A). The top 20 KEGG pathways include PI3K-AKT signaling pathway, focal adhesion, proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, pathways in cancer, endocrine resistance, Rap1 signaling pathway, chemical carcinogenesis-receptor activation, platelet activation, prostate cancer, pancreatic cancer, human cytomegalovirus infection, melanoma, non-small cell lung cancer, platinum drug resistance, regulation of actin cytoskeleton, Kaposi sarcoma-associated herpesvirus infection, ErbB signaling pathway, calcium signaling pathway and colorectal cancer (Figure 2B). Notably, the PI3K-AKT signaling pathway is an important pathway

that contains several identified core targets, such as HSP90AA1, EGFR, MTOR, PIK3CA, ERBB2, and AKT1 (Figure 2C).

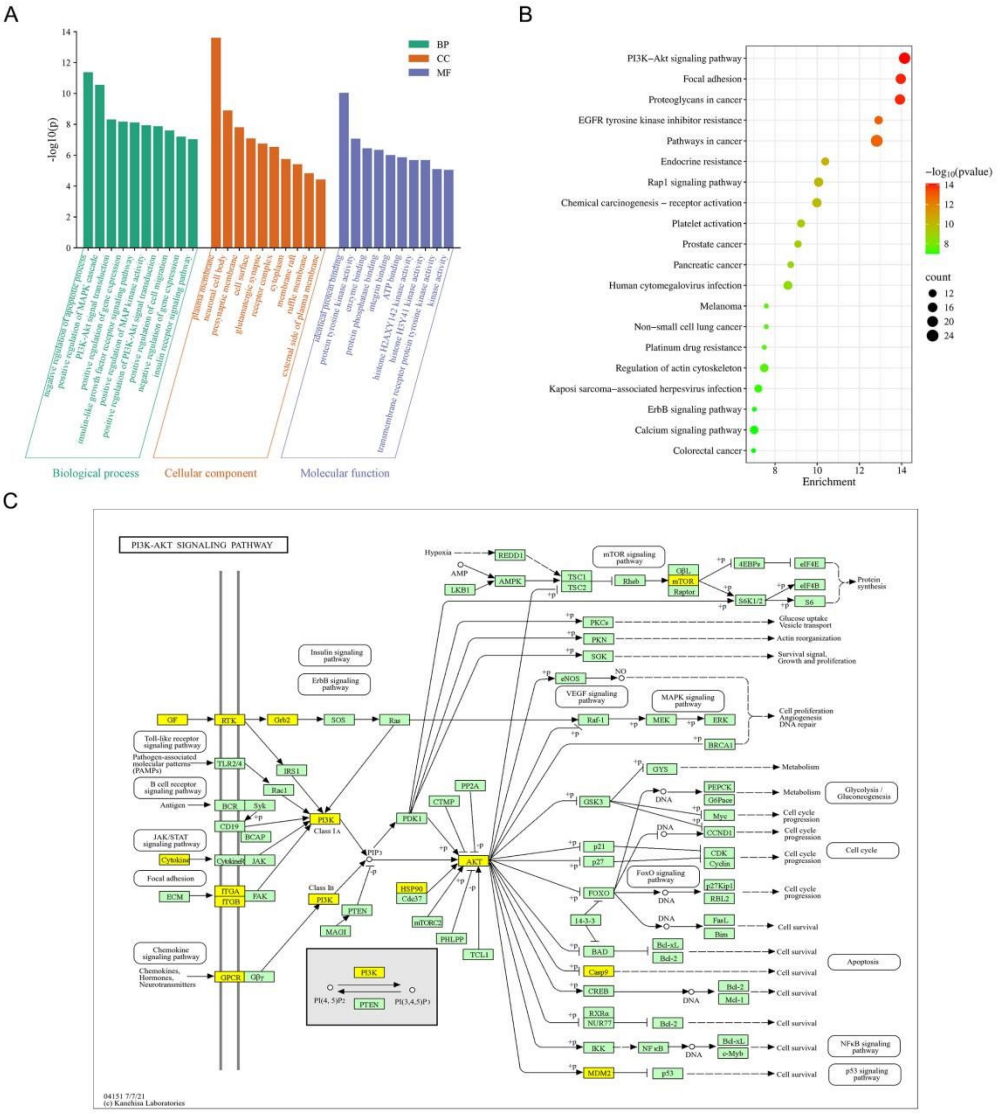


Figure 2. GO and KEGG analysis results. (A) Histogram of the top 10 GO terms. (B) Bubble diagram of the top 20 KEGG pathways. (C) Distribution of the therapeutic targets of AS-IV for CHF in the PI3K-AKT signaling pathway, yellow nodes represent potential therapeutic targets.

4.1.3 Molecular docking

As shown in Figure 3A, all 10 core target proteins can spontaneously bind to AS-IV, and all of them have binding energies less than -5 kcal/mol. AS-IV has the strongest and most stable binding affinity toward AKT1 and PIK3CA, followed by STAT3, MTOR, CASP3, EGFR, HSP90AA1, MAPK8, SRC, and ERBB2. Notably, molecular docking analysis revealed that AS-IV might bind to PIK3CA through the formation of hydrogen bonds via PRO-1011 (distance: 2.8 Å), GLN-809 (distance: 2.1 Å), ASP-806 (distance: 2.7 Å), and ARG-808 (distance: 2.2 Å) (Figure 3B). AS-IV has the potential to bind to AKT1 through the formation of hydrogen bonds via GLN-352 (distance: 2.3 Å), ARG-

346 (distance: 2.2 Å), LEU-347 (distance: 2.4 Å), TYR-340 (distance: 2.7 Å), ARG-367 (distance: 2.3 Å), and GLU-341 (distance: 2.6 Å) (Figure 3C).

