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Study of the Mechanism by Which Shenfu Injection Regulates Cardiomyocyte Hypertrophy Based on Dopamine Receptor D2–Mediated Mitochondrial Fission

ABSTRACT

Background: Shenfu Injection (SFI) has been widely utilized in the treatment of cardiovascular diseases in China for over 3 decades. In recent years, the therapeutic role of SFI in cardiovascular diseases has garnered significant attention. This study aims to elucidate the effects and possible mechanism of SFI using in vitro experiments and network pharmacology analysis.

Methods: Hypertrophy was induced by Angiotensin II (Ang II) treatment of HL-1 and isoprenaline (ISO) treatment of AC16. First, cell viability was detected upon different concentrations of SFI to screen the safe dosage of SFI. The effect of SFI on cell functions was evaluated by cell size, atrial natriuretic peptide/brain type natriuretic peptide (ANP/BNP) levels, mitochondrial morphology, and Ser616/Ser579 or Ser637/Ser600 levels. The potential targets of SFI against cardiomyocyte hypertrophy were predicted by network pharmacology, and then experimentally validated by qRT-PCR.

Results: Shenfu Injection treatment significantly reduced cell size, ANP expression, BNP expression, and mitochondrial injury compared to Ang and ISO groups ($P < .001$). Western blot analysis showed that SFI decreased the expression of Dynamin-related protein 1 (Drp1) phosphorylation at Ser616/Ser579 and increased the expression of Drp1 phosphorylation at Ser637/Ser600 in Ang- and ISO-induced cells. Network pharmacology analysis and cell experiments showed SFI decreased dopamine receptor D2 (DRD2) and increased protein kinase A levels in induced cardiomyocytes.

Conclusion: In summary, the findings demonstrate that SFI may alleviate cardiomyocyte hypertrophy by targeting DRD2 and inhibiting mitochondrial fission.

Keywords: Cardiomyocyte hypertrophy, DRD2, mitochondrial fission, Shenfu Injection

INTRODUCTION

Cardiomyocyte hypertrophy is defined as an adaptive response in which cardiomyocytes increase in size rather than number. This process is usually triggered by mechanical stress, neurohormonal activation, or pathological stimuli (e.g. hypertension, myocardial infarction). The short-term maintenance of cardiac function is a consequence of this process; however, its long-term persistence may lead to pathological remodeling and the eventual development of heart failure.[1,2] The upregulation of atrial natriuretic peptide (ANP) and brain type natriuretic peptide (BNP) in ventricular cardiomyocytes is a hallmark of cardiac hypertrophy.[3] The expression and release of ANP and BNP are primarily triggered by cardiac mechanical stress. Pro-ANP knockout mice exhibited a proportional relationship between the degree of ANP reduction and the severity of resulting hypertension and cardiac hypertrophy.[3] Similar to ANP, BNP expression increases in cardiomyocytes during pressure or volume overload. In recent years, studies on cardiomyocyte hypertrophy have concentrated on the following areas: molecular mechanisms, signaling pathways, and organelle function.[4-6] Mitochondrial dysfunction, triggered by dynamin-related protein 1 (Drp1)-dependent mitochondrial fission, is an established contributor to the development of cardiomyocyte hypertrophy.[7]

ORIGINAL INVESTIGATION

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As the core of cellular energy metabolism, mitochondria play a pivotal role in cardiomyocyte hypertrophy. Mitochondria are not only responsible for adenosine triphosphate (ATP) production but also play critical roles in apoptosis, calcium ion homeostasis, and reactive oxygen species generation.[8] Mitochondrial dynamics (comprising division and fusion) represent a pivotal mechanism by which to maintain their function, with mitochondrial fission primarily mediated by Drp1. This process plays a pivotal role in cellular stress, metabolic demands, and organelle quality control.[9,10] The Drp1 exhibits dual regulation of mitochondrial fission through phosphorylation, with Ser616 promoting and Ser637 inhibiting the process, respectively.[11] As demonstrated in the extant literature, there is a demonstrable correlation between mitochondrial fission and several concomitant pathologies, including oxidative stress, energy metabolism, inflammation, and metabolic reprogramming of cardiomyocytes.[12-15] Dopamine-mediated dopamine receptor D2 (DRD2) antagonists can inhibit protein kinase A (PKA) activation by elevating intracellular cyclic adenosine monophosphate concentration and PKA phosphorylation of Drp1, which decreases Drp1 activity.[16] This coordinated regulation of the DRD2/PKA/Drp1 axis represents a critical pharmacological pathway.

Traditional Chinese medicine (TCM), with its extensive historical background, confers distinctive benefits in the management of cardiovascular diseases. Shenfu Injection (SFI), a classic Chinese medicinal injection, is derived from the traditional Chinese medicine formula Shenfu Decoction. Composed of *Panax ginseng C.A.Mey.* and *Aconitum carmichaelii Debx.* extracts, SFI exhibits multiple pharmacological effects, including cardiotonic, anti-inflammatory, antioxidant, and microcirculation-improving properties.[17,18] In recent years, the application of SFI in the treatment of cardiovascular diseases, particularly cardiac hypertrophy, has garnered increasing attention.[19] Studies have demonstrated that SFI alleviates pathological processes by regulating mitochondrial function, suppressing oxidative stress, and enhancing energy metabolism.[20-22] Nevertheless, further investigation is required to elucidate the mechanism by which SFI ameliorates cardiomyocyte hypertrophy.

Network pharmacology serves as a powerful tool for studying TCM by analyzing multi-target mechanisms and system-level interactions.[23] It enables the prediction of pharmacodynamic material bases and mechanistic pathways, making it highly suitable for revealing the modern scientific foundations of TCM formulas.[24] Therefore, this study aims to analyze the active constituents, potential candidates, and potential molecular mechanisms of SFI in treating cardiomyocyte hypertrophy using an integration of network pharmacology and cellular experiments.

