

Original Research

Growth Differentiation Factor 15 Inhibits Cardiac Fibrosis, Oxidative Stress, Inflammation, and Apoptosis in a Rat Model of Heart Failure with Preserved Ejection Fraction

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Abstract

Background: Heart failure with preserved ejection fraction (HFpEF) is a systemic syndrome primarily associated with fibrosis, oxidative stress, inflammation, and cellular apoptosis. Growth differentiation factor 15 (GDF15), a biomarker commonly used in clinical studies, exhibits protective effects on the myocardium. Therefore, the focus of the present study is to determine the mechanism by which GDF15 protects cardiac function in HFpEF. **Methods:** We conducted functional enrichment analysis and protein-protein interaction network analysis on genes highly expressed in HFpEF but lowly expressed in normal samples. We established an HFpEF rat model by feeding the rats with a high-fat diet and administering N-omega-nitro-L-arginine-methyl ester (L-NAME) in their drinking water and silenced GDF15 by tail vein injection of lentivirus (L3110). After 12 weeks of feeding, echocardiographic examinations were performed. Following euthanasia of the rats, blood and heart tissue samples were collected. Heart tissue sections were stained using Masson's trichrome and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining methods. Western blot (WB) analysis was employed to determine the concentrations of relevant proteins. **Results:** The echocardiographic results showed that compared with the HFpEF + MOCK group, the HFpEF+silencing GDF15 (siGDF15) group exhibited more severe cardiac dysfunction, with significant decreases in ejection fraction ($p < 0.05$) and E/A ratio ($p < 0.001$). WB results demonstrated that, compared with the HFpEF + MOCK group, the HFpEF+siGDF15 group exhibited increased expression of cardiac fibrosis-associated proteins, including collagen I ($p < 0.01$), collagen III ($p < 0.01$), and α -smooth muscle actin (α -SMA) ($p < 0.01$). Additionally, oxidative stress-associated biomarkers such as myeloperoxidase (MPO) ($p < 0.01$) and oxidized low-density lipoprotein (ox-LDL) ($p < 0.01$), inflammation-associated biomarkers, including interleukin-1 beta (IL-1 β) ($p < 0.01$), interleukin-6 (IL-6) ($p < 0.01$), interleukin-8 (IL-8) ($p < 0.01$), and tumor necrosis factor α (TNF α) ($p < 0.01$), and apoptosis-associated biomarkers like cleaved caspase-3 ($p < 0.01$) and BCL2-associated X (BAX) ($p < 0.01$) were also upregulated in HFpEF+siGDF15 group. **Conclusions:** Our research indicates that GDF15 preserves cardiac function by inhibiting myocardial fibrosis, reducing myocardial cell oxidative stress, alleviating cardiac inflammation, and suppressing myocardial cell apoptosis.

Keywords: growth differentiation factor 15; heart failure with preserved ejection fraction; cardiac fibrosis; oxidative stress; inflammation; apoptosis

1. Introduction

Heart failure (HF) is a significant public health challenge worldwide. HF with preserved ejection fraction (HFpEF) constitutes more than half of all HF instances, whose prevalence and mortality are continuously increasing along with the aging population and an escalating comorbidity burden [1,2]. HFpEF is a systemic syndrome primarily associated with mechanisms such as fibrosis, oxidative stress, inflammation, and cellular apoptosis [3,4]. Several studies have found that inhibiting myocardial fibrosis in HFpEF can improve cardiac function, accompanied by alleviation of oxidative stress, inflammation, and cellular apoptosis [5–7]. Currently, due to the complexity of pathophysiological

mechanism and very limited therapeutical options of HFpEF, it presents a major challenge for clinical practice.

Growth differentiation factor 15 (GDF15) is a peptide hormone that is a member of the transforming growth factor beta (TGF β) superfamily [8,9]. It is known to be upregulated under cellular stress conditions, such as tissue injury and inflammation [8,9]. Researches has indicated that GDF15 serves a protective function in the myocardium [9]. Specifically, in models of ischemia/reperfusion myocardial cell injury [10,11], diabetic cardiomyopathy [12], and severe heart failure [13], GDF15 has been found to be upregulated and exert cardioprotective effects. Clinical studies have found that GDF15 is a unique indicator linked directly



to both overall mortality and nonfatal events in individuals with chronic heart failure, outperforming N-terminal pro-B-type natriuretic peptide (NT-proBNP) as a biomarker [14–17]. However, the potential beneficial effects of GDF15 on HFpEF remain poorly understood. Therefore, we hypothesized that GDF15 can protect cardiac function in HFpEF.

Our research demonstrates that GDF15 is upregulated in HFpEF, and it protects cardiac function by inhibiting myocardial fibrosis, reducing myocardial cell oxidative stress, alleviating cardiac inflammation, and suppressing myocardial cell apoptosis.

2. Materials and Methods

2.1 Network Module Genes of HFpEF

The dataset GSE126062, which relates to HFpEF, was obtained from the Gene Expression Omnibus (GEO) database available at <http://www.ncbi.nlm.nih.gov/geo/> and was generated using the GPL11154 platform. This dataset comprises 3 HFpEF tissue samples and 3 normal tissue samples, utilized for pinpointing differentially expressed genes (DEGs) associated with HFpEF. We utilized the R package “limma” (version R4.2.0) to conduct probe summarization and background correction on the gene expression data in GSE126062. To adjust the initial *p*-values, we applied the Benjamini-Hochberg method, and calculated the fold change (FC) to assess the significance of differences in gene expression. The false discovery rate (FDR) served as the criterion for determining significance. DEGs were defined as those with a *p*-value < 0.05 and FC > 1.5. A volcano plot was created to visually represent the DEGs. We identified a total of 3625 DEGs from the gene expression data of GSE126062 related to HFpEF.

By conducting functional enrichment analysis and building and examining a protein-protein interaction (PPI) network, we identified 5 hub genes that exhibited elevated expression in HFpEF samples compared to normal samples, which showed lower expression levels of these genes. To further explore diseases linked to these hub genes, we entered them into the Comparative Toxicogenomics Database (CTD) database and found that all 5 genes are related to HF, cardiac diseases, coronary artery disease, and inflammation.

2.2 Animals

The Animal Ethics Committee of Beijing Hospital granted approval for this study (No. 00001857). A model of HFpEF was established in eight-week-old Dextran Sulfate Sodium (DSS) rats [SPF Biotechnology Co., Ltd., Beijing, China] by administering a high-fat diet and N-omega-nitro-L-arginine-methyl ester (L-NAME, N5751-1G, Shanghai North Connaught bio technology Co., Ltd, Shanghai, China)-infused drinking water. A total of 40 rats were utilized for the creation of this HFpEF model. Following a week of adaptive feeding, the rats were randomly assigned into four groups, and the experiments

were carried out for a period of 12 weeks: (1) blank control group (n = 10), (2) HFpEF group (n = 10): a high fat diet (HFD, 60% calories from lard) + L-NAME (0.5 g/L in drinking water), (3) HFpEF + silencing GDF15 group (HFpEF+siGDF15 group, n = 10): HFD (60% calories from lard) + L-NAME (0.5 g/L in drinking water), received lentivirus tail vein injection (2×10^8 TU/mL; siRNA/miRNA Small Nucleic Acid Transfection Reagent; CAT: L3110; Solarbio, Beijing, China), and (4) HFpEF + MOCK group (n = 10): HFD (60% calories from lard) + L-NAME (0.5 g/L in drinking water), received empty viral vector plasmid tail vein infection. The sequences of siGDF15 and MOCK are as follows: GDF15 (GUGUGU-CACUGCAGACUUAUG UAAGUCUGCAGUGA-CACACCA), MOCK (5′–3′) UUCUCCGAACGUGU-CACGUU CGUGACACGUUCGGAGAACA (Suzhou GenePharma Co., Ltd., Suzhou, China). At the conclusion of the experiment, the rats were euthanized through continuous inhalation of 3%–4% isoflurane for 5–10 minutes.

