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Research article

Fibroblast growth factor 20 ameliorates cardiac hypertrophy via activation ErbB2

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ABSTRACT

Fibroblast growth factor 20 (FGF20) is a member of the fibroblast growth factor family and involved in embryonic development and cardiac repair. This study aimed to explore the role of FGF20 in cardiac hypertrophy and the underlying molecular mechanisms. FGF20 improved cardiac hypertrophy *in vivo* and *in vitro*. Furthermore, FGF20 increased expression of erythroblastic leukemia viral oncogene homolog 2 (ErbB2), which was negatively correlated with expression of the cardiac hypertrophy markers atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). In addition, FGF20 effectively protected cardiomyocytes against apoptosis and oxidative stress. To further investigate whether protective effect of FGF20 is mediated by ErbB2, neonatal rat cardiomyocytes (NRCMs) were treated with lapatinib, an inhibitor of ErbB2. Lapatinib largely abrogated the anti-hypertrophic effect of FGF20, accompanied by increases in cardiomyocyte apoptosis and oxidative stress. In summary, this study reveals that FGF20 prevents cardiac hypertrophy by inhibiting apoptosis and oxidative stress via activating ErbB2 and may be a promising therapeutic strategy for cardiac hypertrophy.

1. Introduction

Due to their limited proliferative capacity, adult mammalian cardiomyocytes undergo compensatory hypertrophic growth in response to stress [1,2]. However, continued stress leads to pathological cardiac hypertrophy, characterized by cardiomyocyte hypertrophy and cardiac remodeling and dysfunction, which eventually results in heart failure and even death [3]. Cardiac hypertrophy is fundamental to the progression of many cardiovascular diseases, which are the major cause of mortality and morbidity worldwide [4]. The pathogenesis and pathological process of cardiac hypertrophy are multifactorial and complicated, and there are no effective therapeutic approaches [5]. Thus, it is urgent to deeply understand the mechanisms underlying cardiac hypertrophy and further

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explore effective strategies for clinical treatment.

The fibroblast growth factor (FGF) family, which consists of 23 members, functions in the development of the heart during the embryonic stage as well as cardiac homeostasis after birth [6,7]. FGFs play crucial roles in cardiac hypertrophy. For instance, FGF18 and FGF21 protect against cardiac remodeling and dysfunction in a murine model of cardiac hypertrophy [8,9]. FGF20, which was originally discovered in *Xenopus laevis* embryos, shares 62–73 % overall homology with FGF9 and FGF16, and was subsequently regarded as a novel member of the FGF9 subfamily [10,11]. FGF20 has been reported to be highly expressed in the brain, therefore, previous research mainly focused on its biological functions in dopaminergic neuronal injury and neurological disorders including Parkinson's disease [12,13]. In addition, growing evidence indicates that FGF20 is also involved in hair follicle formation, cochlear hair cell differentiation, and inflammatory bowel disease [14–17]. FGF20 is detected in the endocardium at E10.5 and E12.5, and is required for embryonic heart development [18]. Although our previous study revealed that FGF20 plays a crucial role in pressure overload (PO)-induced pathological cardiac hypertrophy [19], its precise effect on cardiac hypertrophy and the underlying molecular mechanism warrant further exploration.

ErbB2 (Her2/EGFR2), a crucial member of the epidermal growth factor receptor (EGFR) family, participates in various biological processes including cellular differentiation, growth, proliferation, programmed cell death, and survival [20]. ErbB2 is widely expressed in various tumor tissues and its overexpression or amplification is closely associated with the poor prognosis of cancer patients [21]. Consequently, ErbB2 is an important target for clinical treatment of cancers such as breast cancer [22]. Interestingly, the function of ErbB2 in heart tissue has garnered increasing scientific attention owing to cardiac dysfunction in cancer patients receiving ErbB2-targeted antibody therapy [23]. It is worth noting that ErbB2 exhibits prominent expression in the embryonic heart, and mice with conditional depletion of ErbB2 develop severe dilated cardiomyopathy and even heart failure after birth, suggesting that ErbB2 is indispensable to prevent cardiac hypertrophy [24]. Geiger et al. also reported that ErbB2 is essential for cardiomyocytes, which have a limited proliferative capacity, to counteract pathological stress [25]. Therefore, ErbB2 has been recognized as a promising therapeutic target for cardiac hypertrophy.

Thus, this study aimed to evaluate the role of FGF20 in cardiac hypertrophy and the underlying molecular mechanisms. Furthermore, it explored whether ErbB2 is involved in the cardioprotective effect of FGF20.

2. Methods

2.1. Animals and procedures

All animal experiments conducted in the study followed the guidelines of the Ethics Committee for Laboratory Animals of Ningbo University and Xiamen Medical College (Approval No.20230424002), and followed national and institutional guidelines (Approval No.12645). C57BL/6 male mice aged 6–8 weeks old were obtained from Beijing Vitalstar Biotechnology Co. Ltd (Beijing, China) and then kept in an environmentally controlled room for 1 week to adapt to the environment before experimentation. Mice were housed in SPF barrier conditions, which included filtered-air enclosures, a stable ambient temperature of 21 °C ± 2 °C, relative humidity maintained at 50 % ± 15 %, and a consistent light-dark cycle of 12 h, with water and food available albidum. To establish an animal model of cardiac hypertrophy, mice were subjected to transverse aortic constriction (TAC) surgery, as previously described [8,19]. To overexpress FGF20 in the myocardium, mice were injected with 1×10^{12} vg AAV9 harboring FGF20 RNA under the control of the cTnT promoter (AAV-FGF20) or a negative control vector (AAV-LacZ) via the tail vein, and subsequently underwent TAC or sham surgery 2 weeks later.

2.2. Histological analysis

At the end of the experiments, the body weight (BW) of mice was measured. After euthanasia, hearts were harvested and then heart weight (HW) was immediately measured. Then, HW/BW, an index of cardiac hypertrophy, was calculated. Heart tissue samples was fixed in 4 % paraformaldehyde for 24 h, and subjected to dehydration in ethanol gradient (70–100 %) and paraffin embedding. For morphological assessments, heart sections (5 μm) were deparaffinized and then stained with hematoxylin and eosin (H&E) according to the manufacturer's instructions. Finally, a Nikon Eclipse Ti-S microscope was used to captured the images of the stained sections.

2.3. Isolation, culture, and treatment of NRCMs

NRCMs were isolated from the hearts of Sprague-Dawley (SD) rats aged 1–3-day-old, following previously established methods [8,26]. Briefly, after surface sterilization of SD rats aged 1–3-day-old with a 75 % ethanol solution, the hearts were dissected and immediately minced into 0.5–1 mm^3 pieces under sterile conditions. Next, the heart pieces were digested in 0.08 % trypsin medium in a 37 °C bath with magnetic agitation for 8 min each time until the pieces disappeared. Then, the digestion solution was collected, filtered with 200 μm nylon, and centrifuged at 3000 rpm for 30 min. For removal of fibroblasts and endothelial cells, the cells were resuspended and incubated in Dulbecco's Modified Eagle's Medium (DMEM)/F12 medium (Gibco, 11330032) supplemented with 10 % fetal bovine serum (FBS, Gibco, 10270-106) and 1 % penicillin/streptomycin (Solarbio, P1400) for 1–3 h in an incubator containing 95 % air and 5 % CO_2 at 37 °C. After incubation, NRCMs were obtained from the non-adherent cell suspension and then seeded onto collagen-coated 6-well plates in fresh DMEM/F12 medium supplemented with 0.1 mM 5-bromodeoxyuridine (BrdU) to suppress fibroblast proliferation.

NRCMs were stimulated with 10 μM isoproterenol (ISO) (Sigma, I5627) for 48 h to establish an *in vitro* model of cardiac

hypertrophy and then with 100 ng/mL recombinant human FGF20. To further investigate whether the protection of FGF20 against cardiac hypertrophy is mediated by ErbB2, NRCMs were pretreated with 1 μM lapatinib (Selleck, GW-572016), an inhibitor of ErbB2 [27], before ISO and FGF20 treatment, according to our previous study [28].