HIGHLIGHTS

- Shenfu Injection (SFI) attenuates cardiomyocyte hypertrophy.
- Shenfu Injection modulates mitochondrial fission by balancing p-Drp1 (S616↓, S637↑).
- Dopamine receptor D2 may be a new therapeutic target for SFI.

METHODS

Materials and Reagents

Shenfu Injection (lot #Z51021920) was purchased from China Resources Sanjiu Pharmaceutical Co., Ltd. (Ya'an, Sichuan, China). Angiotensin II (Ang II) and isoprenaline (ISO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The CCK-8 assay kit and BCA protein quantification kit were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). PrimeScript RT Reagent Kit was obtained from Takara Bio Inc. (Shiga, Japan). Enhanced chemiluminescence (ECL) substrate was acquired from Suzhou New Saimei Biotechnology Co., Ltd. (Suzhou, Jiangsu, China). The protein extraction kit and sodium dodecyl-sulfate polyacrylamide gel electrophoresis gel preparation kit were purchased from Beyotime Biotechnology (Shanghai, China). For immunoblot analyses, primary antibodies against phosphorylated Drp1 at Ser637 (#6319) and Ser616 (#4494) were obtained from Cell Signaling Technology (Danvers, MA, USA). MitoTracker Red CMXRos was acquired from Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals were analytical-grade reagents.

Cell Culture and Treatment

The HL-1 cells and AC16 cells were derived from American Type Culture Collection (Manassas, VA, USA). HL-1 cells were cultured in minimal essential medium Eagle α medium, which was enriched with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured in a humidity-regulated milieu with 5% CO_2 at 37°C. HL-1 cells were randomly assigned to 3 experimental groups: (1) untreated control, (2) Ang II (20 µM)-treated, and (3) Ang II + SFI-treated groups.[18]

AC16 human cardiomyocytes underwent cultivation in Dulbecco's Modified Eagle Medium (DMEM) to which 10% fetal FBS was added, and the cultivation was conducted under the same conditions (37°C, 5% CO_2). The AC16 cells were divided into: (1) vehicle control, (2) ISO (10 µmol/L)-treated, and (3) ISO + SFI-treated groups.

Cell Viability

Cell viability was assessed using the Cell Counting Kit-8 according to the manufacturer's protocol. HL-1 cells were exposed to SFI (0, 80, 100, 120 µg/mL) for 24 hours, while AC16 cells received 0, 100, 150, and 200 µg/mL. Following this, 10 µL CCK-8 reagent was added per well, and cells were maintained in an incubation state for 2 hours at 37°C. Subsequently, with the utilization of a microplate reader (Bio Tek, Winooski, VT, USA), absorbance was recorded at a wavelength of 450 nm.

Cell Size

Cells were trypsinized, washed with phosphate buffered saline, and pelleted by centrifugation at 800 × g for 5 minutes at 4°C before resuspension in DMEM supplemented with 20% FBS. Cell suspensions were centrifuged at 800 × g for 15 minutes, resuspended in fresh medium, and seeded onto glass slides. Analysis was performed using an inverted

phase-contrast microscope (Olympus IX73; ×400 magnification) with ImageJ software (NIH, Bethesda, MD, USA) for quantifying ≥100 cells per experimental group.[25]

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from HL-1 and AC16 cells with TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA). RNA purity and concentration were determined. First-strand cDNA was synthesized using the PrimeScript RT Reagent Kit according to the instructions. Quantitative reverse transcription PCR (qRT-PCR) was conducted, and relative mRNA expression was calculated via the $2^{-\Delta\Delta CT}$ method.

Western Blotting Assay

Cellular proteins were extracted from HL-1 and AC16 cells. Protein concentrations were determined using bicinchoninic acid (BCA) assay, and samples were resolved using 10% SDS-polyacrylamide gels and electrophoretically transferred to polyvinylidene fluoride membranes. Membranes were blocked with nonfat dry milk, then followed by incubation with rabbit anti-pDrp1 (Ser616 and Ser637) primary antibody (1:1000) overnight at 4°C. The secondary antibody was incubated at room temperature for 2 hours. Finally, immunoreactive bands were visualized using enhanced chemiluminescence (ECL) and band strength was analyzed using ImageJ software.

Mitochondrial Morphology

Transmission electron microscopy (TEM) and Mito Tracker staining were employed to observe mitochondrial injury in cells.

After treatment, the cells were immediately transferred into pre-cooled 2.5% glutaraldehyde (prepared in 0.1 M phosphate buffer, pH 7.4) and fixed overnight at 4°C. Following 3 washes with phosphate buffer, post-fixation was performed using 1% osmium tetroxide for 2 hours. Dehydration was carried out using a graded acetone series, followed by stepwise infiltration with epoxy resin. Ultrathin sections of 60 nm thickness were prepared, double-stained with uranyl acetate and lead citrate, and subsequently observed under transmission electron microscopy for mitochondrial examination.

Live cells were loaded with MitoTracker Red CMX Ros (50 nM, molecular probe) and confocal images were acquired using an LSM 510 META system (Zeiss LSM510 META). The specific process was referenced in previous literature.[26]

Network Pharmacology

Collection and Screening of Shenfu Injection Sctive Ingredients

Using The Encyclopedia of Traditional Chinese Medicine 2.0 (ETCM2.0), the active ingredients of SFI were searched for and collected based on the quantitative estimate of drug-likeness (QED)>0.49.

Screening of Compound Targets

The potential targets of the active chemical components were collected from ETCM2.0 and HERB databases.