2.3 Masson's Trichrome Staining of Heart Tissue

After euthanasia of the rats, their heart samples were isolated and immersed in 10% phosphate-buffered formalin (PBD999, Scytex, Shanghai, China) for a duration of 24 hours for fixation. Following this, the samples were embedded in paraffin and sectioned into slices of 4–5 micrometers in thickness. The histologic sections of the tissues underwent staining procedures using Masson's trichrome stains (G1006-1 to G1006-6, Servicebio, Wuhan, China). Imaging of these sections was conducted using the NanoZoomer Digital Pathology RS system (E800, Nikon, Tokyo, Japan). Computerized planimetry was employed to quantify the mean cross-sectional area of the cardiomyocytes and to assess the degree of fibrosis present.

2.4 TUNEL Staining for Apoptosis

Upon euthanasia, heart tissue samples of rats were excised and fixed in 10% phosphate-buffered formalin for 24 hours. Subsequently, the fixed samples were embedded in paraffin and sectioned into slices with a thickness of 4–5 μ m. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining was performed using the Tetramethylrhodamine (red) TUNEL Cell Apoptosis Detection Kit (G1502-100T, Servicebio, Wuhan, China) following the manufacturer's instructions. In this procedure, apoptotic nuclei were labeled with red fluorescein, and all cardiomyocyte nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, G1012, Servicebio, Wuhan, China). Visualization of the heart tissue sections was achieved through confocal microscopy (LSM700, Zeiss, Jena, Germany). The apoptosis rate was determined by calculating the ratio of TUNEL-positive nuclei to the total number of DAPI-stained nuclei.

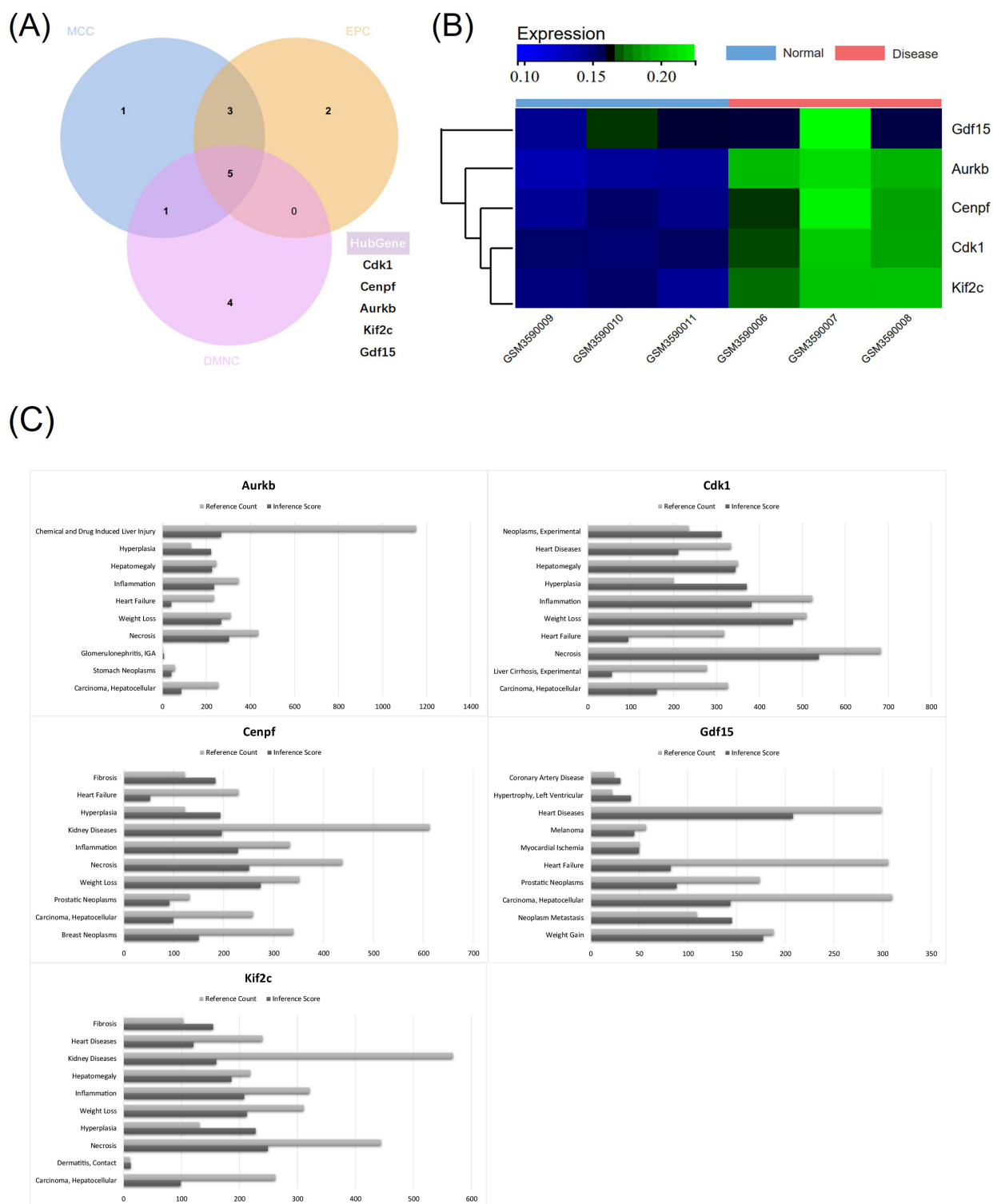


Fig. 1. Network module genes of heart failure with preserved ejection fraction (HFpEF). (A) Identify hub genes. (B) The hub genes were all highly expressed in HFpEF samples and exhibited low expression in normal samples. (C) Reference count and inference score of hub genes in different diseases.

2.5 Western Blotting (WB) Analysis

Proteins were extracted from rat heart tissue, and the total protein concentration was measured using Bicin-

chonic Acid (BCA, G2026-200T, Servicebio, Wuhan, China). Following protein denaturation, gel formation, sample application, electrophoresis, gel excision, mem-