2.4. Western blot analysis

NRCMs were lysed in RIPA buffer (Solarbio, R0010) on ice for 30 min to extract total proteins. After quantification, equal amounts of the protein lysate (30 μg) were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, IPVH00010), followed by which were subsequently blocked with 5 % bovine serum albumin (BSA, Solarbio, A8020) prepared in TBST for 2 h at room temperature (~25 °C). The PVDF membranes were incubated with corresponding primary antibodies overnight at 4 °C and then placed in solution containing horseradish peroxidase (HRP)-goat anti-mouse (TransGen, HS201, 1:10000) or HRP-goat anti-rabbit secondary antibodies (TransGen, HS101, 1:10000) for at least 1 h at room temperature. Finally, the chemical signals of protein bands were detected by a ChemiDoc MP Imaging System using Western Bright ECL HRP Substrate (Advansta, K-12045-D20). The relative protein levels were quantified using ImageJ software.

The primary antibodies were used in this study are listed as follow: anti-ErbB2 (Santa Cruz, sc-7301, 1:1000); anti-ANP (Santa Cruz, sc-515701, 1:1000); anti-Catalase (Santa Cruz, sc-271358, 1:1000); anti-Bax (CST, #2772, 1:2000); anti-Cleaved Caspase-3 (C-CAS-3) (CST, #9661, 1:2000); anti-HO-1 (CST, #43966, 1:2000), anti-Sod2 (Proteintech, 24127-1-AP, 1:1000), Bcl-2 (Proteintech, 68103-1-Ig, 1:1000); anti-BNP (Affinity, DF6902, 1:800) and anti-Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Abcam, ab9485, 1:2000). GAPDH was used as a loading control.

2.5. RT-qPCR analysis

In brief [29], total RNA was isolated from heart tissue and NRCMs using RNA-easy Isolation Reagent (Vazyme, R701-01) and quantified using Nanodrop 3100. Subsequently, 1 μg RNA was reverse-transcribed into cDNA using a Hiscript® III Reverse Transcriptase Kit (Vazyme, R302-01). Quantitative PCR was performed on an ABI7500 Real-Time PCR System using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711-02). Relative expression of target genes was quantified using the $2^{-\Delta\Delta Cq}$ comparative threshold cycle method, with GAPDH as the endogenous normalization control. The primer sequences used for amplification are presented in Table 1.

2.6. Transcriptomic analysis

After isolation and quantification, RNA integrity was assessed using a Bioanalyzer 2100 analyzer and verified by agarose gel electrophoresis. After establishing sequencing libraries, each sample was subjected to 2 × 150 bp paired-end sequencing (PE150) using an Illumina Novaseq 6000 instrument in accordance with manufacturer's instructions. Reads were aligned to *Rat* genome using Hisat2, and calculated with feature Counts. Next, differential gene expression (DEG) was analyzed using the DESeq2 R-package. KEGG pathway and gene ontology (GO) term enrichment analyses of DEGs were conducted using an online tool (https://www.omicstudio.cn/). If the *P*-value was <0.05, the KEGG pathways and GO terms were considered statistically significant.

2.7. Lactate dehydrogenase (LDH) assay

Cardiomyocytes releases LDH into the culture medium upon hypertrophic stimulation [30], therefore, LDH release from NRCMs was detected in this study. NRCMs were cultured in 96-well plates and then underwent the treatment as described above. After treatment, the culture supernatant of NRCMs was gathered, and the amount of LDH released was measured using a LDH assay kit (Beyotime, P0028) and measuring absorbance at 490 nm using a microplate reader, as described by the manufacturer's instructions.

2.8. Immunofluorescence

Briefly, NRCMs were fixed in a 4 % paraformaldehyde solution for 15 min and permeabilized with 0.5 % Triton X-100 (Solarbio, P1080) in PBS for 30 min at room temperature. After blocking with 5 % BSA for 1 h at room temperature, NRCMs were incubated with

Table 1
Details of the primers used in RT-qPCR.

Primer	Forward	Reverse
ANP (Rat)	5′- ATCTGATGGATTTCAACAACC -3′	5′- CTCTGAGACGGGTTGACTTC -3′
BNP (Rat)	5′- GGGCTGTAACGCACTGAAGTT -3′	5′- GTGGATTGTTCTGGAGACTG -3′
ErbB2 (Rat)	5′- GCTGGCTCCGATGTGTTTGATGG-3	5-GTTCTCTGCAGTGGGGGTCCCTTC-3
GAPDH(Rat)	5′- ATCAAGAAGGTGGTGAAGCA -3′	5′- AAGGTGGAAGAATGGGAGTTG -3′
ANP(Mouse)	5′- TGCTTCCTCAGTCTGCTC -3′	5′- CAACACAGATCTGATGGATTTCA -3′
BNP(Mouse)	5′- GGGCTGTAACGCACTGAAGTT -3′	5′- AGTTTGTGCTCCAAGATAAGA -3′
ErbB2(Mouse)	5′-GAGACAGAGCTAAGGAAGCTGA-3′	5′- ACGGGGATTTTCACGTTCTCC -3′
GAPDH(Mouse)	5′- AGGTCGGTGTGAACGGATTTG -3′	5′- TGTAGACCATGTAGTTGAGGTCA -3′

rabbit anti-cardiac troponin I (cTnT, the cardiomyocytes marker) (Abcam, ab196384, 1:200) at 4 °C overnight and then incubated with an Alexa Fluor 647-conjugated anti-rabbit IgG secondary antibody (Abcam, ab150075) for 1 h at room temperature. Finally, nuclei of NRCMs were labeled with DAPI. Images were obtained using a confocal laser scanning microscope, and cardiomyocyte size was measured with ImageJ software.

2.9. Intracellular reactive oxygen species (ROS) measurement

ROS production in NRCMs was detected by Dihydroethidium (DHE) staining. NRCMs were plated on 6-well plates and then underwent the treatment as described above. After removal of the culture medium, 5 μM DHE (Beyotime, S0063) was added to the culture plates and reacted in the dark for 30 min at 37 °C. The intensity of DHE fluorescence was visualized using a fluorescence microscope.

2.10. Statistical analysis

Data were expressed as mean ± standard deviation (SD) from at least 3 independent experiments. The statistical significance of each sample was analyzed with one-way analysis of variance (ANOVA) for multiple groups. If the *P*-value was <0.05, the result was considered statistically significant.

3. Results

3.1. FGF20 protects against cardiac hypertrophy in vivo and in vitro

To investigated the role of FGF20 in cardiac hypertrophy *in vivo*, mice were injected with AAV-FGF20 via the tail vein to overexpress FGF20 in the myocardium and then subjected to TAC surgery to establish an animal model of cardiac hypertrophy. The results demonstrated that TAC significantly induced increases in heart size and the HW/BW ratio, which were significantly inhibited by FGF20 overexpression (Fig. 1A and B). In addition, the mRNA levels of ANP and BNP, the markers of cardiac hypertrophy, were obviously increased in TAC-operated mice, but these increases were reversed by FGF20 overexpression (Fig. 1C). Consistently, FGF20 also inhibited the TAC-induced increases in ANP and BNP protein levels (Fig. 1D and E). These results suggested that FGF20 improves TAC-induced cardiac hypertrophy *in vivo*.

To further evaluate the effect of FGF20 on cardiac hypertrophy *in vitro*, NRCMs were stimulated with ISO to establish an *in vitro* model of cardiac hypertrophy. As expected, immunofluorescence staining of cTnT showed that ISO significantly induced cardiomyocyte hypertrophy, evidenced by increased surface area of NRCMs, which was inhibited by co-treatment with FGF20 (Fig. 2A and B). Consistent with our *in vivo* results, FGF20 treatment also effectively mitigated the ISO-induced upregulation of ANP and BNP at both the mRNA and protein levels (Fig. 2C and D). Moreover, co-treatment with FGF20 substantially decreased the robust LDH release induced by ISO in NRCMs (Fig. 2F). Overall, these results suggested that FGF20 improves ISO-induced cardiac hypertrophy *in vitro*.