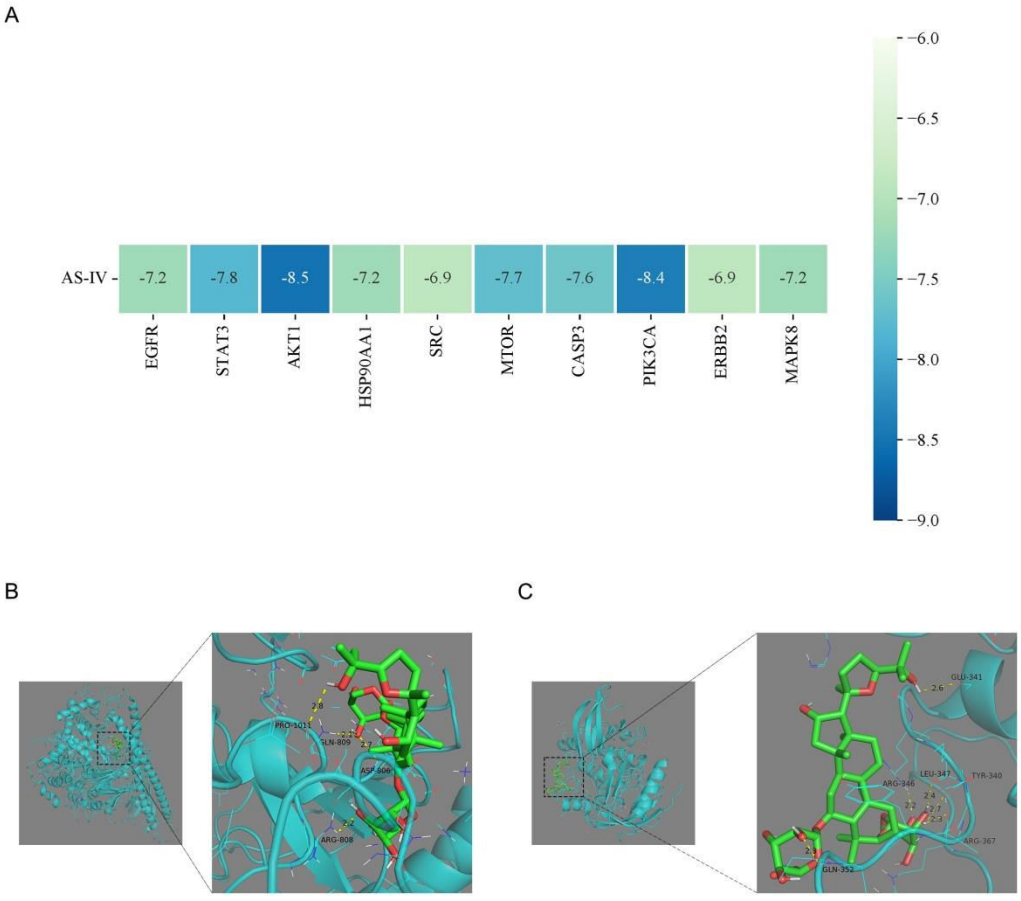


Figure 3. Molecular docking results. (A) The binding energies of AS-IV and 10 core target proteins. (B) Molecular models of the binding of AS-IV to PIK3CA. (C) Molecular models of the binding of AS-IV to AKT1.

4.2 Validation of animal experimental studies

4.2.1 AS-IV Improves Cardiac Dysfunction and Reduces Biomarker Levels in CHF Rats

As shown in Figure 4A, the LVIDd and LVIDs in the CHF group were significantly higher than those in the Control group, while the EF and FS were significantly decreased, indicating that the rat model of CHF was successfully established. After treatment with AS-IV, there was a significant decrease in the LVIDd and LVIDs, along with a distinct increase in EF and FS. In this study, ANP and BNP were also measured. Normally, ANP and BNP are expressed and secreted by myocardial cells primarily in the atrium and ventricle, and their concentrations are minimal. [57,58] The increase in ANP and BNP levels is usually considered as diagnostic and predictive biomarkers for CHF. [59] As shown in Figure 4B, compared with the Control group, the levels of ANP and BNP were significantly increased in the CHF group, but AS-IV treatment reversed these changes.