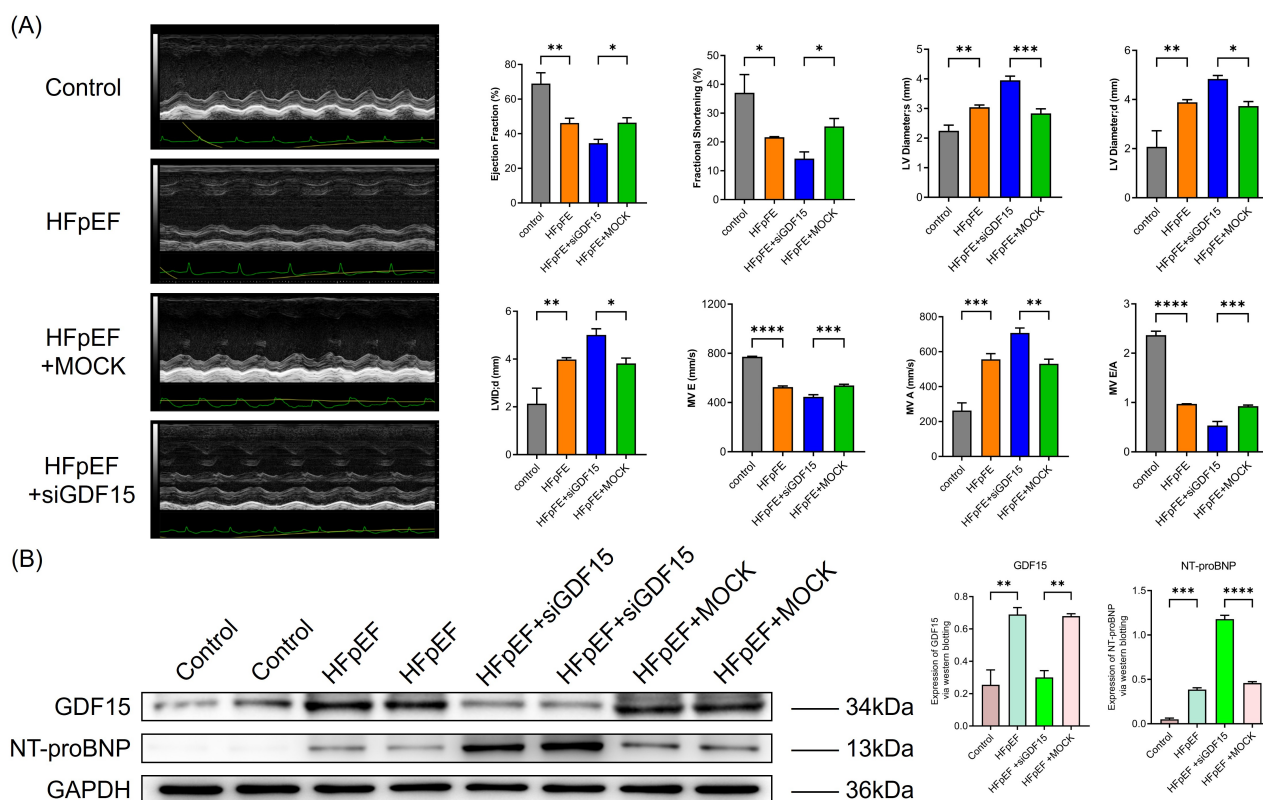


Fig. 2. Growth differentiation factor 15 (GDF15) had cardioprotective effects in HFpEF. (A) Silencing GDF15 further exacerbates cardiac structural and functional damage in HFpEF. Typical echocardiogram images are shown in the figure. Quantified of ejection fraction, fraction shortening, left ventricular (LV) dimension systole, LV dimension diastole, LV internal dimension diastole (LVIDd), mitral orifice peak E blood flow velocity (MV E), mitral orifice peak A blood flow velocity (MV A) and MV E/A are shown in the bar graph. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. (B) Expression of cardiac remodeling-associated biomarkers, including GDF15 and N-terminal pro-Btype natriuretic peptide (NT-proBNP). The original western blot (WB) images are organized in **Supplementary Western Blot Raw Image**. Relative protein expression was quantified. $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. siGDF15, silencing GDF15; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MOCK refers to HFpEF rats receiving empty viral vector plasmid tail vein infection.

brane transfer, blocking, and antibody incubation, the membrane was visualized and imaged. The primary antibodies utilized included rabbit anti-GDF15 antibody (1:2000; 27455-1-AP; Proteintech; Wuhan, China), mouse anti-NT-proBNP antibody (1:1000; ab13115; abcam; Cambridge, UK), rabbit anti-Collagen I antibody (1:1000; 14695-1-AP; Proteintech; Wuhan, China), rabbit anti-Collagen III antibody (1:300; 22734-1-AP; Proteintech; Wuhan, China), rabbit anti- α -smooth muscle actin (α -SMA) antibody (1:1000; 14395-1-AP; Proteintech; Wuhan, China), rabbit anti-Myeloperoxidase (MPO) antibody (1:1000; 22225-1-AP; Proteintech; Wuhan, China), rabbit anti-oxidized low-density lipoprotein (ox-LDL) antibody (1:500; Ys-1698R; YaJi Biotechnology Co., Ltd; Shanghai, China), rabbit anti-Interleukin-8 (IL8) antibody (1:500; 27095-1-AP; Proteintech; Wuhan, China), rabbit anti-Interleukin-6 (IL6) antibody (1:500; 21865-1-AP; Proteintech; Wuhan, China), rabbit anti-Tumor Necrosis Factor α (TNF α) antibody (1:500; 17590-1-AP; Proteintech; Wuhan, China),

rabbit anti-Interleukin-1 beta (IL-1 β) antibody (1:500; 16806-1-AP; Proteintech; Wuhan, China), rabbit anti-Cleaved Caspase-3 antibody (1:1000; 66470-2-Ig; Proteintech; Wuhan, China), and rabbit anti-BCL2-associated X (Bax) antibody (1:2000; 50599-2-Ig; Proteintech; Wuhan, China). The bands' grayscale values were quantified using ImageJ software (version 1.52; National Institute of Health, Bethesda, MD, USA).

2.6 Echocardiographic Assessment

After a 12-week feeding period, echocardiography was conducted using the Vevo 1100 High-Resolution Imaging System (Visual Sonics Inc.; Toronto, Canada). The animals were anesthetized with isoflurane (RWD Life Science Co., Ltd; Shenzhen, China) at a concentration of 1.5% to 2.0% and promptly placed on a thermostat set to 37 °C to maintain normal body temperature. The ultrasound beam's position and orientation were meticulously adjusted to acquire clear echocardiographic images of the left ventricle.