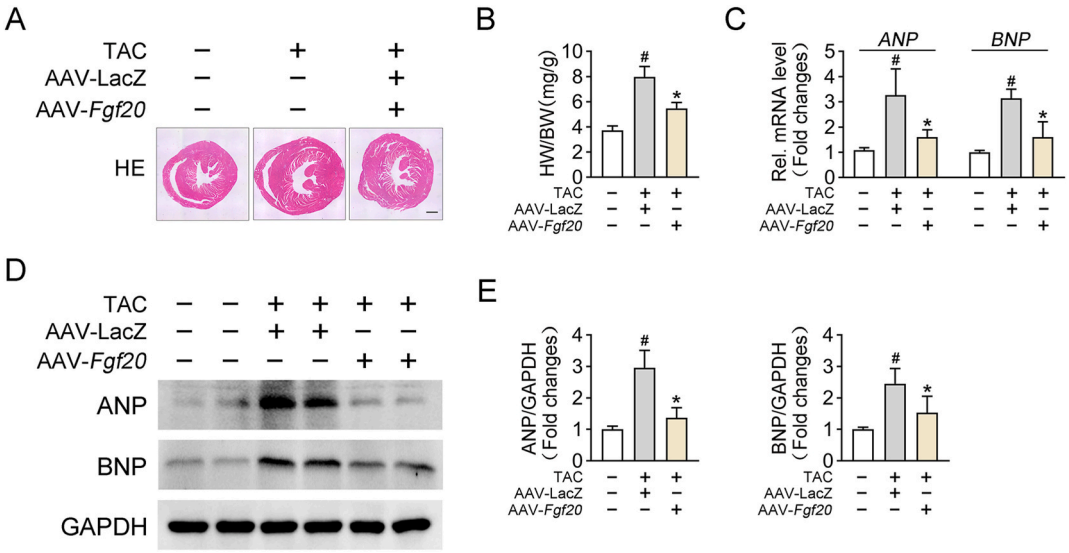

Fig. 1. FGF20 protects against TAC-induced cardiac hypertrophy *in vivo*. Mice were transfected with AAV-*Fgf20* or AAV-LacZ and then subject with TAC or sham surgery. (A) HE staining was performed to detect heart size of mice, scale bars = 400 μm. (B) Quantitative analysis of HW/BW of mice. (C) RT-qPCR analysis was performed to measure the mRNA levels of *ANP* and *BNP* in the hearts. (D–E) Western blot was performed and quantitatively analyzed to determine the protein levels ANP, BNP and ErbB2 in the hearts. $^{\#}P < 0.05$ versus Sham group, $^{*}P < 0.05$ versus TAC + AAV-LacZ group.

3.2. FGF20 upregulates ErbB2 expression in vitro and in vivo

To explore the mechanism by which FGF20 protects against cardiac hypertrophy, RNA-sequencing was performed following treatment with FGF20 plus ISO and ISO alone. KEGG enrichment analysis showed there were significant changes in the ErbB signaling pathway after FGF20 treatment under the hypertrophic state (Fig. 3A). More importantly, FGF20 upregulated expression of ErbB2, which was negatively correlated with ANP and BNP expression (Fig. 3B and C). Subsequently, we validated that FGF20 regulated ErbB2 expression *in vitro* and *in vivo*. As depicted in Fig. 3D and E, the protein and mRNA levels of ErbB2 were both decreased in response to ISO stimulation in NRCMs, while FGF20 treatment markedly increased ErbB2 expression. Similarly, FGF20 overexpression significantly increased expression of ErbB2 in TAC-operated mice (Fig. 3F and G). These findings indicated that ErbB2 is involved in the protective effect of FGF20 against cardiac hypertrophy.

3.3. FGF20 ameliorates apoptosis and oxidative stress in hypertrophic NRCMs

Biological progression analysis of GO enrichment demonstrated that DEGs (ISO + FGF20 group *vs.* ISO group) were associated with apoptosis and oxidative stress (Fig. 4A). Cardiomyocyte apoptosis and oxidative stress are crucial events in the pathological process of cardiomyocyte hypertrophy [3]. Consequently, the regulatory effects of FGF20 on apoptosis and oxidative stress were verified in hypertrophic NRCMs.

ISO significantly induced cardiomyocyte apoptosis, as evidenced by increases in cleaved caspase-3 (C-CAS-3) protein level and the ratio of Bax/Bcl-2, which were repressed by FGF20 treatment (Fig. 4B and C). In addition, DHE staining revealed that ISO markedly induced ROS overproduction in NRCMs, which was significantly decreased by FGF20 treatment (Fig. 5A and B). Catalase, superoxide dismutase 2 (Sod2), and heme oxygenase-1 (HO-1) are intracellular anti-oxidant defense enzymes and play crucial roles in maintaining cellular redox balance [31]. Therefore, the protein levels of these enzymes were also detected. The protein levels of catalase, Sod2, and HO-1 were dramatically reduced by ISO, but significantly increased by FGF20 (Fig. 5C–E). These data confirmed that FGF20 prevents cardiac hypertrophy by inhibiting apoptosis and oxidative stress *in vitro*.

3.4. Inhibition of ErbB2 reverses the protective effect of FGF20 against cardiac hypertrophy in vitro

Next, we further explored whether ErbB2 is responsible for FGF20-mediated protection against cardiac hypertrophy. NRCMs were pretreated with lapatinib (an ErbB2 inhibitor) and subsequently the anti-apoptotic and anti-oxidant capacities of FGF20 was investigated. In line with the results shown in Fig. 3, FGF20 attenuated the ISO-induced increases of the level of C-CAS-3 and the Bax/Bcl-2 ratio. Conversely, pretreatment with lapatinib markedly increased C-CAS-3 protein level and the Bax/Bcl-2 ratio (Fig. 6A and B), indicating that ErbB2 mediates the anti-apoptotic effect of FGF20 upon cardiac hypertrophy. In addition, lapatinib effectively abolished the FGF20-mediated upregulation of catalase, Sod2, and HO-1 (Fig. 6C–E). DHE staining also showed that ISO-induced ROS accumulation was decreased by FGF20 treatment but elevated by lapatinib treatment (Fig. 6F and G), further indicating that the anti-oxidant effect of FGF20 upon cardiac hypertrophy is mediated by ErbB2.

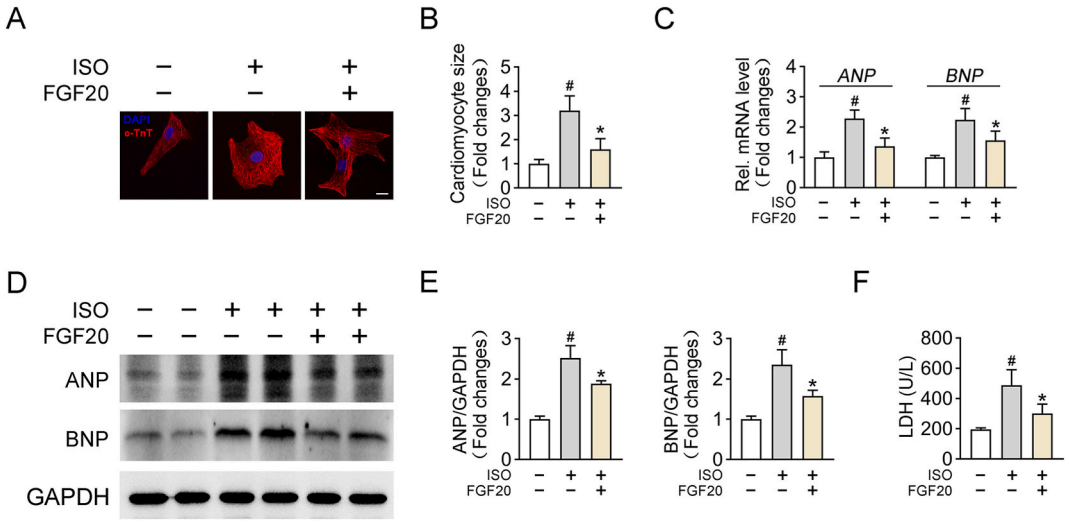

Fig. 2. FGF20 protects against ISO-induced cardiomyocyte hypertrophy *in vitro*. NRCMs were treated with or without ISO for 48 h in the presence or absence of FGF20. (A–B) Surface area of NRCMs was detected by cTnT immunofluorescence (red for cTnT protein, blue for the nuclei) and then quantitatively analyzed, scale bars = 60 mm. (C) RT-qPCR analysis was performed to measure the mRNA levels of *ANP* and *BNP* in NRCMs. (D–E) Western blot was performed and quantitatively analyzed to determine the protein levels of ANP, BNP and ErbB2 in NRCMs. (F) LDH assay was performed to measure the LDH release from NRCMs. $^{\#}P < 0.05$ versus CON group, $^{*}P < 0.05$ versus ISO group.