Network Construction of Drug-Active Ingredients-Targets

The acquired targets and bioactive compounds were imported into Cytoscape 3.9.1 to construct an injection-active ingredient-target network. This network visualization enabled systematic analysis of the active ingredients in SFI, their corresponding targets, and interaction relationships. Furthermore, network topology analysis was conducted using the built-in Network Analyzer tool.

Construction of the Protein-Protein Interaction Network and Pathway Analysis

The curated targets were subsequently submitted to the STRING database (https://string-db.org/), with species restriction to Homo sapiens and a confidence threshold >0.4. Non-interacting nodes were systematically filtered. The resultant protein-protein interaction (PPI) data were then imported into Cytoscape 3.9.1 for topological analysis and network visualization. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was subsequently conducted. Finally, the top 10 enriched pathways (FDR < 0.05) were visualized using the pathview R package (v1.40.0).

Hub Genes Analysis

The constructed PPI network was subsequently analyzed using the Maximal Clique Centrality (MCC) algorithm within the CytoHubba plugin in Cytoscape. The MCC analysis identified the top 10 hub genes based on their node degree scores, which were prioritized for downstream functional characterization.

Drawing of Venn Diagram

Genes associated with the identified hub genes and those annotated to the cAMP signaling pathway in KEGG analysis were systematically compared using Venny (http://bioinformatics.psb.ugent.be/webtools/Venn/). The intersection subset containing overlapping genes was subsequently identified.

Verification of Target Expression Level

The qRT-PCR is utilized to detect the expression levels of DRD2 and PKA mRNA in HL-1 and AC16 cells. The process is performed according to the previous literature.[27]

Statistical Analysis

All values are expressed as mean ± SD. The assumption of normality for the continuous outcome variables within each group was assessed prior to conducting parametric comparisons. This was evaluated using the Shapiro–Wilk test. Following assessment of normality, for comparisons across more than 2 independent groups meeting these assumptions, 1-way ANOVA was applied, followed by post-hoc tests where significant (Bonferroni, Dunnett's, or Tukey's post hoc, as appropriate). If the normality assumption was violated, the non-parametric Mann–Whitney *U*-test (for 2 groups) or Kruskal–Wallis test with Dunn's post hoc (for more than 2 groups) was used instead. When $P < .05$, the difference was considered significant.

RESULTS

Selection of Non-Cytotoxic Concentration of Shenfu Injection by CCK-8 Method

As shown in Figure 1A, SFI treatment demonstrated concentration-dependent effects on HL-1 cardiomyocyte viability.