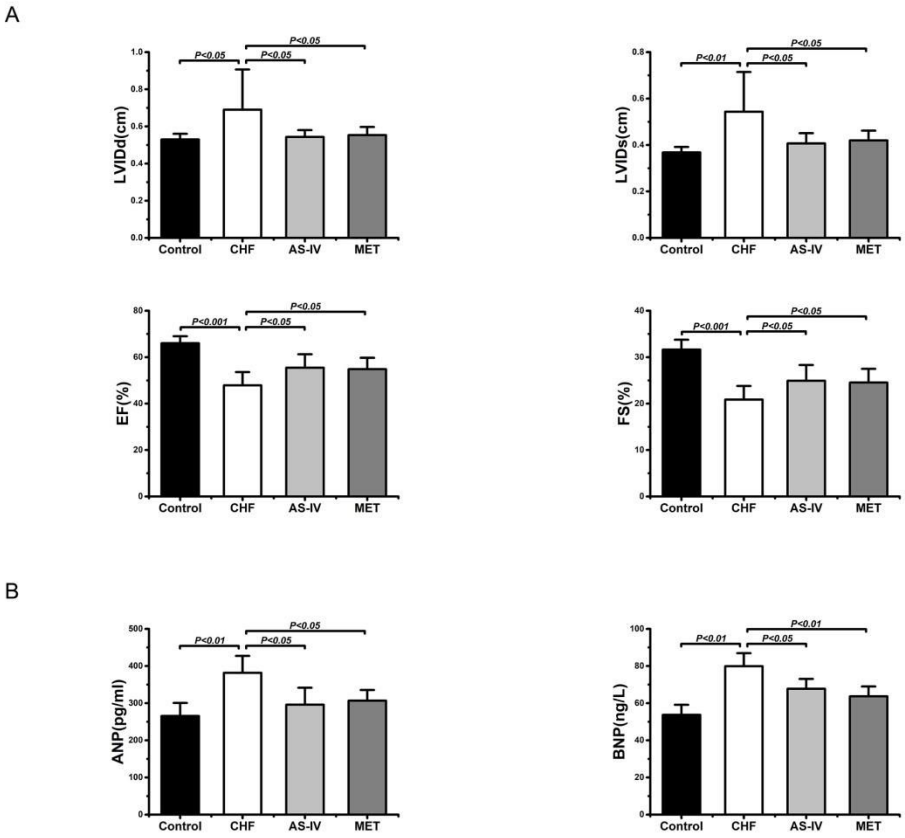


Figure 4. AS-IV improved cardiac function and reduced biomarker levels. (A) Quantitative analysis of cardiac function: LVIDd, LVIDs, EF, and FS. (B) Quantitative analysis of ANP and BNP.

4.2.2 AS-IV Alleviates Cardiac Hypertrophy and Attenuates Myocardial Pathological Changes in CHF Rats

As shown in Figure 5A and 5B, the rats in the CHF group showed significant cardiac hypertrophy, manifested by increased heart size and elevated HWI. After intervention with AS-IV, both the heart size and HWI were significantly reduced. As shown in Figure 5C, the Control group exhibited well-arranged myocardial fibers with normal diameters and interstitial gaps. However, swollen and necrotic myocardial cells, disordered myocardial fibers, hyperplastic fibrous connective tissue, and infiltrated inflammatory cells were observed in the CHF group. After treatment with AS-IV, these pathological changes were obviously reversed.