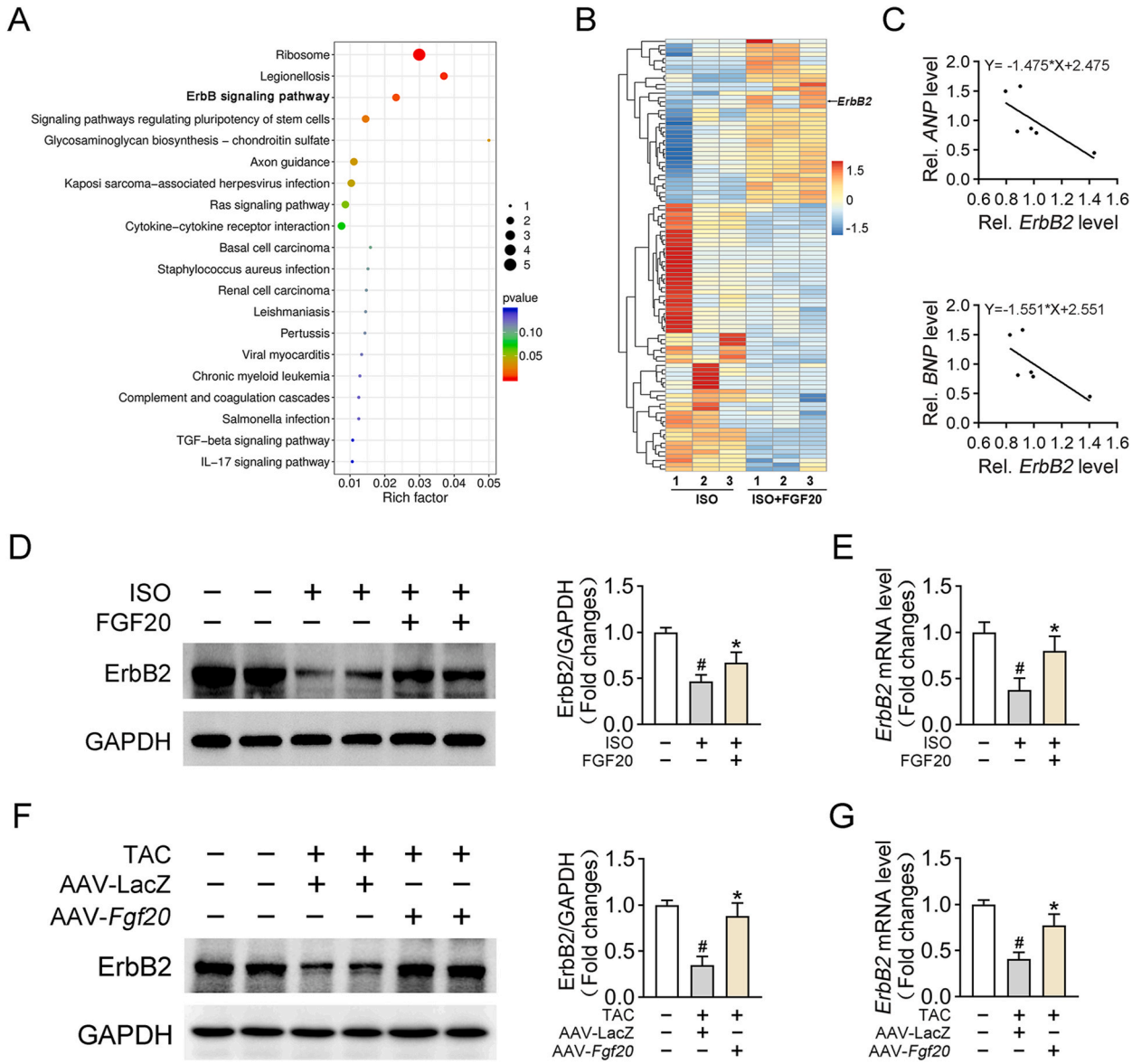

Fig. 3. FGF20 activated ErbB2 in hypertrophic NRCMs and heart. (A) RNA sequence was carried out in NRCMs between FGF20+ISO group and ISO group (n = 3 each group), top twenty enriched items of KEGG analysis for differentially gene expression (DEGs). (B) Heat map of DEGs in NRCMs between FGF20+ISO group and ISO group. (C) Correlation analysis of *ErbB2* with *ANP* and *BNP*. (D) Western blot was performed and quantitatively analyzed to determine the protein levels of ErbB2 in NRCMs treated with or without ISO for in the presence or absence of FGF20. (E) RT-qPCR was performed to measure the mRNA levels of *ErbB2* in NRCMs treated with or without ISO in the presence or absence of FGF20, $^{\#}P < 0.05$ versus CON group, $^{*}P < 0.05$ versus ISO group. (F) Western blot was performed and quantitatively analyzed to determine the protein levels of ErbB2 in the heart of mice transfected with AAV-*Fgf20* or AAV-LacZ and underwent TAC or sham surgery, (G) RT-qPCR was performed to measure the mRNA levels of *ErbB2* in the heart of mice transfected with AAV-*Fgf20* or AAV-LacZ and underwent TAC or sham surgery, $^{\#}P < 0.05$ versus Sham group, $^{*}P < 0.05$ versus TAC + AAV-LacZ group.

The anti-hypertrophic effect of FGF20 was also investigated. FGF20 significantly inhibited ISO-induced cardiomyocyte hypertrophy, which was dramatically reversed by lapatinib, as evidenced by immunofluorescence staining of cTnT (Fig. 7A and B). Moreover, lapatinib significantly abrogated FGF20-mediated downregulation of ANP and BNP at both the mRNA and protein levels, accompanied by an increase of LDH release (Fig. 7C–F), suggesting that the anti-hypertrophic effect of FGF20 is greatly dependent on ErbB2. Taken together, these findings supported the notion that FGF20 mitigates cardiac hypertrophy by suppressing oxidative stress and apoptosis via activating ErbB2.

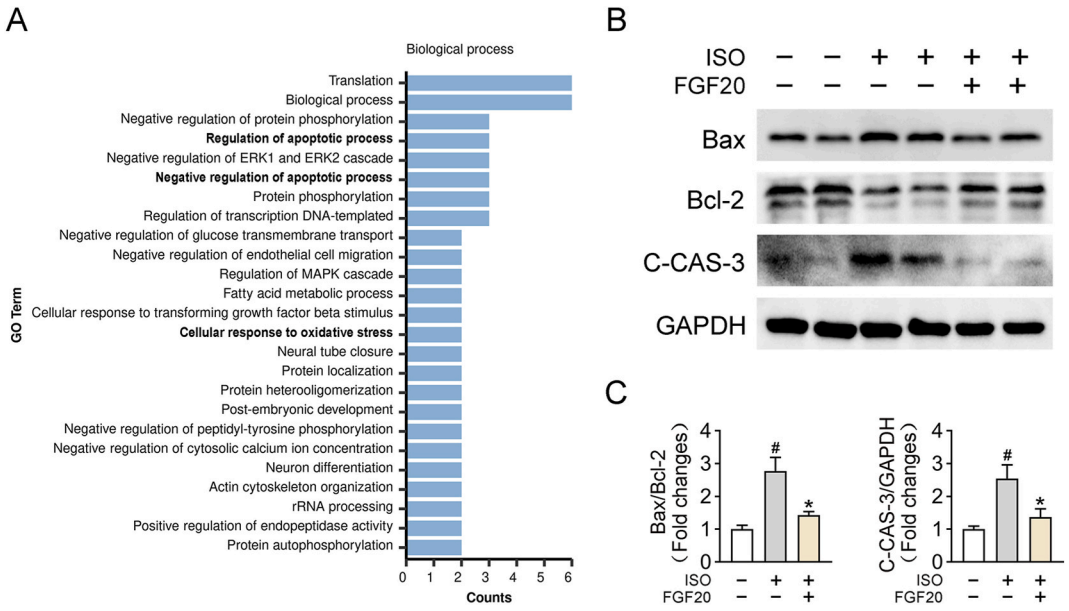

Fig. 4. FGF20 alleviated ISO-induced apoptosis in NRCMs. (A) The enriched biological processes in GO analysis for DEGs in NRCMs between FGF20+ISO group and ISO group. (B–C) Western blots was performed and quantitatively analyzed to determine the apoptotic protein levels of bax, bcl-2 and cleaved caspase 3 (C-CAS-3) in NRCMs treated with or without FGF20 in the presence or absence of ISO.