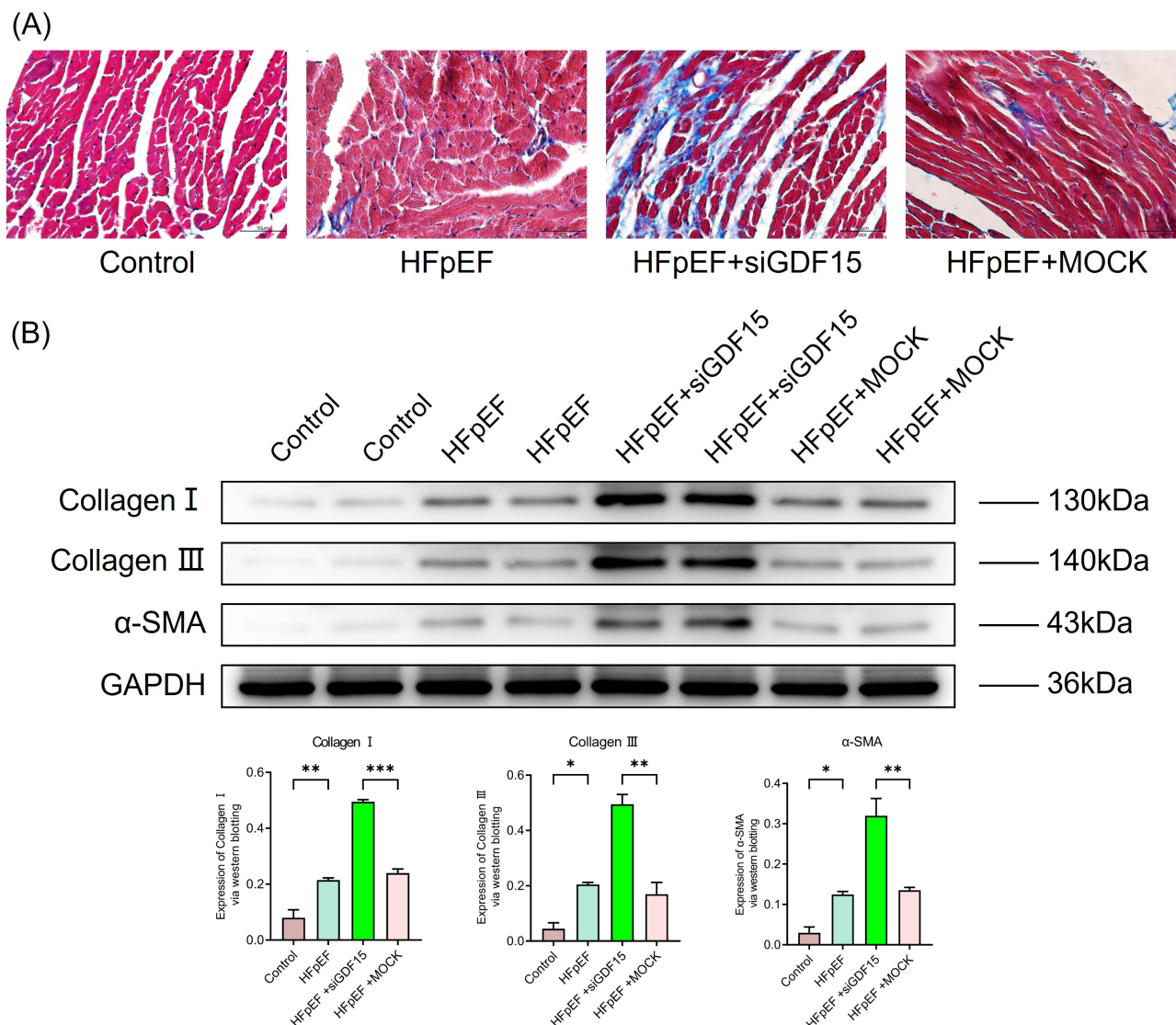


Fig. 3. GDF15 inhibited cardiac fibrosis in HFpEF. (A) Masson's trichrome staining for collagen deposition in the cardiac tissue (scale bar: 50 μ m). (B) Expression of cardiac fibrosis-associated biomarkers, including Collagen I, Collagen III, and α -smooth muscle actin (α -SMA). The original western blot (WB) images are organized in **Supplementary Western Blot Raw Image**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. siGDF15, silencing GDF15; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MOCK refers to HFpEF rats receiving empty viral vector plasmid tail vein infection.

Subsequently, M-mode images were captured for the assessment of left ventricular functional parameters.

2.7 Statistical Analysis

The data are reported as mean $\pm$ SEM. For statistical comparisons among multiple groups, one-way analysis of variance (ANOVA) was employed, followed by Tukey's post-hoc test. A p value < 0.05 was deemed statistically significant for all comparisons. SPSS Statistics 21.0 (IBM Corp., New York, NY, USA) and GraphPad Prism 9.0.0 (GraphPad Software, LLC, Boston, MA, USA) were used to perform all statistical analyses.

3. Results

3.1 GDF-15 is Essential in the Mechanism Underlying HFpEF

In our proteomics analysis of HFpEF rat cardiac tissue, we identified 3625 DEGs, which were primarily enriched in functions related to immune response, inflammatory response, and cell death, as determined by functional enrichment analysis. We analyzed the intersection of hub genes by maximum clique centrality (MCC), edge percolated component (EPC) and density of maximum neighborhood component (DMNC) algorithms to extract more core targets, which revealed significant involvement of cyclin-dependent kinase 1 (CDK1), centromere protein F

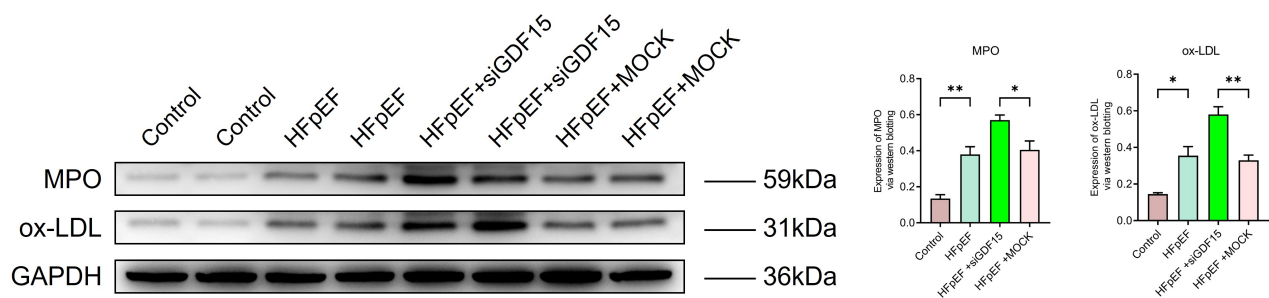


Fig. 4. GDF15 decreased oxidative stress in HFpEF. Expression of oxidative stress-associated biomarkers, Myeloperoxidase (MPO) and oxidized low-density lipoprotein (ox-LDL). The original western blot (WB) images are organized in **Supplementary Western Blot Raw Image**. $*p < 0.05$, $**p < 0.01$. siGDF15, silencing GDF15; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MOCK refers to HFpEF rats receiving empty viral vector plasmid tail vein infection.

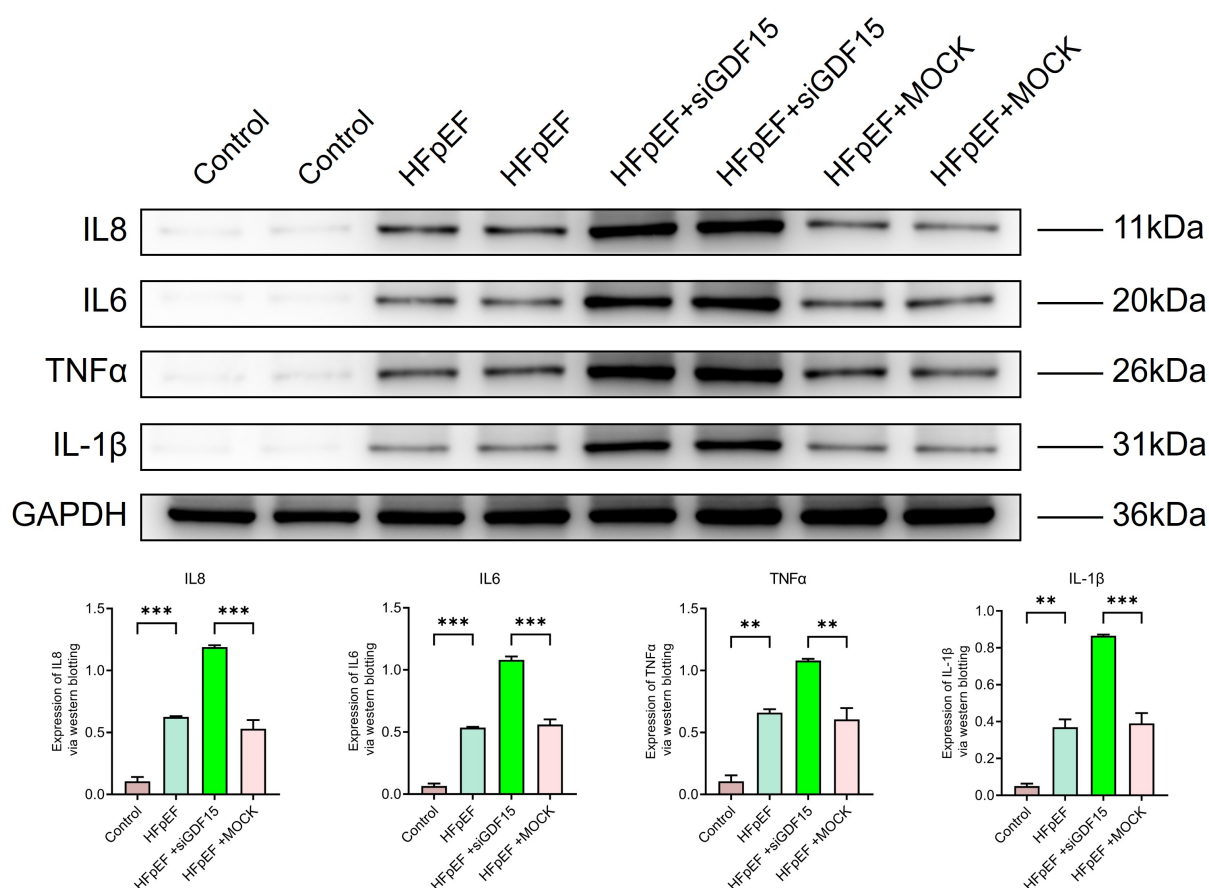


Fig. 5. GDF15 reduces cardiac inflammatory responses in HFpEF. Expression of inflammation-associated biomarkers, Interleukin-1 beta (IL-1β), Interleukin-6 (IL6), Interleukin-8 (IL8) and Tumor Necrosis Factor α (TNFα). The original western blot (WB) images are organized in **Supplementary Western Blot Raw Image**. $**p < 0.01$, $***p < 0.001$. siGDF15, silencing GDF15; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MOCK refers to HFpEF rats receiving empty viral vector plasmid tail vein infection.