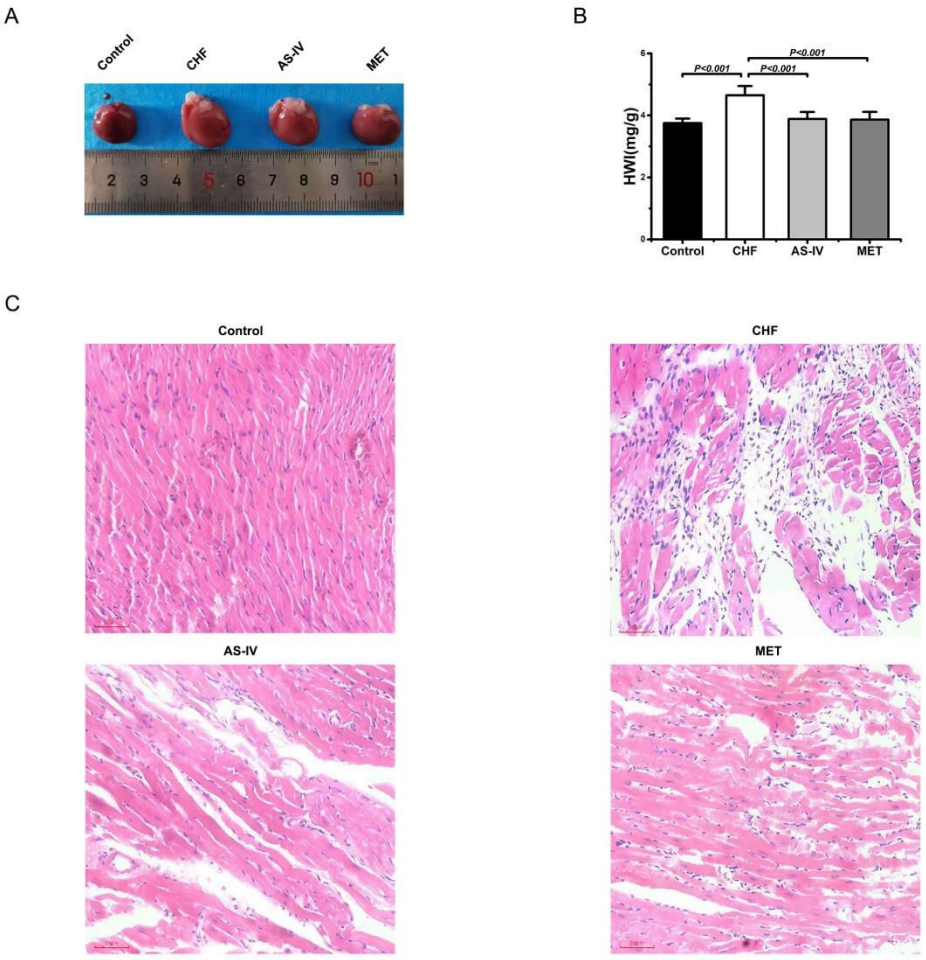


Figure 5. AS-IV alleviated cardiac hypertrophy and attenuated myocardial pathological changes. (A) Macroscopic morphological images of hearts. (B) Quantitative analysis of HWI results. (C) HE staining images of cardiac tissue.

4.2.3 AS-IV Inhibits Myocardial Apoptosis and Activates PI3K-AKT Signaling Pathways in CHF Rats

Network pharmacology analysis revealed that the apoptotic process and the PI3K-Akt signaling pathway might be pivotal biological events related to AS-IV in CHF treatment. Therefore, we evaluated the effect of AS-IV on these two regulatory mechanisms. After Hoechst 33258 staining, normal myocardial nuclei exhibit uniformly pale blue fluorescence, whereas apoptotic nuclei display densely bright blue fluorescence. As shown in Figure 6A and 6B, the number of apoptotic cells was significantly increased in the CHF group compared with the Control group. After treatment with AS-IV, the apoptotic cells were significantly reduced. Next, to verify the effect of AS-IV on the PI3K-Akt signaling pathway, the protein expressions of P-PI3K and P-AKT were examined by Western blotting analysis. As shown in Figure 6C and 6D, the levels of P-PI3K and P-AKT proteins in the CHF group were significantly lower than those in the Control group. After treatment with AS-IV, the expression levels of these proteins were significantly up-regulated.