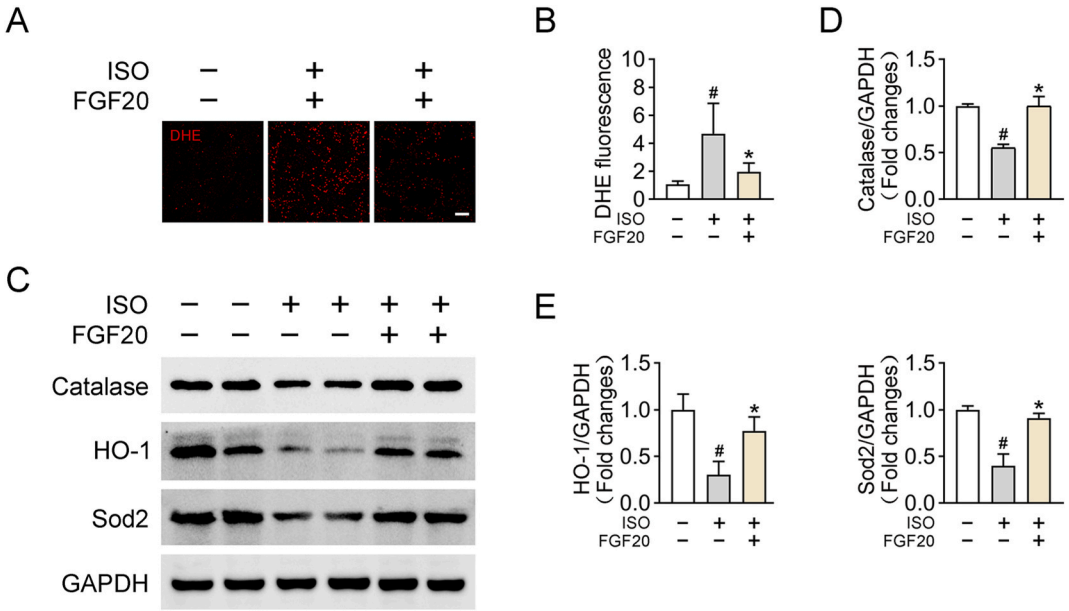

Fig. 5. FGF20 attenuates oxidative stress in ISO-treated NRCMs. NRCMs were treated with or without ISO for 48 h in the presence or absence of FGF20. (A) DHE staining was performed to measure intracellular ROS (red) in NRCMs, scale bars = 1 mm. (B) Quantitative analysis of intensity of DHE fluorescence. (C) Western blot was performed to determine the protein levels of catalase, HO-1 and sod2 in NRCMs. (D–E) Quantitative analysis of the protein level of catalase, HO-1 and sod2. $^{\#}P < 0.05$ versus CON, $^{*}P < 0.05$ versus ISO.

4. Discussion

There is emerging evidence that FGF20 and ErbB2 play crucial roles in maintaining cardiac homeostasis after injury [19,32]. Nevertheless, no study has focused on the relationship between FGF20 and ErbB2 in cardiomyocyte hypertrophy. In this study, we provide evidence that: (i) FGF20 improves cardiac hypertrophy *in vitro* and *in vivo*; (ii) FGF20 increases expression of ErbB2, which is negatively correlated with expression of the cardiac hypertrophy markers ANP and BNP; (iii) ErbB2 inhibition reverses the protective

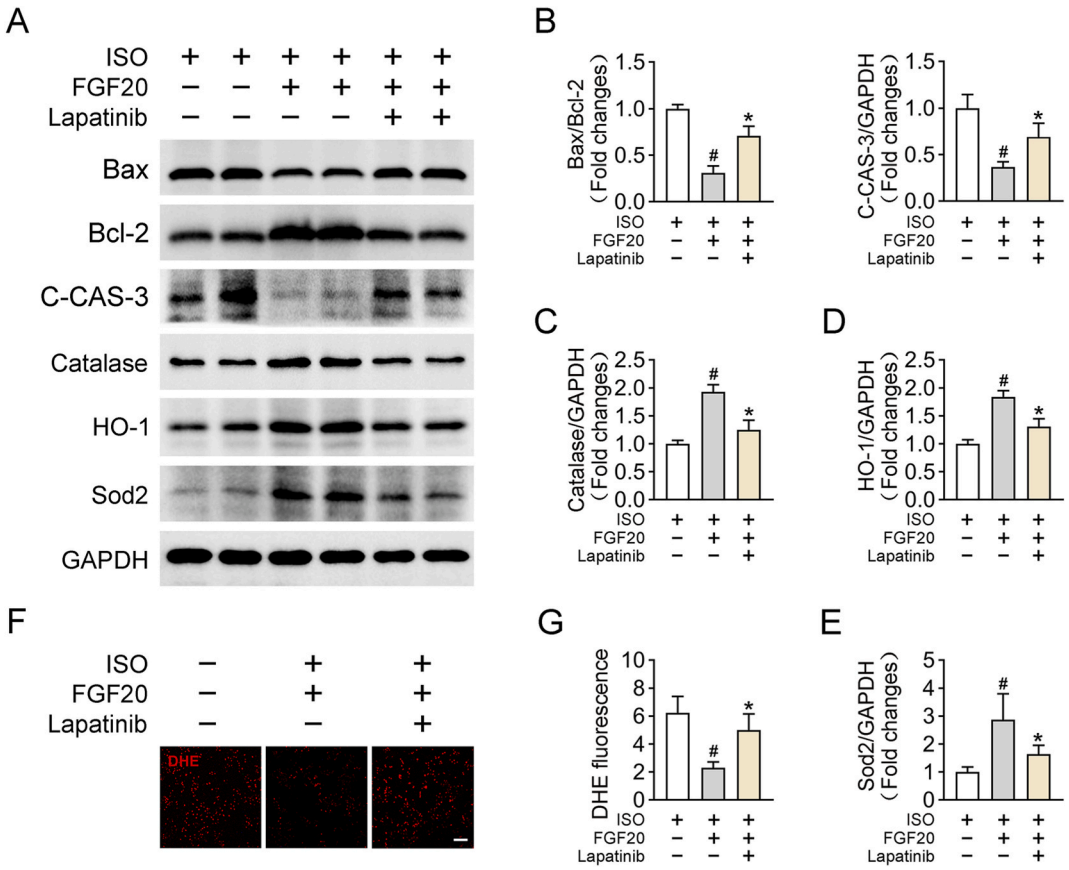

Fig. 6. ErbB2 inhibitor weakens the anti-oxidative and anti-apoptotic effects of FGF20 in ISO-treated NRCMs. NRCMs were pretreated with or without Lapatinib for 2 h and then incubated with or without FGF20 for 48 h in the presence of ISO. (A) Western blot was performed to detect the protein levels of bax, bcl-2, cleaved caspase 3 (C-CAS-3) catalase, HO-1 and sod2. (B) Quantitative analysis of bax/bcl-2 ratio and the protein levels of C-CAS-3. (C–E) Quantitative analysis of the protein levels of catalase, HO-1 and sod2. (F) DHE staining was performed to measure intracellular ROS (red) in NRCMs, scale bars = 1 mm. (G) Quantitative analysis of intensity of DHE fluorescence. $^{\#}P < 0.001$ versus ISO group, $^{*}P < 0.05$versus ISO + FGF20 group.

effect of FGF20 against cardiomyocyte apoptosis and oxidative stress. Therefore, our study reveals that FGF20 alleviates cardiac hypertrophy by activating ErbB2.