(CENFP), aurora kinase B (AURKB), kinesin family member 2C (KIF2C), and notably growth differentiation factor 15 (GDF15) (Fig. 1A). Subsequently, the reference value of the core target for different diseases (including heart failure) was analyzed (Fig. 1B,C). Given its strong association with

cardiac diseases and its significantly elevated expression in the hearts of HFpEF rats, GDF15, which is recognized for its cardioprotective effects, is implicated as a key protective mechanism in the pathogenesis of HFpEF.

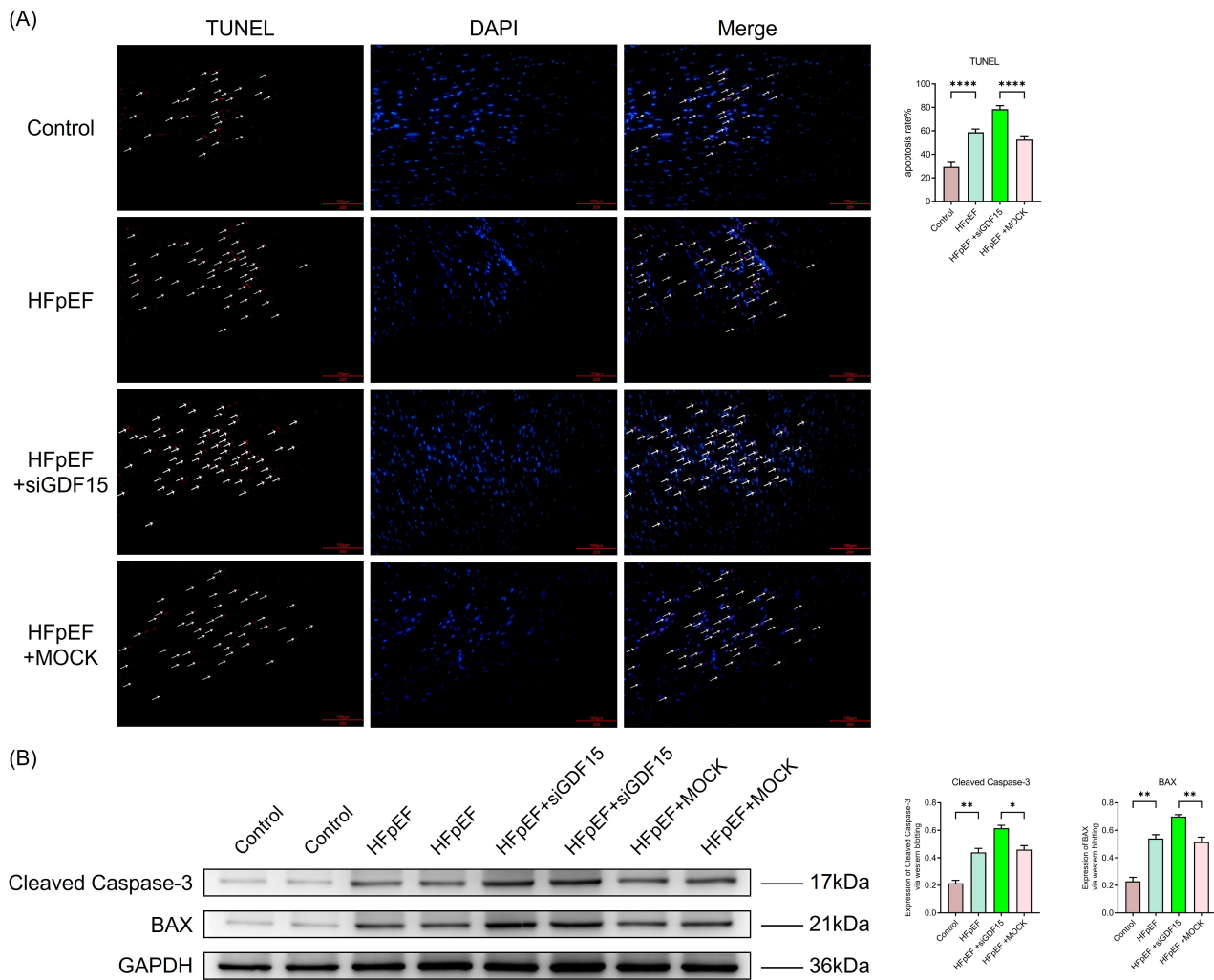


Fig. 6. GDF15 suppresses apoptosis in cardiomyocytes in HFpEF. (A) Representative photographs of TUNEL staining in heart sections (scale bar: 100 μ m). The arrows are used for positive marking. **** $p < 0.0001$. (B) Expression of apoptosis-associated biomarkers, Cleaved Caspase-3 and BAX. The original western blot (WB) images are organized in **Supplementary Western Blot Raw Image**. * $p < 0.05$, ** $p < 0.01$. siGDF15, silencing GDF15; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MOCK refers to HFpEF rats receiving empty viral vector plasmid tail vein infection.

3.2 GDF15 Exhibited Cardioprotective Effects in HFpEF

To verify the cardioprotective effects of GDF15 in HFpEF, a model of HFpEF in rats was established, and the expression level of GDF15 was regulated through lentivirus transfection. Compared with the blank control group, the ejection fraction (EF) of the HFpEF group decreased significantly (Fig. 2A), and plasma NT-proBNP levels were significantly elevated (Fig. 2B), confirming the successful establishment of the HFpEF model. In comparison to the HFpEF + MOCK group, GDF15 expression levels in the cardiac tissue of si-GDF15 rats were significantly reduced, indicating the successful establishment of the si-GDF15 rat model (Fig. 2B). Upon the silencing of GDF15, the EF and E/A ratio in HFpEF rats further decreased (Fig. 2A), and plasma NT-proBNP levels dramatically increased (Fig. 2B). These findings suggest that cardiac GDF15 plays a protec-

tive role in the cardiac function of HFpEF rats, particularly in preserving diastolic function.

3.3 GDF15 Inhibited Cardiac Fibrosis in HFpEF

Due to HFpEF, primarily characterized by diastolic dysfunction, cardiac tissue fibrosis is markedly evident, and GDF15 has been reported to possess antifibrotic effects. Therefore, the extent of cardiac fibrosis in rats from each group was assessed. Consistent with previous literature [18–21], HFpEF rats exhibited significantly increased cardiac fibrosis and elevated expression of fibrosis-related proteins compared with the blank control group (Fig. 3A,B). Rats in the HFpEF + si-GDF15 group demonstrated more pronounced cardiac fibrosis and significantly higher expression of fibrosis-related proteins compared with the HFpEF + MOCK group (Fig. 3A,B). These findings suggest

that the improvement in diastolic function observed in HFpEF rats treated with GDF15 is associated with its antifibrotic effects.

3.4 GDF15 Decreased Oxidative Stress in HFpEF

Severe oxidative stress in cardiac tissue has been reported in association with HFpEF, and GDF15 has been shown to possess antioxidant properties. Therefore, we assessed the levels of oxidative stress markers in the cardiac tissue of rats from each group. Similar to the previous findings [22–24], HFpEF rats exhibited markedly elevated levels of ox-LDL and MPO in their cardiac tissue (Fig. 4). Following the silencing of GDF15 in cardiomyocytes, these oxidative stress markers further increased, indicating that the protective role of GDF15 in HFpEF may be related to its ability to reduce oxidative stress, particularly lipid oxidation.