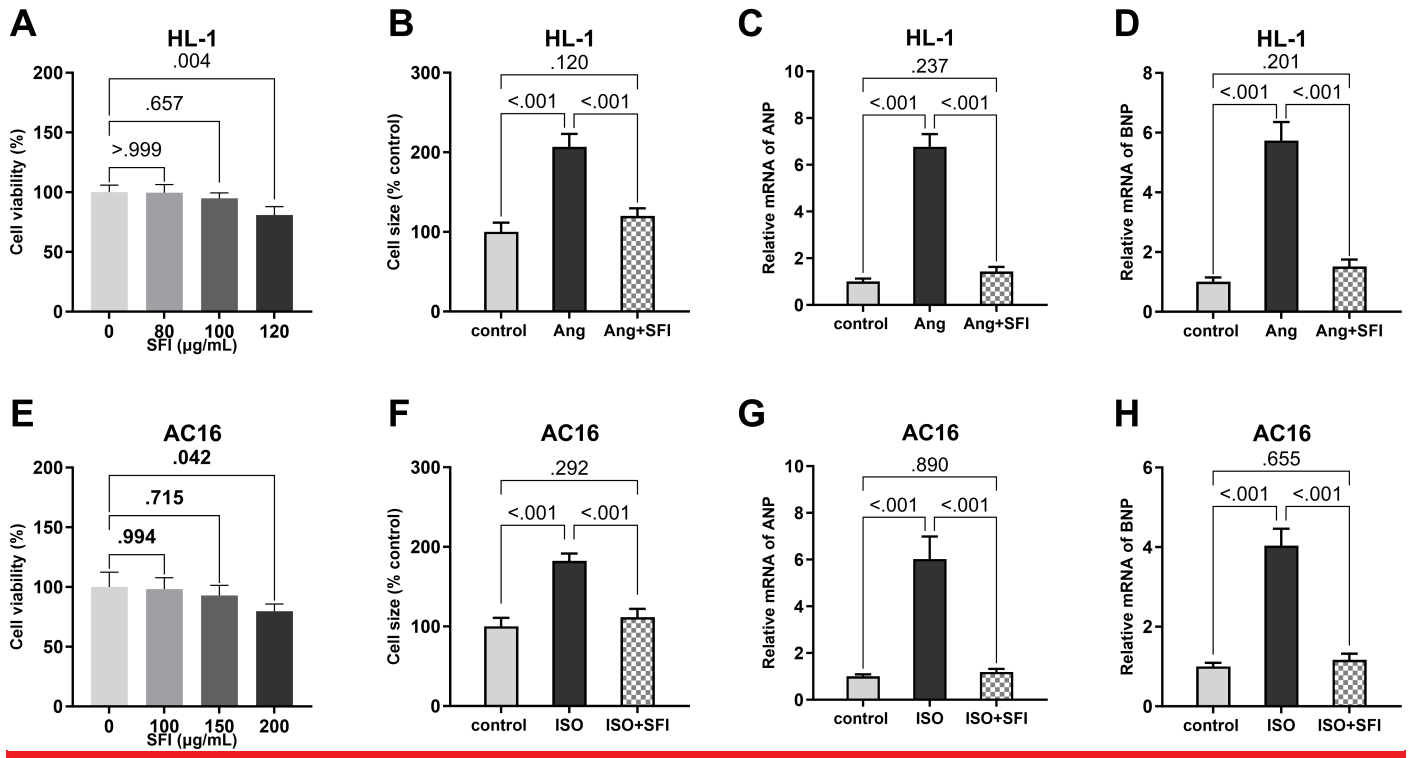

Figure 1. SFI alleviates Ang II-induced hypertrophy of HL-1 cells and ISO-induced hypertrophy of AC16 cells. A, the cell viability of HL-1 cells with different doses of SFI was measured. B, the cell viability of AC16 cells with different doses of SFI was measured. C, cell size of HL-1 cells was measured after Ang II treatment for 24 or 48 hours (n = 4). D, cell size of AC16 cells was measured after ISO treatment for 24 or 48 hours. E, F RT-qPCR analysis was conducted to detect the mRNA expression of ANP and BNP in HL-1 cells. G, H, RT-qPCR analysis was conducted to detect the mRNA expression of ANP and BNP in AC16 cells. n = 4. Results were expressed as mean ± SD. SFI, Shenfu Injection.

No significant cell viability was observed at 80 and 100 μg/mL. However, the cell viability was markedly decreased at 120 μg/mL. Consequently, 100 μg/mL of SFI was utilized for subsequent HL-1 cell experiments. Similarly, in AC16 cells (Figure 1B), compared with the control group, there was no significant difference in the activity of AC16 cells at SFI concentrations up to 150 μg/mL. When SFI was 200 μg/mL, the cell viability was significantly decreased, so 150 μg/mL SFI was selected as the concentration of subsequent assays.

Shenfu Injection Attenuates Cardiomyocyte Hypertrophy

In HL-1 cardiomyocytes, the therapeutic potential of SFI was investigated against Ang II-induced hypertrophy through cell size and mRNA expression. As shown in Figure 1C, Ang II treatment promoted cardiomyocyte cell size compared to the control group. Notably, the adverse effects of Ang II can be reversed by SFI. It was also found that ISO treatment promoted cardiomyocyte hypertrophy, and SFI significantly attenuated the adverse effects of ISO (Figure 1D). Quantitative reverse transcription PCR was used to detect the mRNA expression of ANP and BNP. The results showed that SFI significantly reversed the increased levels of ANP and BNP in HL-1 cells induced by Ang II in HL-1 (Figure 1E and F). The results of AC16 cells were consistent with those of HL-1 cells. The results of the qRT-PCR analysis demonstrated that SFI significantly suppressed ISO-induced upregulation of ANP and BNP mRNA expression in AC16 (Figure 1G and H).

Shenfu Injection Inhibits Mitochondrial Fission in Cardiomyocyte Hypertrophy

The TEM mitochondrial morphology changes HL-1 and AC16 cells revealed that Ang II and ISO treatment caused marked mitochondrial ultrastructural injury, while SFI treatment relieves this mitochondrial damage (Figure 2A). Moreover, MitoTracker staining showed that SFI significantly suppressed Ang II-induced fragmented mitochondria (Figure 2B). Similarly, it also significantly inhibited ISO-induced fragmented mitochondria (Figure 2C). The mitochondrial fission function of Drp1 in humans is controlled by phosphorylation of Ser616 and Ser637 (Ser579 and Ser600 in mice).[28] Western blot analysis demonstrated that pathological stimulation significantly increased Drp1 phosphorylation at Ser616 (Ser579) while decreasing phosphorylation at Ser637 (Ser600) in both Ang II- and ISO-treated groups; the expression of p-Drp1 Ser616 (Ser579) was significantly reduced, whereas the expression of p-Drp1 Ser637 (Ser600) was significantly increased (Figure 2D-I).

Network Pharmacology-Based Analysis

Network pharmacology analysis was performed using Cytoscape 3.7.2 to construct the "herb-active component-target" network (Figure 3), comprising 2 medicinal materials, 27 bioactive compounds, and 78 potential targets.

The highest-scoring module in the PPI network was identified. MCC analysis prioritized 10 hub genes: ESR1, NR3C1,