Due to discoveries about its biological structure and function, the role of FGF20 in heart tissue has garnered substantial attention. For example, Morrisey et al. reported that FGF20 promotes cardiomyocyte proliferation and repair of the heart [33], which may be beneficial for adult cardiomyocytes encountering pathological stress. Surprisingly, few studies have focused on the effect of FGF20 on cardiac hypertrophy and the underlying molecular mechanism. In the present study, we demonstrated that FGF20 improves TAC-induced cardiac hypertrophy in mice and NRCMs, which is consistent with our previous research [19]. Thus, our results suggest that FGF20 protects against cardiac hypertrophy.

In recent years, the function of ErbB2 in cardiac homeostasis has been extensively studied. Expression of ErbB2 is decreased in pathological cardiac tissue [34,35]. Furthermore, activation of ErbB2 protects against myocardial infraction, cardiac hypertrophy, and heart failure [25,36,37]. Data from a phase II clinical trial showed that recombinant human neuregulin-1 (NRG1), an activator of ErbB2, improved the cardiac function of patients with chronic heart failure [38]. Therefore, ErbB2 may be a promising therapeutic target for heart diseases. In this study, we employed transcriptomics to investigate the mechanism by which FGF20 protects against cardiomyocyte hypertrophy. DEGs after FGF20 treatment were enriched in the ErbB signaling pathway. Expression of ErbB2 was increased by FGF20 and was negatively related to expression of ANP and BNP, indicating that ErbB2 is a downstream target of FGF20 upon cardiac hypertrophy. Consistently, western blotting and RT-qPCR analyses detected significant decreases in the protein and mRNA levels of ErbB2 in response to hypertrophic stimulation *in vivo* and *in vitro*, which were conversely increased by FGF20 administration. Hence, our data suggest that FGF20 protects against cardiac hypertrophy by activating ErbB2.

Cardiomyocyte apoptosis and oxidative stress are considered major causes of cardiac hypertrophy [9,39]. Therefore, anti-oxidant and anti-apoptosis therapies are effective approaches to treat cardiac hypertrophy [40]. Moreover, FGF20 is a regulatory factor with anti-oxidant and anti-apoptotic activities. Alvarez et al. reported that FGF20 promotes expression of anti-oxidant enzymes such as Sod2 and thereby facilitates scavenging of excess ROS following acute ionizing irradiation, which ultimately increases the overall survival of

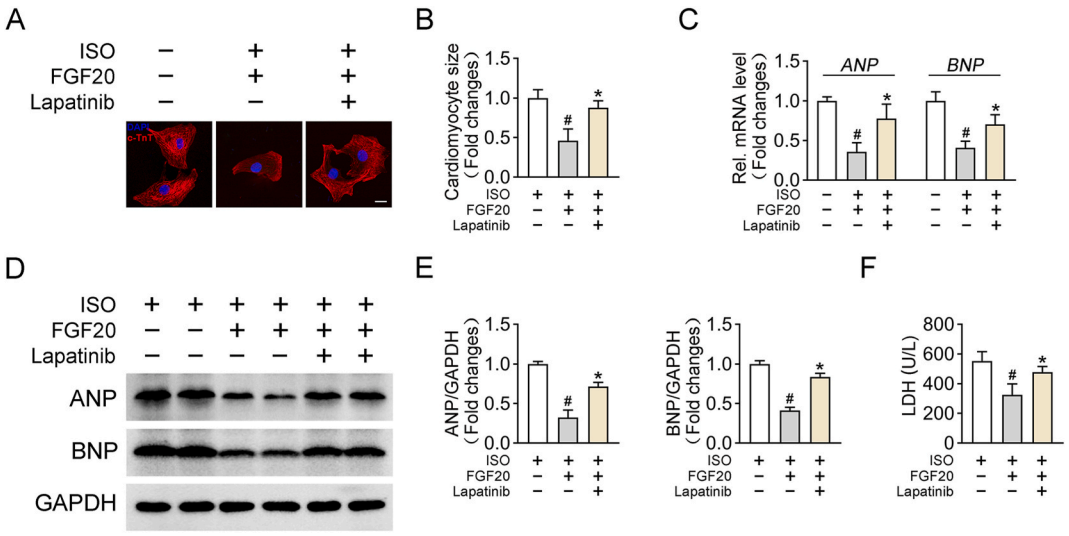

Fig. 7. ErbB2 inhibitor abrogated the anti-hypertrophic effect of FGF20 in ISO-treated NRCMs. NRCMs were pretreated with or without Lapatinib for 2 h and then incubated with or without FGF20 for 48 h in the presence of ISO. (A–B) Surface area of NRCMs was detected by cTnT immunofluorescence (red for cTnT protein, blue for the nuclei) and then quantitatively analyzed, scale bars = 60 μm. (C) RT-qPCR analysis was performed to measure the mRNA levels of *ANP* and *BNP*. (D–E) Western blot was performed and quantitatively analyzed to determine the protein levels of ErbB2. (F) LDH assay was performed to measure the LDH release from NRCMs. $^{\#}P < 0.001$ versus ISO group, $^{*}P < 0.05$versus ISO + FGF20 group.

mice [41]. In addition, FGF20 elicits a protective effect in Alzheimer's disease by inhibiting apoptosis [42]. By analyzing GO enrichment, we found that the protective effect of FGF20 was associated with apoptosis and oxidative stress. We further demonstrated that FGF20 treatment suppressed apoptosis and oxidative stress in hypertrophic cardiomyocytes. Taken together, these findings indicate that FGF20 alleviates cardiac hypertrophy by inhibiting apoptosis and oxidative stress.

The question arose of whether FGF20-mediated prevention of cardiomyocyte apoptosis and oxidative stress is associated with FGF20-mediated activation of ErbB2. ErbB2 functions as a cardioprotective factor by inhibiting apoptosis and oxidative stress. For example, NRG1 improves myocardial infraction by relieving cardiomyocyte apoptosis [43]. Furthermore, Gabrielson et al. demonstrated that overexpression of ErbB2 ameliorates doxorubicin-induced cardiomyopathy and that this effect is mediated by inhibition of oxidative stress [44]. Importantly, our previous research showed that ErbB2 deletion results in cardiomyocyte apoptosis and oxidative stress [28]. Thus, based on the anti-oxidant and anti-apoptotic capacities of ErbB2 in cardiomyocytes and our results described above, we speculated that FGF20-mediated prevention of cardiomyocyte apoptosis and oxidative stress in cardiac hypertrophy is attributable to activation of ErbB2. To investigate this hypothesis, ErbB2 was inhibited in cardiomyocytes using lapatinib. As expected, lapatinib abrogated the protective effect of FGF20 against cardiomyocyte apoptosis and oxidative stress. In addition, lapatinib effectively eliminated the anti-hypertrophic effect of FGF20. Thus, our results confirm that FGF20 improves cardiac hypertrophy by suppressing apoptosis and oxidative stress in an ErbB2-dependent manner.

However, this study has limitations. First, although it demonstrated the protective effect of FGF20 against cardiac hypertrophy and the underlying ErbB2-dependent mechanism, this mechanism must be further validated *in vivo*. Second, the effects of ErbB2 on cellular biological processes are largely mediated by its phosphorylation. Therefore, it is important to clarify whether ErbB2 phosphorylation is involved in FGF20-mediated ErbB2 activation in future studies. Finally, further research is expected to reveal the mechanism underlying FGF20-mediated ErbB2 activation.

In conclusion, this study provides the first evidence that FGF20 inhibits apoptosis and oxidative stress by activating ErbB2, thereby alleviating cardiac hypertrophy.