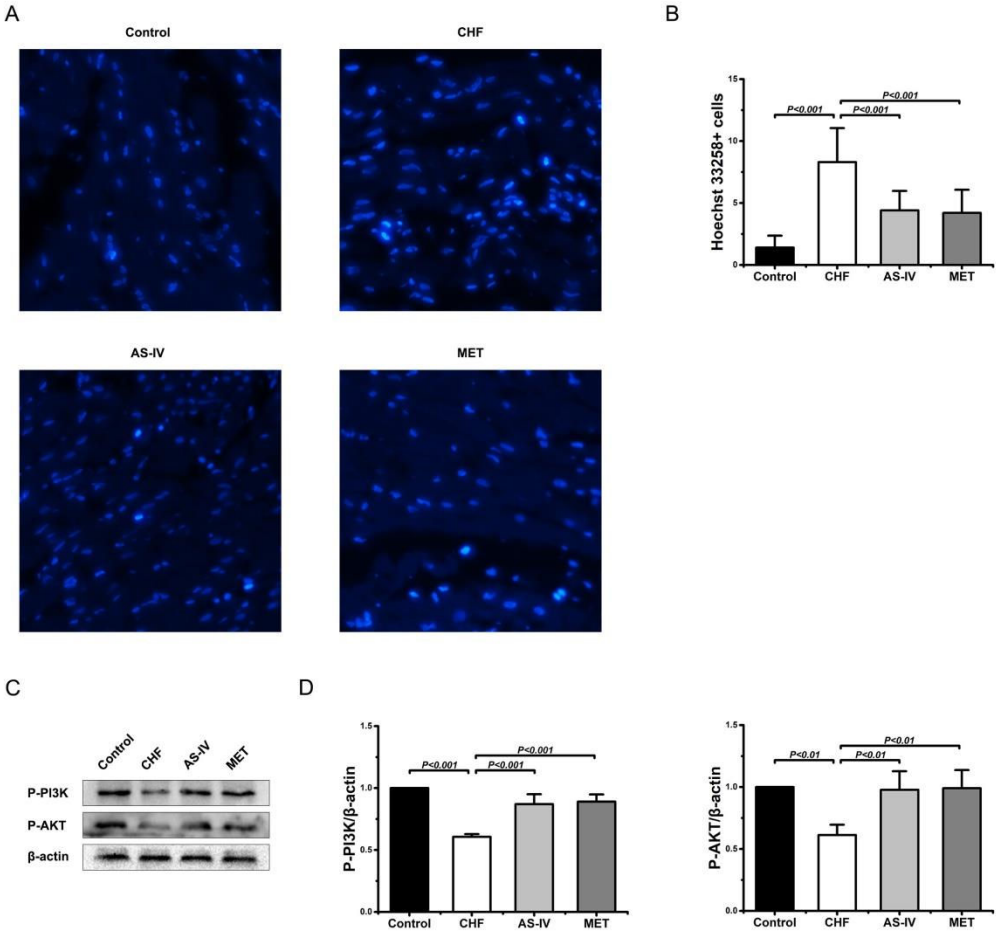


Figure 6. AS-IV inhibited myocardial apoptosis and activated the PI3K-AKT signaling pathway. (A) Hoechst 33258 staining images of cardiac tissue. (B) Quantitative analysis of Hoechst-positive cells. (C) Western blotting images of P-PI3K and P-AKT. (D) Quantitative analysis of the protein expression levels.

5. Discussion

Pharmacotherapy serves as both the cornerstone of current CHF treatment and the main direction for future advancements. [60] Over the past few decades, researchers have devoted significant efforts to developing natural pharmaceuticals and their active components based on TCM theory for the treatment of CHF. [61] AS-IV is considered the primary active compound derived from *Astragalus membranaceus* for CHF treatment, [62] yet the precise mechanism underlying its effects remains to be elucidated. To ensure the safe clinical application of AS-IV, several scholars have conducted toxicity-related studies. It was found that AS-IV does not cause obvious hepatic or renal toxicity after oral administration of 10 mg/kg/day for 14 weeks. [63] In pregnant rats, AS-IV does not cause obvious reproductive toxicity at doses of 0.25–1.0 mg/kg/day. However, at a dose of 1.0 mg/kg/day, the offspring rats showed some delays in fur development, eye opening, and cliff parry reflex. [64] As for the heart, no research to date has found evidence of significant cardiac toxicity associated with AS-IV. In this study, we systematically analyzed its therapeutic mechanisms in CHF by combining

network pharmacology and animal experimental validation.

Firstly, we identified 64 overlapped targets presented in genes related to AS-IV and CHF by database screening. PPI network analysis identified the top 10 hub genes, including *EGFR, STAT3, AKT1, HSP90AA1, SRC, MTOR, CASP3, PIK3CA, ERBB2,* and *MAPK8*. Next, we conducted molecular docking to evaluate the interaction intensity of AS-IV with the 10 core targets. The results showed that AS-IV had a relatively high binding affinity for all 10 core targets, particularly AS-IV-AKT1 and AS-IV-PIK3CA, which exhibited the best binding effects.