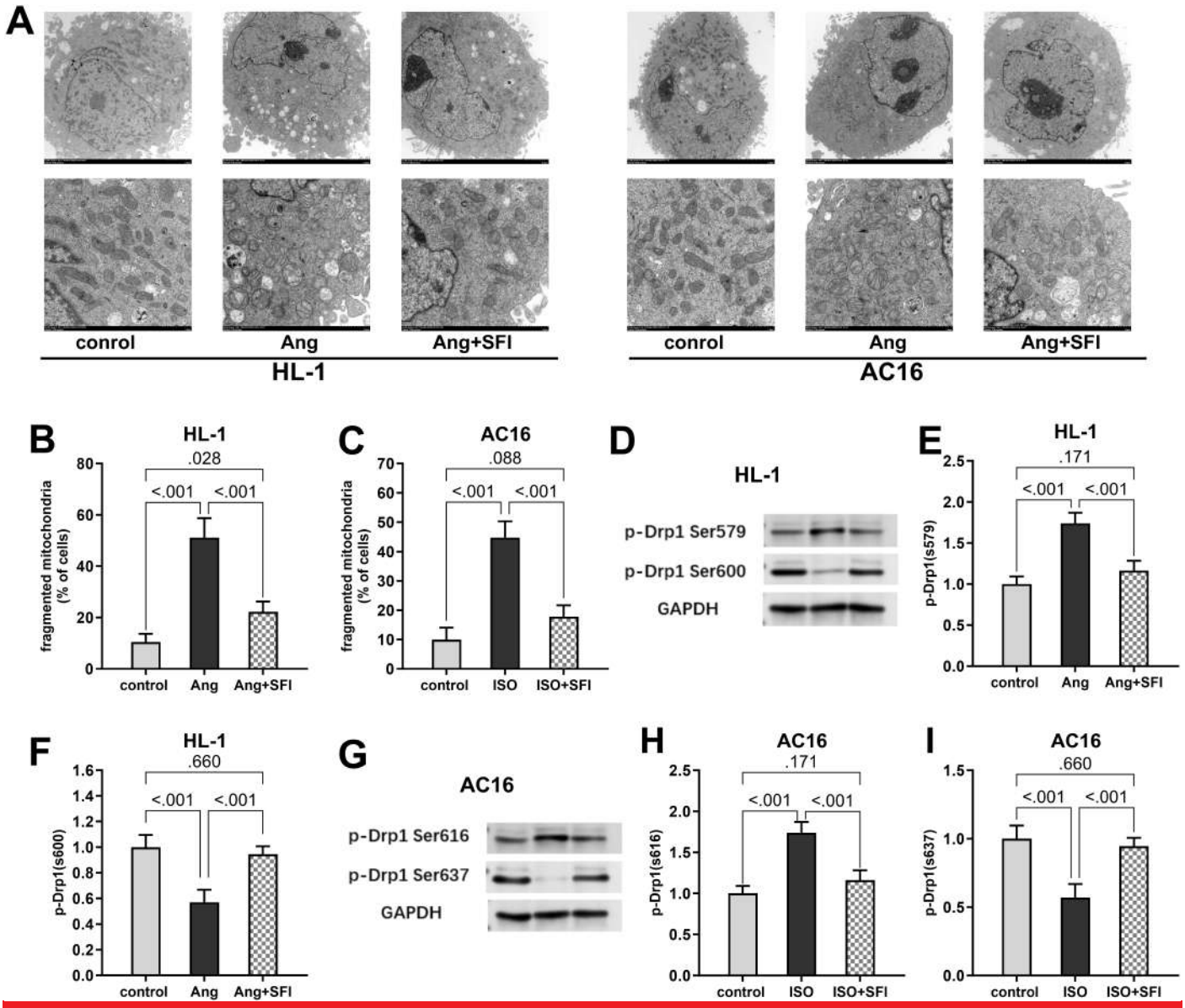

Figure 2. SFI attenuated the mitochondrial fission triggered by Ang II in HL-1 cells and that induced by ISO in AC16 cells. A, Representative electron photomicrographs of mitochondria from HL-1 and AC16 cells. B, The ratio of fragmented mitochondria in HL-1 cells was calculated by Mito Tracker Red staining. C, The ratio of fragmented mitochondria in AC16 cells was calculated by Mito Tracker Red staining. D, E, F, Representative images and chart plots of the phosphorylation expression of Drp1 in HL-1 cells were assessed by western blotting. G, H, I Representative images and chart plots of the phosphorylation expression of Drp1 in AC16 cells were assessed by western blotting. n = 4. The data are expressed as the mean ± SD; SFI, Shenfu Injection.

SLC6A4, MAOA, PTGS2, CASP3, SLC6A3, MAOB, DRD2, and TH (Figure 4A). KEGG pathway enrichment analysis revealed SFI targets were significantly enriched in the cAMP pathway (Supplementary Figure 1A). As illustrated in Supplementary Figure 1B, the cAMP pathway has been shown to regulate a variety of genes, including adenylate cyclase (AC), protein kinase A (PKA), G protein-coupled receptor (GPCR), phosphodiesterase (PDE), stress-activated protein kinase (JNK), and others. These factors have been demonstrated to be involved in a multitude of responses through signaling. Venn analysis of top-ranked targets and cAMP pathway components identified DRD2 as the key convergent node (Figure 4B).

Shenfu Injection Inhibits Cardiomyocyte Hypertrophy by Targeting DRD2 and PKA

As shown in Figure 5, DRD2 and PKA subunit mRNA levels were quantified using qRT-PCR. Compared with the control group, pathological stimulation with Ang II and ISO significantly upregulated DRD2 expression while downregulating PKA levels. Notably, SFI co-treatment reversed these pathological alterations, significantly reducing DRD2 expression, with concomitant PKA upregulation.

DISCUSSION

The investigation is to explore the mechanism of SFI in the treatment of cardiomyocyte hypertrophy through network