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Data availability statement

All data supporting the findings of this study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Yunjie Chen: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization. **Xuan Zhou:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Xu Wang:** Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Yuanbin Zhang:** Methodology, Investigation. **Jiayi Song:** Methodology, Investigation, Funding acquisition, Conceptualization. **Yan Cai:** Writing – original draft, Investigation, Conceptualization. **Yizhuo Zhao:** Methodology, Investigation, Funding acquisition, Conceptualization. **Lin Mei:** Writing – review & editing, Investigation, Funding acquisition. **Suyan Zhu:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Xueqin Chen:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] F. Weinberger, T. Eschenhagen, Cardiac regeneration: new hope for an old dream, Annu. Rev. Physiol. 83 (2021) 59–81, https://doi.org/10.1146/annurev-physiol-031120-103629.
[2] L. Jin, X. Shi, J. Yang, Y. Zhao, L. Xue, L. Xu, J. Cai, Gut microbes in cardiovascular diseases and their potential therapeutic applications, Protein Cell 12 (2021) 346–359, https://doi.org/10.1007/s13238-020-00785-9.
[3] M. Nakamura, J. Sadoshima, Mechanisms of physiological and pathological cardiac hypertrophy, Nat. Rev. Cardiol. 15 (2018) 387–407, https://doi.org/10.1038/s41569-018-0007-y.
[4] T.G. Martin, M.A. Juarros, L.A. Leinwand, Regression of cardiac hypertrophy in health and disease: mechanisms and therapeutic potential, Nat. Rev. Cardiol. 20 (2023) 347–363, https://doi.org/10.1038/s41569-022-00806-6.
[5] J. Liu, W. Li, K.Q. Deng, S. Tian, H. Liu, H. Shi, Q. Fang, Z. Liu, Z. Chen, T. Tian, S. Gan, F. Hu, M. Hu, X. Cheng, Y.X. Ji, P. Zhang, Z.G. She, X.J. Zhang, S. Chen, J. Cai, H. Li, The E3 ligase TRIM16 is a key suppressor of pathological cardiac hypertrophy, Circ. Res. 130 (2022) 1586–1600, https://doi.org/10.1161/circresaha.121.318866.
[6] L. Chen, L. Fu, J. Sun, Z. Huang, M. Fang, A. Zinkle, X. Liu, J. Lu, Z. Pan, Y. Wang, G. Liang, X. Li, G. Chen, M. Mohammadi, Structural basis for FGF hormone signalling, Nature 618 (2023) 862–870, https://doi.org/10.1038/s41586-023-06155-9.
[7] F. Khosravi, N. Ahmadvand, S. Bellusci, H. Sauer, The multifunctional contribution of FGF signaling to cardiac development, homeostasis, disease and repair, Front. Cell Dev. Biol. 9 (2021) 672935, https://doi.org/10.3389/fcell.2021.672935.
[8] G. Chen, N. An, J. Shen, H. Chen, Y. Chen, J. Sun, Z. Hu, J. Qiu, C. Jin, S. He, L. Mei, Y. Sui, W. Li, P. Chen, X. Guan, M. Chu, Y. Wang, L. Jin, K. Kim, X. Li, W. Cong, X. Wang, Fibroblast growth factor 18 alleviates stress-induced pathological cardiac hypertrophy in male mice, Nat. Commun. 14 (2023) 1235, https://doi.org/10.1038/s41467-023-36895-1.
[9] S. Li, Z. Zhu, M. Xue, X. Yi, J. Liang, C. Niu, G. Chen, Y. Shen, H. Zhang, J. Zheng, C. Zhao, Y. Liang, W. Cong, Y. Wang, L. Jin, Fibroblast growth factor 21 protects the heart from angiotensin II-induced cardiac hypertrophy and dysfunction via SIRT1, Biochim. Biophys. Acta, Mol. Basis Dis. 1865 (2019) 1241–1252, https://doi.org/10.1016/j.bbadis.2019.01.019.
[10] C. Koga, N. Adati, K. Nakata, K. Mikoshiba, Y. Furuhata, S. Sato, H. Tei, Y. Sakaki, T. Kurokawa, K. Shiokawa, K.K. Yokoyama, Characterization of a novel member of the FGF family, XFGF-20, in Xenopus laevis, Biochem. Biophys. Res. Commun. 261 (1999) 756–765, https://doi.org/10.1006/bbrc.1999.1039.
[11] S. Wang, Y. Li, C. Jiang, H. Tian, Fibroblast growth factor 9 subfamily and the heart, Appl. Microbiol. Biotechnol. 102 (2018) 605–613, https://doi.org/10.1007/s00253-017-8652-3.
[12] J. Chen, X. Wang, J. Hu, J. Du, C. Dordoe, Q. Zhou, W. Huang, R. Guo, F. Han, K. Guo, S. Ye, L. Lin, X. Li, FGF20 protected against BBB disruption after traumatic brain injury by upregulating junction protein expression and inhibiting the inflammatory response, Front. Pharmacol. 11 (2020) 590669, https://doi.org/10.3389/fphar.2020.590669.
[13] U. Rudakou, E. Yu, L. Krohn, J.A. Ruskey, F. Asayesh, Y. Dauvilliers, D. Spiegelman, L. Greenbaum, S. Fahn, C.H. Waters, N. Dupré, G.A. Rouleau, S. Hassin-Baer, E.A. Fon, R.N. Alcalay, Z. Gan-Or, Targeted sequencing of Parkinson's disease loci genes highlights SYT11, FGF20 and other associations, Brain 144 (2021) 462–472, https://doi.org/10.1093/brain/awaa401.
[14] L.C. Biggs, O.J. Mäkelä, S.M. Myllymäki, R. Das Roy, K. Närhi, J. Pispa, T. Mustonen, M.L. Mikkola, Hair follicle dermal condensation forms via Fgf20 primed cell cycle exit, cell motility, and aggregation, Elife 7 (2018) e36468, https://doi.org/10.7554/eLife.36468.
[15] Y. Su, L.M. Yang, D.M. Ornitz, FGF20-FGFR1 signaling through MAPK and PI3K controls sensory progenitor differentiation in the organ of Corti, Dev Dyn 250 (2021) 134–144, https://doi.org/10.1002/dvdy.231.
[16] M. Jeffers, W.F. McDonald, R.A. Chillakuru, M. Yang, H. Nakase, L.L. Deegler, E.D. Sylander, B. Rittman, A. Bendele, R.B. Sartor, H.S. Lichenstein, A novel human fibroblast growth factor treats experimental intestinal inflammation, Gastroenterology 123 (2002) 1151–1162, https://doi.org/10.1053/gast.2002.36041.
[17] X. Cai, W. Tao, L. Li, Glioma cell-derived FGF20 suppresses macrophage function by activating β-catenin, Cell. Signal. 89 (2022) 110181, https://doi.org/10.1016/j.cellsig.2021.110181.
[18] K.J. Lavine, K. Yu, A.C. White, X. Zhang, C. Smith, J. Partanen, D.M. Ornitz, Endocardial and epicardial derived FGF signals regulate myocardial proliferation and differentiation in vivo, Dev. Cell 8 (2005) 85–95, https://doi.org/10.1016/j.devcel.2004.12.002.
[19] Y. Chen, N. An, X. Zhou, L. Mei, Y. Sui, G. Chen, H. Chen, S. He, C. Jin, Z. Hu, W. Li, Y. Wang, Z. Lin, P. Chen, L. Jin, X. Guan, X. Wang, Fibroblast growth factor 20 attenuates pathological cardiac hypertrophy by activating the SIRT1 signaling pathway, Cell Death Dis. 13 (2022) 276, https://doi.org/10.1038/s41419-022-04724-w.
[20] A.I. Fernandez, M. Liu, A. Bellizzi, J. Brock, O. Fadare, K. Hanley, M. Harigopal, J.M. Jorns, M.G. Kuba, A. Ly, M. Podoll, K. Rabe, M.A. Sanders, K. Singh, O. L. Snir, T.R. Soong, S. Wei, H. Wen, S. Wong, E. Yoon, L. Pusztai, E. Reisenbichler, D.L. Rimm, Examination of low ERBB2 protein expression in breast cancer tissue, JAMA Oncol. 8 (2022) 1–4, https://doi.org/10.1001/jamaoncol.2021.7239.