3.5 GDF15 Reduces Cardiac Inflammatory Responses in HFpEF

In further detection of inflammatory cytokines in cardiac tissues revealed a significant increase in IL-1 β , IL-6, IL-8, and TNF α in HFpEF rats (Fig. 5), which is consistent with previous reports [25]. This finding suggests that the occurrence of HFpEF is associated with abnormal cardiac inflammatory responses. Furthermore, this inflammatory response was exacerbated after silencing GDF15, indicating that the protective effect of GDF15 on HFpEF cardiac function may be attributed to its anti-inflammatory properties, as previously confirmed in liver and kidney tissues [26,27].

3.6 GDF15 Suppresses Apoptosis in Cardiomyocytes in HFpEF

To further explore whether the protective effect of GDF15 on HFpEF is associated with its anti-apoptotic function, TUNEL staining was also performed on cardiac tissues from each group of rats (Fig. 6A). The results indicated a significant increase in TUNEL-positive cells in the heart tissue of HFpEF rats compared to the blank control group. This increase was even more pronounced after GDF15 was silenced, suggesting that GDF15 exerts an anti-apoptotic effect in the context of HFpEF. Further WB analysis revealed that apoptotic proteins such as Cleaved Caspase-3 and Bax in the cardiomyocytes of HFpEF rats were significantly upregulated (Fig. 6B), and this upregulation was exaggerated after GDF15 silencing. These findings confirm that the cardioprotective effect of GDF15 is mediated, at least in part, through its anti-apoptotic action.

4. Discussion

In this study, we demonstrated that GDF15 was upregulated and protected cardiac function in HFpEF rats. We also found that the mechanism by which GDF15 preserves cardiac function in HFpEF is related to inhibiting fibrosis,

reducing oxidative stress, regulating the inflammatory response, and alleviating cardiomyocyte apoptosis.

Myocardial fibrosis, marked by the presence of perivascular and interstitial fibrosis, constitutes a crucial pathological hallmark of HFpEF and underlies LV diastolic dysfunction in patients with this condition [28]. Recent studies have explored diverse anti-fibrotic therapies for HFpEF, encompassing angiotensin receptor-neprilysin inhibitor (ARNI), sodium-dependent glucose transporters 2 (SGLT-2) inhibitors, and statins [6,29,30]. The cardiac extracellular matrix (ECM) is predominantly structured by cardiac collagen, which consists mainly of 85% Collagen I and 11% Collagen III [31]. Myofibroblasts, a distinct subset of activated fibroblasts characterized by α -SMA expression, are responsible for producing and depositing collagen [32]. The TGF- β 1/Smads pathway serves as an essential modulator of collagen synthesis and fibroblast activation [32]. As a stress response protein, GDF15 has been shown to have anti-fibrotic effects in mice models of liver fibrosis, in mice models of acute kidney injury and kidney fibrosis, and in rat models of isoproterenol (ISO)-induced myocardial fibrosis [26,33,34]. Our study found that GDF15 plays an anti-fibrotic role in HFpEF, and this is an important mechanism by which it exerts its cardioprotective effects.

The onset of HFpEF has been confirmed to be linked to metabolic reprogramming and mitochondrial dysfunction, leading to severe oxidative stress [35]. Consistent with previous finding, our study also observed excessive oxidative stress in patients with HFpEF. This oxidative stress is, in part, a consequence of mitochondrial dysfunction, which induces GDF-15 expression through increased reactive oxygen species (ROS) production [36]. GDF15 is believed to possess antioxidative effects in various cell types, including cardiomyocytes, osteoblasts, and tumor cells [37–39]. Further supporting its protective role, research by Gao *et al.* [11] demonstrated that GDF15 can protect cardiac function by inhibiting oxidative stress in a myocardial ischemia-reperfusion injury (MIRI) animal model. In line with these findings, our study revealed that GDF15 significantly reduces inappropriate oxidative stress in cardiomyocytes in HFpEF.

Heart failure has been considered a systemic inflammatory response [40]. Some studies suggest that abnormal inflammatory responses also contribute to the pathogenesis of HFpEF [41,42]. In our study, we observed significant infiltration of inflammatory cells in the cardiac tissues of HFpEF rats compared to normal myocardium, and further WB analysis revealed significant upregulation of various pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, TNF α , and MPO, which were consistent with the findings of Yang *et al.* [43]. As an important stress-response protein, GDF15 has been reported to inhibit inflammatory responses [26,44,45]. Our study results demonstrate that downregulation of GDF15 expression significantly reduces diastolic function in HFpEF hearts, accompanied by signif-

icant upregulation of the aforementioned pro-inflammatory cytokines in myocardial tissue. This indicates that the anti-inflammatory effects of GDF15 may be one of its mechanisms for protecting cardiac function.

Due to the involvement of various forms of programmed cell death in the pathogenesis of HFpEF, including cardiomyocyte apoptosis considered as a major mechanism [46], our study revealed a significant upregulation of cardiomyocyte apoptosis levels in HFpEF compared to healthy hearts. Research on GDF15's role in inhibiting programmed cell death is well-documented. It has been shown to restrain ferroptosis in cardiomyocytes in a myocardial ischemia-reperfusion injury rat model [11], protect airways by inhibiting necroptosis in obese asthmatic mice models [47], and preserve pancreatic β -cell function by suppressing apoptosis in type 1 diabetes mice models [48]. In our study, we found that downregulation of GDF15 led to a significant increase in cardiomyocyte apoptosis levels, indicating that GDF15's protective role in HFpEF cardiac function is associated with inhibiting cardiomyocyte apoptosis.

In summary, our research indicates that GDF15 can protect cardiac function in HFpEF through various mechanisms. However, there are corresponding shortcomings that deserve consideration. First, we have only completed experiments after silencing GDF15, and the next step is to conduct rescue experiments after silencing GDF15, as well as experiments involving GDF15 overexpression. Second, we did not obtain human tissue samples for experimental research.

5. Conclusions

In conclusion, this current study not only confirms the involvement of myocardial fibrosis, oxidative stress, excessive inflammatory responses, and cardiomyocyte apoptosis in the pathogenesis of HFpEF but also demonstrates that GDF15 can protect against HFpEF through multiple mechanisms including inhibition of myocardial fibrosis, oxidative stress, excessive inflammatory responses, and cardiomyocyte apoptosis. This may provide new strategies for the treatment of HFpEF.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

FW, XW and XM designed the research study. YL, LM, CX, and CY performed the research. YL and LM analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Animal Experimentation Committee of Beijing Hospital, China, and conducted in accordance with ethical guidelines (protocol number: No. 00001857).

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL26857>.