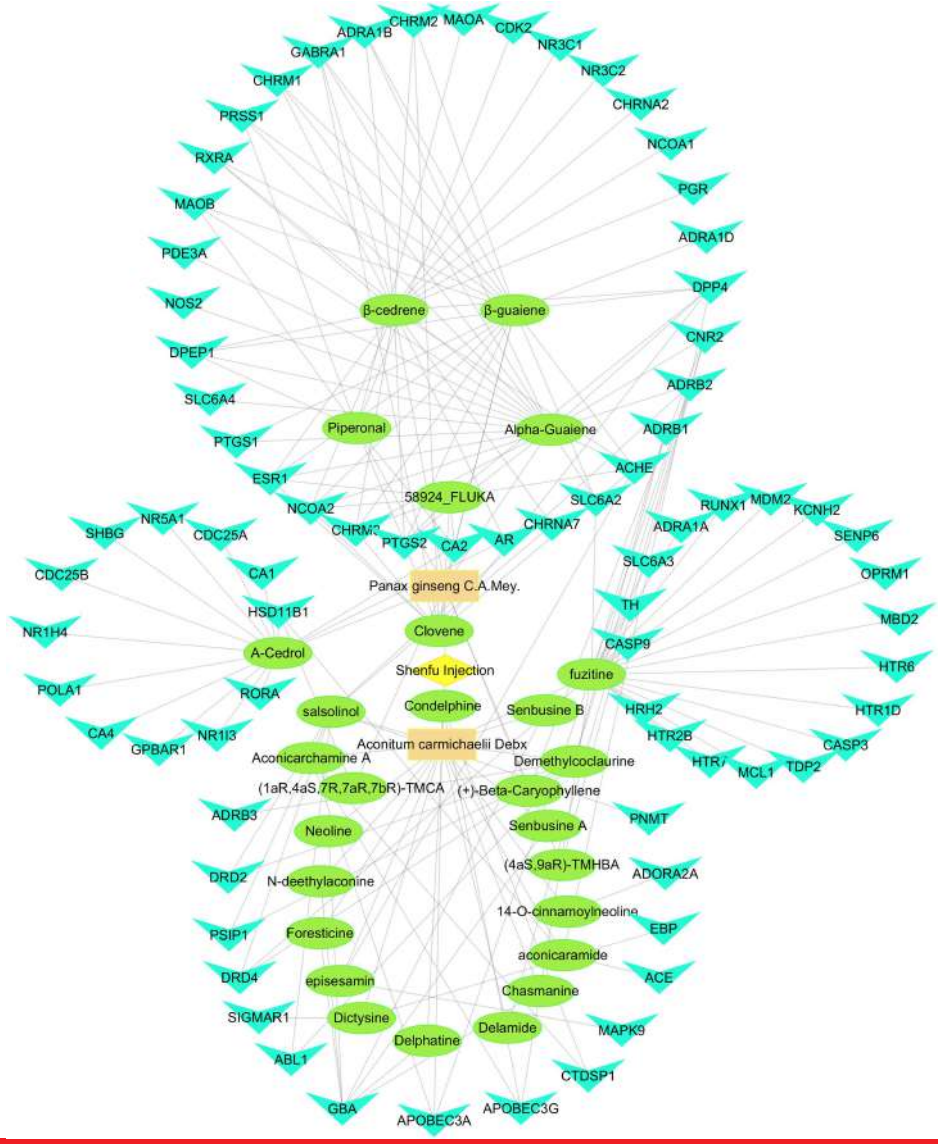

Figure 3. Visualization of drug-compound-target interaction network. The yellow rhombus is the Shenfu Injection, the orange rectangle is the herbs Shenfu Injection, the green ellipses are active compounds, the blue arrows are the target genes, and the line represents the interaction between each item.

pharmacology combined with cell experiments, uncovering the close relationship between SFI and DRD2-mediated mitochondrial fission. These findings establish an innovative conceptual framework for developing targeted therapies for cardiomyocyte hypertrophy.

The experimental data conclusively established that SFI substantially attenuated Ang II/ISO-induced hypertrophic responses in both murine HL-1 and human AC16 cardiomyocytes, as evidenced by attenuated cell size and downregulation of hypertrophy-associated markers ANP and BNP. These findings align with existing pharmacological evidence,[29,30] confirming that SFI has a stable anti-myocardial hypertrophy effect in different sources of cardiomyocytes and different induction factors.

Dysregulated mitochondrial dynamics, particularly excessive fission, constitute a pivotal mechanism in pathological cardiac hypertrophy. In the context of physiological conditions, mitochondria maintain a dynamic equilibrium between fission and fusion processes to preserve cellular bioenergetic homeostasis.[31] The data demonstrate that the Ang II/ISO challenge markedly exacerbated mitochondrial fragmentation, concomitant with augmented Drp1 phosphorylation at Ser616 and diminished Ser637 phosphorylation. As demonstrated in previous studies, Ang II and ISO have been shown to induce Drp1-mediated mitochondrial fission in cardiomyocytes, consistent with the results of the present study.[26,32] The intervention of SFI can make the phosphorylation of mitochondrial fission-related proteins (Drp1) tend to be normal, and the mitochondrial fragmentation decreases. This indicates that SFI may mitigate pathological hypertrophy progression via modulation of mitochondrial fission specifically through regulation of Drp1 phosphorylation states.