GO enrichment analysis revealed that AS-IV might potentially play a role in the treatment of CHF by regulating apoptosis and PI3K-AKT signal transduction. Apoptosis serves as the principal cellular pathway leading to myocardial cell death. [65] Excessive loss of myocardial cells leads to both cardiac dysfunction and structural lesions, thereby increasing the risk of CHF. [66] Currently, inhibiting myocardial apoptosis has been widely recognized as one of the critical strategies for managing CHF. [7] Additionally, the KEGG results indicated that the PI3K-AKT signaling pathway was one of the main enriched pathways and ranked as the highest among the pathways related to AS-IV and CHF. Furthermore, the key targets in this pathway, such as PIK3CA and AKT1, are also the core targets determined through the PPI network and molecular docking. The proteins encoded by AKT1 and PIK3CA are respectively key isoforms of AKT and PI3K, and these proteins are the key components in the PI3K-AKT signaling pathway. [67,68] The PI3K-AKT signaling pathway has been proven to play a critical role in the occurrence, development, and pathologic formation of CHF. [69] Activation of the PI3K-AKT signaling pathway can inhibit myocardial apoptosis and protect the myocardium. [70] Recent studies have demonstrated that the PI3K-AKT signaling pathway has become an important and promising target for TCM in the prevention and treatment of CHF. [71] TCM can delay ventricular remodeling and protect heart function by modulating the PI3K-AKT signaling pathway and suppressing cell apoptosis. [72,73] Therefore, the PI3K-AKT signaling pathway, as a potential mechanism, was chosen for further experimental verification.