References

- [1] Omote K, Verbrugge FH, Borlaug BA. Heart Failure with Preserved Ejection Fraction: Mechanisms and Treatment Strategies. *Annual Review of Medicine*. 2022; 73: 321–337. <https://doi.org/10.1146/annurev-med-042220-022745>.
- [2] Roger VL. Epidemiology of Heart Failure: A Contemporary Perspective. *Circulation Research*. 2021; 128: 1421–1434. <https://doi.org/10.1161/CIRCRESAHA.121.318172>.
- [3] Campbell P, Rutten FH, Lee MM, Hawkins NM, Petrie MC. Heart failure with preserved ejection fraction: everything the clinician needs to know. *Lancet (London, England)*. 2024; 403: 1083–1092. [https://doi.org/10.1016/S0140-6736\(23\)02756-3](https://doi.org/10.1016/S0140-6736(23)02756-3).
- [4] Gharagozloo K, Mehdizadeh M, Heckman G, Rose RA, Howlett J, Howlett SE, *et al.* Heart Failure With Preserved Ejection Fraction in the Elderly Population: Basic Mechanisms and Clinical Considerations. *The Canadian Journal of Cardiology*. 2024; 40: 1424–1444. <https://doi.org/10.1016/j.cjca.2024.04.006>.
- [5] Zhao L, Qin Y, Liu Y, An L, Liu W, Zhang C, *et al.* The total xanthenes extracted from *Gentianaella acuta* alleviates HFpEF by activating the IRE1 α /Xbp1s pathway. *Journal of Cellular and Molecular Medicine*. 2024; 28: e18466. <https://doi.org/10.1111/jcmm.18466>.
- [6] Shi YJ, Yang CG, Qiao WB, Liu YC, Liu SY, Dong GJ. Sacubitril/valsartan attenuates myocardial inflammation, hypertrophy, and fibrosis in rats with heart failure with preserved ejection fraction. *European Journal of Pharmacology*. 2023; 961: 176170. <https://doi.org/10.1016/j.ejphar.2023.176170>.
- [7] Ni XY, Feng XJ, Wang ZH, Zhang Y, Little PJ, Cao Y, *et al.* Empagliflozin and liraglutide ameliorate HFpEF in mice via augmenting the Erbb4 signaling pathway. *Acta Pharmacologica Sinica*. 2024; 45: 1604–1617. <https://doi.org/10.1038/s41401-024-01265-0>.

- [8] Assadi A, Zahabi A, Hart RA. GDF15, an update of the physiological and pathological roles it plays: a review. *Pflugers Archiv: European Journal of Physiology*. 2020; 472: 1535–1546. <https://doi.org/10.1007/s00424-020-02459-1>.
- [9] Emmerson PJ, Duffin KL, Chintharlapalli S, Wu X. GDF15 and Growth Control. *Frontiers in Physiology*. 2018; 9: 1712. <https://doi.org/10.3389/fphys.2018.01712>.
- [10] Dogon G, Rigal E, Potel E, Josse M, Rochette L, Bejot Y, *et al.* Growth/differentiation factor 15 (GDF15) expression in the heart after myocardial infarction and cardioprotective effect of pre-ischemic rGDF15 administration. *Scientific Reports*. 2024; 14: 12949. <https://doi.org/10.1038/s41598-024-63880-5>.
- [11] Gao Q, Li C, Zhong P, Yu Y, Luo Z, Chen H. GDF15 restrains myocardial ischemia-reperfusion injury through inhibiting GPX4 mediated ferroptosis. *Aging*. 2024; 16: 617–626. <https://doi.org/10.18632/aging.205402>.
- [12] Chan JSF, Tabatabaei Dakhili SA, Lorenzana-Carrillo MA, Gopal K, Pulente SM, Greenwell AA, *et al.* Growth differentiation factor 15 alleviates diastolic dysfunction in mice with experimental diabetic cardiomyopathy. *Cell Reports*. 2024; 43: 114573. <https://doi.org/10.1016/j.celrep.2024.114573>.
- [13] Takaoka M, Tadross JA, Al-Hadithi ABAK, Zhao X, Villena-Gutiérrez R, Tromp J, *et al.* GDF15 antagonism limits severe heart failure and prevents cardiac cachexia. *Cardiovascular Research*. 2024; cvae214. <https://doi.org/10.1093/cvr/cvae214>.
- [14] Anand IS, Kempf T, Rector TS, Tapken H, Allhoff T, Jantzen F, *et al.* Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial. *Circulation*. 2010; 122: 1387–1395. <https://doi.org/10.1161/CIRCULATIONAHA.109.928846>.
- [15] Chan MMY, Santhanakrishnan R, Chong JPC, Chen Z, Tai BC, Liew OW, *et al.* Growth differentiation factor 15 in heart failure with preserved vs. reduced ejection fraction. *European Journal of Heart Failure*. 2016; 18: 81–88. <https://doi.org/10.1002/ejhf.431>.
- [16] Sharma A, Stevens SR, Lucas J, Fuzat M, Adams KF, Whellan DJ, *et al.* Utility of Growth Differentiation Factor-15, A Marker of Oxidative Stress and Inflammation, in Chronic Heart Failure: Insights From the HF-ACTION Study. *JACC: Heart Failure*. 2017; 5: 724–734. <https://doi.org/10.1016/j.jchf.2017.07.013>.
- [17] Javaheri A, Ozcan M, Moubarak L, Smoyer KE, Rossulek MI, Revkin JH, *et al.* Association between growth differentiation factor-15 and adverse outcomes among patients with heart failure: A systematic literature review. *Heliyon*. 2024; 10: e35916. <https://doi.org/10.1016/j.heliyon.2024.e35916>.
- [18] Reed AL, Tanaka A, Sorescu D, Liu H, Jeong EM, Sturdy M, *et al.* Diastolic dysfunction is associated with cardiac fibrosis in the senescence-accelerated mouse. *American Journal of Physiology: Heart and Circulatory Physiology*. 2011; 301: H824–H831. <https://doi.org/10.1152/ajpheart.00407.2010>.
- [19] Friebe J, Weithauser A, Witkowski M, Rauch BH, Savvatis K, Dörner A, *et al.* Protease-activated receptor 2 deficiency mediates cardiac fibrosis and diastolic dysfunction. *European Heart Journal*. 2019; 40: 3318–3332. <https://doi.org/10.1093/eurheartj/ehz117>.
- [20] Kanagala P, Arnold JR, Khan JN, Singh A, Gulsin GS, Chan DCS, *et al.* Plasma Tenascin-C: a prognostic biomarker in heart failure with preserved ejection fraction. *Biomarkers: Biochemical Indicators of Exposure, Response, and Susceptibility to Chemicals*. 2020; 25: 556–565. <https://doi.org/10.1080/1354750X.2020.1810319>.
- [21] Yuan S, Wang Z, Yao S, Wang Y, Xie Z, Wang J, *et al.* Knocking out USP7 attenuates cardiac fibrosis and endothelial-to-mesenchymal transition by destabilizing SMAD3 in mice with heart failure with preserved ejection fraction. *Theranostics*. 2024; 14: 5793–5808. <https://doi.org/10.7150/thno.97767>.
- [22] Kumar AA, Kelly DP, Chirinos JA. Mitochondrial Dysfunction in Heart Failure With Preserved Ejection Fraction. *Circulation*. 2019; 139: 1435–1450. <https://doi.org/10.1161/CIRCULATIONAHA.118.036259>.
- [23] Zhou Y, Zhu Y, Zeng J. Research Update on the Pathophysiological Mechanisms of Heart Failure with Preserved Ejection Fraction. *Current Molecular Medicine*. 2023; 23: 54–62. <https://doi.org/10.2174/1566524021666211129111202>.
- [24] Han YL, Yan TT, Li HX, Chen SS, Zhang ZZ, Wang MY, *et al.* Geniposide alleviates heart failure with preserved ejection fraction in mice by regulating cardiac oxidative stress via MMP2/SIRT1/GSK3 β pathway. *Acta Pharmacologica Sinica*. 2024; 45: 2567–2578. <https://doi.org/10.1038/s41401-024-01341-5>.
- [25] Mesquita T, Lin YN, Ibrahim A. Chronic low-grade inflammation in heart failure with preserved ejection fraction. *Aging Cell*. 2021; 20: e13453. <https://doi.org/10.1111/accel.13453>.
- [26] Li X, Huai Q, Zhu C, Zhang X, Xu W, Dai H, *et al.* GDF15 Ameliorates Liver Fibrosis by Metabolic Reprogramming of Macrophages to Acquire Anti-Inflammatory Properties. *Cellular and Molecular Gastroenterology and Hepatology*. 2023; 16: 711–734. <https://doi.org/10.1016/j.jcmgh.2023.07.009>.
- [27] Zhang X, Wang S, Chong N, Chen D, Shu J, Sun J, *et al.* GDF-15 alleviates diabetic nephropathy via inhibiting NEDD4L-mediated IKK/NF- κ B signalling pathways. *International Immunopharmacology*. 2024; 128: 111427. <https://doi.org/10.1016/j.intimp.2023.111427>.
- [28] de Boer RA, De Keulenaer G, Bauersachs J, Brutsaert D, Cleland JG, Diez J, *et al.* Towards better definition, quantification and treatment of fibrosis in heart failure. A scientific roadmap by the Committee of Translational Research of the Heart Failure Association (HFA) of the European Society of Cardiology. *European Journal of Heart Failure*. 2019; 21: 272–285. <https://doi.org/10.1002/ejhf.1406>.
- [29] Zhang X, Wang N, Fu P, An Y, Sun F, Wang C, *et al.* Dapagliflozin Attenuates Heart Failure With Preserved Ejection Fraction Remodeling and Dysfunction by Elevating β -Hydroxybutyrate-activated Citrate Synthase. *Journal of Cardiovascular Pharmacology*. 2023; 82: 375–388. <https://doi.org/10.1097/FJC.0000000000001474>.
- [30] Marunouchi T, Matsumura K, Fuji E, Iwamoto A, Tanonaka K. Simvastatin Attenuates Cardiac Fibrosis under Pathophysiological Conditions of Heart Failure with Preserved Left Ventricular Ejection Fraction by Inhibiting TGF- β Signaling. *Pharmacology*. 2024; 109: 43–51. <https://doi.org/10.1159/000534933>.
- [31] Tallquist MD, Molkentin JD. Redefining the identity of cardiac fibroblasts. *Nature Reviews: Cardiology*. 2017; 14: 484–491. <https://doi.org/10.1038/nrcardio.2017.57>.
- [32] Shi Y, Liu C, Yang C, Qiao W, Liu Y, Liu S, *et al.* A rat model of metabolic syndrome-related heart failure with preserved ejection fraction phenotype: pathological alterations and possible molecular mechanisms. *Frontiers in Cardiovascular Medicine*. 2023; 10: 1208370. <https://doi.org/10.3389/fcvm.2023.1208370>.
- [33] Valiño-Rivas L, Cuarental L, Ceballos MI, Pintor-Chocano A, Perez-Gomez MV, Sanz AB, *et al.* Growth differentiation factor-15 preserves Klotho expression in acute kidney injury and kidney fibrosis. *Kidney International*. 2022; 101: 1200–1215. <https://doi.org/10.1016/j.kint.2022.02.028>.
- [34] Ren Q, Lin P, Wang Q, Zhang B, Feng L. Chronic peripheral ghrelin injection exerts antifibrotic effects by increasing growth differentiation factor 15 in rat hearts with myocardial fibrosis induced by isoproterenol. *Physiological Research*. 2020; 69: 439–450. <https://doi.org/10.33549/physiolres.934183>.
- [35] Bhattarai N, Scott I. In the heart and beyond: Mitochondrial dysfunction in heart failure with preserved ejection fraction (HF-