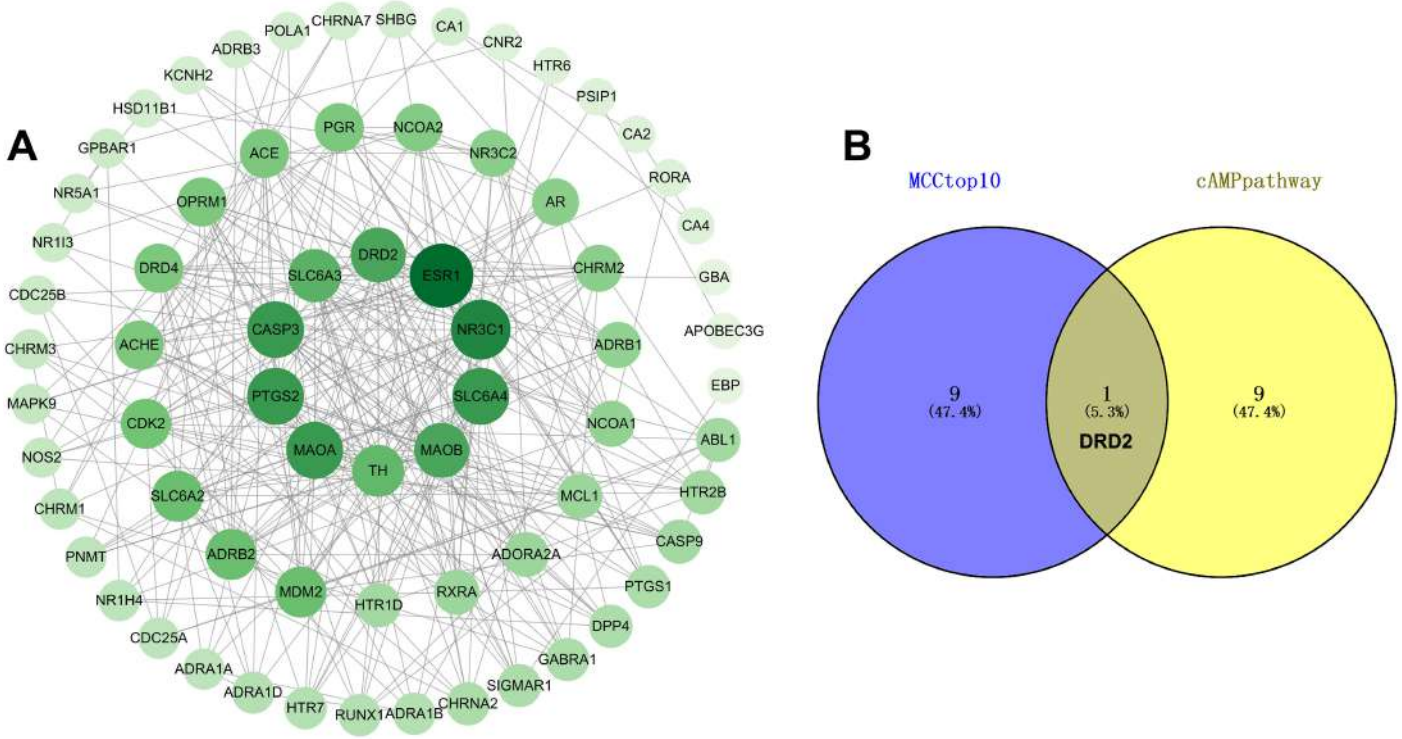

Figure 4. Hub targets for SFI in cardiomyocyte hypertrophy treatment. A, PPI network of targets of SFI in the treatment of cardiomyocyte hypertrophy. Circles represent proteins, and the color (from deep to shallow) indicates the degree of nodes. The larger the node, the higher the corresponding target degree and the more connections with other nodes. B, Venn diagram of the top 10 targets calculated by the MCC method and genes in the cAMP pathway. SFI, Shenfu Injection; PPI, protein-protein interaction.

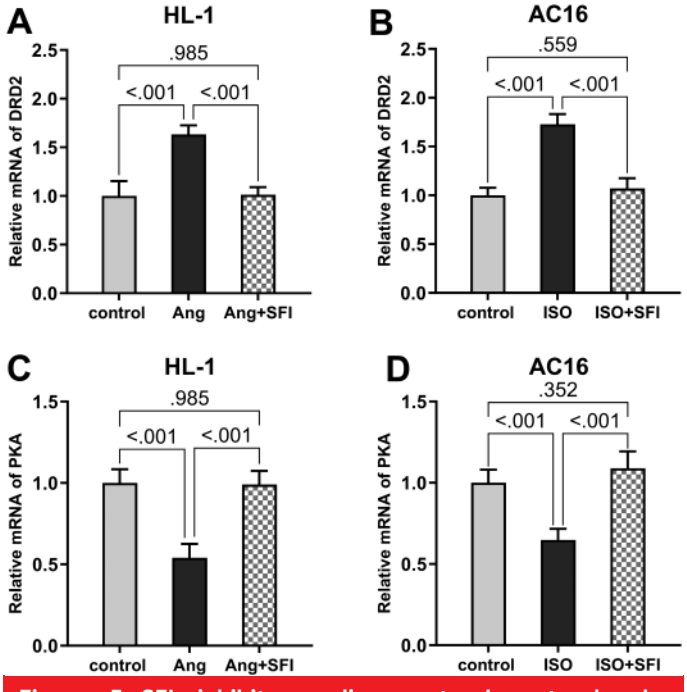

Figure 5. SFI inhibits cardiomyocyte hypertrophy by targeting DRD2 and PKA. A, B, the mRNA expression level of DRD2 in HL-1 cells. C, D, the mRNA expression level of PKA in HL-1 and AC16 cells. n = 4. Data expressed as individual values with mean ± SD. SFI, Shenfu Injection.

As a central executor of mitochondrial fission, Drp1 undergoes spatiotemporal regulation through multiple post-translational modifications, with phosphorylation serving as the predominant regulatory mechanism.[33] The phosphorylation of Drp1 is regulated by a variety of signaling pathways: 1. PKA-mediated Ser637 phosphorylation promotes mitochondrial fusion; 2. CaMKII/ERK1/2-mediated Ser616 phosphorylation drives fission; 3. CDK5-dependent Ser579 modification coordinates fission-autophagy coupling. Phosphorylation at different sites has been shown to have different effects on mitochondrial fission.[34] For example, Ser637 phosphorylation has been demonstrated to promote mitochondrial fusion, while Ser616 phosphorylation has been shown to drive fission[35,36] In addition, Ser579 modification has been found to orchestrate fission-autophagy coupling.[37]