To validate the predicted conclusion, we performed animal experiments. Isoproterenol, a classic agonist of the β-adrenergic receptor, may increase myocardial oxygen consumption and can lead to heart failure when used excessively. [74,75] Previous studies [49,52] have shown that isoproterenol has been widely used to establish experimental models of CHF. MET is a cardioselective β-blocker that improves cardiac function and protects the heart in patients with CHF. [76] Therefore, we also constructed an animal experimental model of CHF induced by isoproterenol and intervened with AS-IV and MET. The experimental results showed that AS-IV improves cardiac dysfunction caused by CHF, reduces biomarker levels, alleviates cardiac hypertrophy, and attenuates myocardial pathological changes. Isoproterenol enhances myocardial contractility by acting on β-adrenergic receptors; however, high-dose use may cause myocardial ischemia and hypoxia, leading to cardiomyocyte apoptosis. [77] Moreover, studies have shown that certain natural compounds can exert cardioprotective effects against myocardial injury caused by overstimulation of the β-adrenergic receptor, by inhibiting myocardial apoptosis through the activation of the PI3K-AKT signaling pathway. [78] Previous studies suggested that AS-IV exerts an inhibitory effect on apoptosis in

isoproterenol-induced hypertrophic myocardial cells. [79,80] Zhao *et al*. [81] found that AS-IV reduces myocardial apoptosis and protects myocardial cells, possibly as a mechanism for treating heart failure. Jia *et al*. [82] found that AS-IV significantly inhibits doxorubicin-induced myocardial apoptosis through activating the PI3K-AKT signaling pathway. This study showed that after treatment with AS-IV, the levels of P-PI3K and P-AKT proteins were significantly up-regulated, and the apoptosis of myocardial cells was significantly reduced. These experimental results indicate that AS-IV can inhibit myocardial apoptosis by activating the PI3K-AKT signaling pathway, thereby playing an important role in the treatment of CHF. In this study, MET was selected as the positive control drug. It was found that AS-IV and MET performed similarly in various measured indices, indicating that both have good therapeutic effects on CHF.

This research has several limitations. We did not, for example, use inhibitors related to the PI3K-AKT signaling pathway for further validation. Additionally, we mainly focused on the regulation of this pathway, while other potential targets and signaling pathways were not verified. These two limitations will be the focus of our subsequent research in order to provide a more comprehensive understanding of the therapeutic effects of AS-IV.

6. Conclusion

This study demonstrates that AS-IV improves cardiac dysfunction, reduces biomarker levels, alleviates cardiac hypertrophy, and attenuates myocardial pathological changes in rats with CHF. These therapeutic effects may be achieved by inhibiting myocardial apoptosis via regulating the PI3K-AKT signaling pathway.

Ethical approval

The animal study protocol was approved by the Medical Ethics Committee of Hunan University of Medicine (Approval number: 2021093043).

Funding

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Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

Ethics in publishing

1. Does your research involve experimentation on animals? :

Yes

If yes; please provide name of the ethical committee approving these experiments and the registration number. :

The animal study protocol was approved by the Medical Ethics Committee of Hunan University of Medicine (Approval number: 2021093043).

If yes; please confirm authors compliance with all relevant ethical regulations. :

Yes

2. Does your study include human subjects?:

No

3. Does your study include a clinical trial?:

No

4. Are all data shown in the figures and tables also shown in the text of the Results section and discussed in the Conclusions?:

Yes

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