- pEF). *Current Opinion in Pharmacology*. 2024; 76: 102461. <https://doi.org/10.1016/j.coph.2024.102461>.
- [36] Nyárády BB, Kiss LZ, Bagyura Z, Merkely B, Dósa E, Láng O, *et al.* Growth and differentiation factor-15: A link between inflammaging and cardiovascular disease. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2024; 174: 116475. <https://doi.org/10.1016/j.biopha.2024.116475>.
- [37] Li X, Sun H, Zhang L, Liang H, Zhang B, Yang J, *et al.* GDF15 attenuates sepsis-induced myocardial dysfunction by inhibiting cardiomyocytes ferroptosis via the SOCS1/GPX4 signaling pathway. *European Journal of Pharmacology*. 2024; 982: 176894. <https://doi.org/10.1016/j.ejphar.2024.176894>.
- [38] Kim KM, Jang WG. NXNL1 negatively regulates osteoblast differentiation via GDF15-induced PP2A C α dependent manner in MC3T3-E1 cells. *BioFactors (Oxford, England)*. 2022; 48: 239–248. <https://doi.org/10.1002/biof.1817>.
- [39] Lin H, Luo Y, Gong T, Fang H, Li H, Ye G, *et al.* GDF15 induces chemoresistance to oxaliplatin by forming a reciprocal feedback loop with Nrf2 to maintain redox homeostasis in colorectal cancer. *Cellular Oncology (Dordrecht, Netherlands)*. 2024; 47: 1149–1165. <https://doi.org/10.1007/s13402-024-00918-w>.
- [40] Kosyakovsky LB, de Boer RA, Ho JE. Screening for Heart Failure: Biomarkers to Detect Heightened Risk in the General Population. *Current Heart Failure Reports*. 2024; 21: 591–603. <https://doi.org/10.1007/s11897-024-00686-6>.
- [41] Krittanawong C, Britt WM, Rizwan A, Siddiqui R, Khawaja M, Khan R, *et al.* Clinical Update in Heart Failure with Preserved Ejection Fraction. *Current Heart Failure Reports*. 2024; 21: 461–484. <https://doi.org/10.1007/s11897-024-00679-5>.
- [42] Liu H, Magaye R, Kaye DM, Wang BH. Heart failure with preserved ejection fraction: The role of inflammation. *European Journal of Pharmacology*. 2024; 980: 176858. <https://doi.org/10.1016/j.ejphar.2024.176858>.
- [43] Yang H, Zhu J, Fu H, Shuai W. Dapansutril Ameliorates Atrial Inflammation and Vulnerability to Atrial Fibrillation in HFpEF Rats. *Heart, Lung & Circulation*. 2024; 33: 65–77. <https://doi.org/10.1016/j.hlc.2023.09.017>.
- [44] Dai C, Zhang H, Zheng Z, Li CG, Ma M, Gao H, *et al.* Identification of a distinct cluster of GDF15^{high} macrophages induced by *in vitro* differentiation exhibiting anti-inflammatory activities. *Frontiers in Immunology*. 2024; 15: 1309739. <https://doi.org/10.3389/fimmu.2024.1309739>.
- [45] von Rauchhaupt E, Klaus M, Ribeiro A, Honarpisheh M, Li C, Liu M, *et al.* GDF-15 Suppresses Puromycin Aminonucleoside-Induced Podocyte Injury by Reducing Endoplasmic Reticulum Stress and Glomerular Inflammation. *Cells*. 2024; 13: 637. <https://doi.org/10.3390/cells13070637>.
- [46] Jankowski J, Kozub KO, Kleibert M, Camlet K, Kleibert K, Cudnoch-Jędrzejewska A. The Role of Programmed Types of Cell Death in Pathogenesis of Heart Failure with Preserved Ejection Fraction. *International Journal of Molecular Sciences*. 2024; 25: 9921. <https://doi.org/10.3390/ijms25189921>.
- [47] Li N, He F, Shang Y. Growth differentiation factor 15 protects the airway by inhibiting cell pyroptosis in obese asthmatic mice through the phosphoinositide 3-kinase/AKT pathway. *International Immunopharmacology*. 2023; 119: 110149. <https://doi.org/10.1016/j.intimp.2023.110149>.
- [48] Wang Y, Chen J, Sang T, Chen C, Peng H, Lin X, *et al.* NAG-1/GDF15 protects against streptozotocin-induced type 1 diabetes by inhibiting apoptosis, preserving beta-cell function, and suppressing inflammation in pancreatic islets. *Molecular and Cellular Endocrinology*. 2022; 549: 111643. <https://doi.org/10.1016/j.mce.2022.111643>.