[21] S. Vranić, S. Beślija, Z. Gatalica, Targeting HER2 expression in cancer: new drugs and new indications, Bosn. J. Basic Med. Sci. 21 (2021) 1–4, https://doi.org/10.17305/bjbms.2020.4908.
[22] D.A. Sabbah, R. Hajjo, K. Sweidan, Review on epidermal growth factor receptor (EGFR) structure, signaling pathways, interactions, and recent updates of EGFR inhibitors, Curr. Top. Med. Chem. 20 (2020) 815–834, https://doi.org/10.2174/1568026620666200303123102.
[23] B.A. Shraim, M.O. Moursi, I.F. Benter, A.M. Habib, S. Akhtar, The role of epidermal growth factor receptor family of receptor tyrosine kinases in mediating diabetes-induced cardiovascular complications, Front. Pharmacol. 12 (2021) 701390, https://doi.org/10.3389/fphar.2021.701390.
[24] A. Negro, B.K. Brar, K.F. Lee, Essential roles of Her2/erbB2 in cardiac development and function, Recent Prog. Horm. Res. 59 (2004) 1–12, https://doi.org/10.1210/rp.59.1.1.
[25] A. Aharonov, A. Shakked, K.B. Umansky, A. Savidor, A. Genzelinakh, D. Kain, D. Lendengolts, O.Y. Revach, Y. Morikawa, J. Dong, Y. Levin, B. Geiger, J. F. Martin, E. Tzahor, ERBB2 drives YAP activation and EMT-like processes during cardiac regeneration, Nat. Cell Biol. 22 (2020) 1346–1356, https://doi.org/10.1038/s41556-020-00588-4.
[26] J. Sun, C. Niu, W. Ye, N. An, G. Chen, X. Huang, J. Wang, X. Chen, Y. Shen, S. Huang, Y. Wang, X. Wang, Y. Wang, L. Jin, W. Cong, X. Li, FGF13 is a novel regulator of NF-κB and potentiates pathological cardiac hypertrophy, iScience 23 (2020) 101627, https://doi.org/10.1016/j.isci.2020.101627.
[27] O. Cooper, V.S. Bonert, J. Rudnick, B.D. Pressman, J. Lo, R. Salvatori, K.C.J. Yuen, M. Fleseriu, S. Melmed, EGFR/ErbB2-Targeting lapatinib therapy for aggressive prolactinomas, J. Clin. Endocrinol. Metab. 106 (2021) e917–e925, https://doi.org/10.1210/clinem/dgaa805.
[28] Y. Chen, S. Zhu, Z. Lin, Y. Zhang, C. Jin, S. He, X. Chen, X. Zhou, Metformin alleviates ethanol-induced cardiomyocyte injury by activating AKT/Nrf2 signaling in an ErbB2-dependent manner, Mol. Biol. Rep. 50 (2023) 3469–3478, https://doi.org/10.1007/s11033-023-08310-x.
[29] L. Lin, S. Han, Z. Gong, F. Ding, Z. Liu, W. Xiao, L-Theanine attenuates heat stress-induced proteotoxicity and alterations in carbohydrate and lipid metabolism via heat shock factor 1, Food Funct. 14 (2023) 6172–6186, https://doi.org/10.1039/d3fo01001e.
[30] N. Nalban, R. Sangaraju, S. Alavala, S.M. Mir, M.K. Jerald, R. Sistla, Arbutin attenuates isoproterenol-induced cardiac hypertrophy by inhibiting TLR-4/NF-κB pathway in mice, Cardiovasc. Toxicol. 20 (2020) 235–248, https://doi.org/10.1007/s12012-019-09548-3.
[31] C.J.A. Ramachandra, S. Cong, X. Chan, E.P. Yap, F. Yu, D.J. Hausenloy, Oxidative stress in cardiac hypertrophy: from molecular mechanisms to novel therapeutic targets, Free Radic. Biol. Med. 166 (2021) 297–312, https://doi.org/10.1016/j.freeradbiomed.2021.02.040.
[32] R. Florido, K.L. Smith, K.K. Cuomo, S.D. Russell, Cardiotoxicity from human epidermal growth factor receptor-2 (HER2) targeted therapies, J. Am. Heart Assoc. 6 (2017) e006915, https://doi.org/10.1161/jaha.117.006915.
[33] Y. Zhang, S. Li, L. Yuan, Y. Tian, J. Weidenfeld, J. Yang, F. Liu, A.L. Chokas, E.E. Morrisey, Foxp1 coordinates cardiomyocyte proliferation through both cell-autonomous and nonautonomous mechanisms, Genes Dev. 24 (2010) 1746–1757, https://doi.org/10.1101/gad.1929210.
[34] R. Matsukawa, Y. Hirooka, K. Ito, N. Honda, K. Sunagawa, Central neuregulin-1/ErbB signaling modulates cardiac function via sympathetic activity in pressure overload-induced heart failure, J. Hypertens. 32 (2014) 817–825, https://doi.org/10.1097/hjh.0000000000000072.
[35] C. Gui, J.A. Wang, N. Li, Y.Y. Wang, L. Chen, Changes of ErbB receptors mRNA expression in left ventricle of myocardial infarction, Zhejiang Da Xue Xue Bao Yi Xue Ban 37 (2008) 381–385, https://doi.org/10.3785/j.issn.1008-9292.2008.04.010.
[36] X. Liu, X. Gu, Z. Li, X. Li, H. Li, J. Chang, P. Chen, J. Jin, B. Xi, D. Chen, D. Lai, R.M. Graham, M. Zhou, Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy, J. Am. Coll. Cardiol. 48 (2006) 1438–1447, https://doi.org/10.1016/j.jacc.2006.05.057.
[37] G. D'Uva, A. Aharonov, M. Lauriola, D. Kain, Y. Yahalom-Ronen, S. Carvalho, K. Weisinger, E. Bassat, D. Rajchman, O. Yifa, M. Lysenko, T. Konfino, J. Hegesh, O. Brenner, M. Neeman, Y. Yarden, J. Leor, R. Sarig, R.P. Harvey, E. Tzahor, ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation, Nat. Cell Biol. 17 (2015) 627–638, https://doi.org/10.1038/ncb3149.
[38] R. Gao, J. Zhang, L. Cheng, X. Wu, W. Dong, X. Yang, T. Li, X. Liu, Y. Xu, X. Li, M. Zhou, A Phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure, J. Am. Coll. Cardiol. 55 (2010) 1907–1914, https://doi.org/10.1016/j.jacc.2009.12.044.
[39] A.K. Shah, S.K. Bhullar, V. Elimban, N.S. Dhalla, Oxidative stress as A mechanism for functional alterations in cardiac hypertrophy and heart failure, Antioxidants 10 (2021) 93, https://doi.org/10.3390/antiox10060931.
[40] C.J. Oldfield, T.A. Duhamel, N.S. Dhalla, Mechanisms for the transition from physiological to pathological cardiac hypertrophy, Can. J. Physiol. Pharmacol. 98 (2021) 74–84, https://doi.org/10.1139/cjpp-2019-0566.
[41] T. Maclachlan, B. Narayanan, V.L. Gerlach, G. Smithson, R.W. Gerwien, O. Folkerts, E.G. Fey, B. Watkins, T. Seed, E. Alvarez, Human fibroblast growth factor 20 (FGF-20; CG53135-05): a novel cytoprotectant with radioprotective potential, Int. J. Radiat. Biol. 81 (2005) 567–579, https://doi.org/10.1080/09553000500211091.
[42] H. Tian, Y. Zhao, N. Chen, M. Wu, W. Gong, J. Zheng, D.G. Fernig, A. Jungbauer, D. Wang, X. Li, C. Jiang, High production in E. coli of biologically active recombinant human fibroblast growth factor 20 and its neuroprotective effects, Appl. Microbiol. Biotechnol. 100 (2016) 3023–3034, https://doi.org/10.1007/s00253-015-7168-y.
[43] M. Shiraishi, A. Yamaguchi, K. Suzuki, Nrg1/ErbB signaling-mediated regulation of fibrosis after myocardial infarction, Faseb j 36 (2022) e22150, https://doi.org/10.1096/fj.202101428RR.
[44] F. Belmonte, S. Das, P. Sysa-Shah, V. Sivakumaran, B. Stanley, X. Guo, N. Paolocci, M.A. Aon, M. Nagane, P. Kuppusamy, C. Steenbergen, K. Gabrielson, ErbB2 overexpression upregulates antioxidant enzymes, reduces basal levels of reactive oxygen species, and protects against doxorubicin cardiotoxicity, Am. J. Physiol. Heart Circ. Physiol. 309 (2015) H1271–H1280, https://doi.org/10.1152/ajpheart.00517.2014.