The integrative network pharmacology approach identified DRD2 as the top-ranked node in SFI's anti-hypertrophic target network. Increase of DRD2 signaling has been reported to inhibit choroidal neovascularization, independently of nigrostriatal pathway lesion.[38] DRD2 antagonists can induce unfolded protein response signaling and suppress the GLI1/OCT4/Nanog axis to induce autophagy-mediated apoptosis and stemness inhibition in renal cell carcinoma cells.[39] The systemic implications of DRD2 targeting extend beyond its central nervous system effects, influencing metabolic, endocrine, and cardiovascular pathways.[40] Off-target effects may arise from structural similarities with other

dopamine receptors (e.g., DRD3) and cross-reactivity with adrenergic receptors due to conserved transmembrane domains. Recent studies have demonstrated that DRD2 can inhibit the development of Ang II-induced hypertrophy in rat cardiomyocytes.[41] DRD2 is an inhibitory G protein (G_i)-coupled receptor. When dopamine binds to DRD2, it is activated, which in turn activates G_i. The α subunit (Giα) of G_i then binds to GDP, thereby inhibiting adenylyl cyclase (AC) activity. The AC is responsible for catalyzing the formation of cAMP from ATP, and thus the inhibition of AC activity leads to a decrease in intracellular cAMP levels. Since PKA activation is highly dependent on cAMP, a decrease in cAMP levels leads to inhibition of PKA activity.[42] The studies demonstrated that SFI treatment resulted in decreased DRD2 expression and increased PKA levels. In addition, SFI treatment also attenuated Ser616 phosphorylation levels, increased Ser637 phosphorylation, and inhibited mitochondrial fission. These findings provide compelling evidence that DRD2 plays a key role in the mechanism by which SFI regulates cardiomyocyte hypertrophy. The investigation revealed that SFI regulates mitochondrial fission through DRD2, thereby reducing myocardial hypertrophy. Through integrative systems pharmacology and experimental validation, SFI's multi-target therapeutic strategy was comprehensively delineated against myocardial hypertrophy. While these findings advance the understanding, several limitations warrant consideration: (1) MitoTracker staining alone was used to indicate mitochondrial fragmentation, which can also result from other processes, such as apoptosis, and is not specific to fission. (2) The upstream regulators and downstream effectors of DRD2 signaling require comprehensive mapping; (3) The pharmacodynamics of SFI components targeting DRD2 remain to be characterized. (4) The findings are derived exclusively from in vitro experiments using cultured cardiomyocytes. While this approach provides a controlled system to elucidate the fundamental molecular mechanisms, it cannot fully recapitulate the complex pathophysiology of cardiac hypertrophy in vivo. Therefore, the translational relevance of the findings requires further validation in animal models and, ultimately, clinical studies.

CONCLUSION AND RECOMMENDATIONS

Collectively, the findings revealed that SFI may inhibit Drp1-mediated mitochondrial fission, thereby improving cardiomyocyte hypertrophy, via regulation of the expression of DRD2 and PKA. This mechanistic elucidation provides both a novel therapeutic strategy targeting mitochondrial dynamics and a pharmacological basis for the precision management of cardiac hypertrophy.

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Declaration of Interests: The authors have no conflicts of interest to declare.

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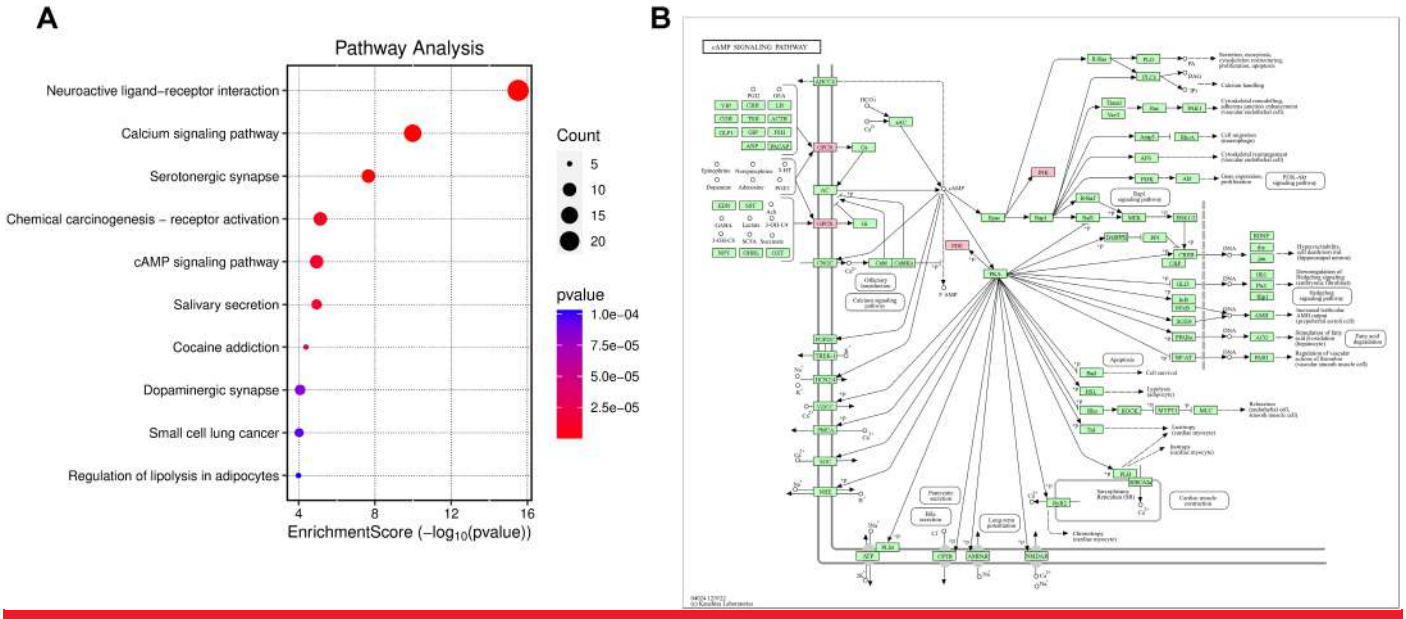

Supplementary Figure 1. KEGG pathway enrichment results and mechanism of action of the cAMP pathway. A, visualization of KEGG pathway enrichment. B, the action of the core target on the cAMP signaling pathway in cardiomyocyte hypertrophy. Pink rectangles represent the key targets.