ETS2 and microRNA-155 regulate the pathogenesis of heart failure through targeting and regulating GPR18 expression

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Received June 13, 2018; Accepted November 29, 2019

DOI: 10.3892/etm.2020.8642

Abstract. Heart failure (HF) is a global pandemic cardiovascular disease with increasing prevalence, but the pathogenesis remains to be elucidated. The present study aimed to investigate the underlying mechanism in heart failure (HF) using bioinformatics and experimental validation. A HF-associated dataset GSE84796 was downloaded from the Gene Expression Omnibus database and differentially expressed genes (DEGs) were screened for using Bayes method in the Limma package. Kyoto Encyclopedia of Genes and Genomes pathway analysis was used to perform pathway enrichment analysis of these DEGs using The Database for Annotation, Visualization and Integrated Discovery. A protein-protein interaction (PPI) network of DEG-encoded proteins was subsequently constructed using the Search tool for the Retrieval of Interacting Genes/Proteins, and a transcription factor (TF)/miRNA-target network was constructed according to the WEB-based Gene Set AnaLysis Tookit. The expression levels of microRNA (miRNA/miR)-155, G-protein coupled receptor 18 (GPR18) and E26 transformation-specific transcription factor 2 (ETS2) were analyzed in clinical HF samples, and functional validations were performed in H9c2 (2-1) cells. A total of 419 DEGs were identified, including 366 upregulated genes and 53 downregulated genes. The upregulated DEGs were significantly enriched in the pathways of ‘cytokine-cytokine receptor interaction’, ‘natural killer cell mediated cytotoxicity’ and ‘primary immunodeficiency’. A total of two functional modules were identified in the PPI network: Module A was enriched in 3 KEGG pathways and module B was enriched in 15 KEGG pathways. Furthermore, a total of three miRNAs and eight TFs were identified in the TF/miRNA-target network. Specifically, GPR18 was discovered to be targeted by both ETS2 and miR-155. Clinical validation revealed that the expression levels of miR-155 were significantly decreased in the HF samples, whereas the expression levels of ETS2 and GPR18 were significantly increased in HF samples. In conclusion, the present study suggested that GPR18 may be a target of ETS2 and miR-155, and miR-155 may regulate cell viability and apoptosis in H9c2 (2-1) cells through targeting and regulating GPR18.

Introduction

Heart failure (HF) is a common health problem worldwide, which leads to disability and reductions in life quality and expectancy (1); however, because HF is prevalent in elderly populations, the management of HF is often associated with a high use of resources and healthcare costs (2). For example, in China it is estimated that HF healthcare has cost ~$5.42 billion per year, accounting for 5.01% of total healthcare costs (3). Although a number of different methods have been investigated to help manage HF, including medical devices, pharmacological agents and telemonitoring, the clinical treatment of HF remains relatively poor (4). Therefore, there is an urgent requirement for clinicians to investigate novel therapies for the management of HF.

The dysregulation of gene expression is a common mechanism that occurs in HF and microRNAs (miRNAs) are suggested to serve critical roles in the metabolic modulation during HF (5). Within the past few decades, concerted efforts have been made to investigate the mechanism of HF. For example, previous studies have revealed that miRNA (miR)-214 is upregulated in HF and suppresses the transcription factor (TF) X-box binding protein 1 (XBP1)-mediated endothelial cell angiogenesis (6,7). Masson et al (8) reported that increased levels of circulating miR-132 improved the hospitalization of patients with HF, whilst miR-21 was observed to negatively regulate T regulatory cells in coronary heart disease via the transforming growth factor-β1/Smad pathway, including HF (9). Moreover, TFs are considered to serve important roles in the regulation of gene expression (10); Bakker et al (11) demonstrated that T-box TF 3 (TBX3) could reprogram mature cardiac myocytes into pacemaker-like cells, whereas the silenced TF interferon regulatory factor 5 (IRF5) was indicated to reprogram the macrophage phenotype and promote infarct healing (12). In addition, cardiomyocyte-specific IκB kinase (IKK)/NF-κB activation reversed inflammation in HF (13). However, despite these findings, the pathogenesis of HF is still not fully understood.

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Key words: heart failure, regulatory network, cell viability, apoptosis, E26 transformation-specific transcription factor 2, G-protein coupled receptor 18
To identify the mechanisms of HF, Chevillard et al have created a dataset (accession no. GSE84796) on the Gene Expression Omnibus (GEO) database to investigate the differential expression of the transcriptome between patients with HF and normal healthy controls; however, the functions and mechanisms of the identified differences were not further elucidated. In the present study, differentially expressed genes (DEGs) in the dataset GSE84796 were also identified and subsequently, the biofunction and regulatory mechanisms of these DEGs were further predicted and validated in clinical samples and cell line experiments. These findings may aid in providing novel information that will aid in understanding and treating HF.

**Materials and methods**

**Data sourcing.** The dataset GSE84796 was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo). This dataset comprised the gene expression data of the left ventricular tissue of 10 end-stage patients with HF that had undergone heart transplantation and the left ventricular tissue of seven healthy hearts from organ donors. Samples included in this dataset were sequenced using the GPL4550 Agilent-028004 SurePrint G Human GE 8x60K Microarray (Agilent Technologies, Inc.) platform.

**Identification of DEGs.** Raw data from the dataset was downloaded and DEGs were identified between the patients with HF and healthy controls using the limma version 3.10.3 package (http://www.bioconductor.org/packages/2.9/bioc/html/limma.html) of R software (14), including background correction and expression value normalization. According to the annotation files, probes were mapped to gene symbols and probes that did not map to gene symbols were removed. For multiple probes mapped to one gene symbol, the average expression value was calculated as the expression level of this gene. According to the expression matrix, DEGs between the disease and control group were isolated using Bayes method in the limma package (15) with adjusted cut-off thresholds of P<0.05 and log2fold change (FC)>2.

**Pathway enrichment analysis for DEGs.** DEGs were then subjected to signaling pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (16). Significantly enriched DEGs in KEGG pathways were analyzed using the The Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool (version 6.8; https://david-d.ncifcrf.gov) (17) using the following criteria: Enriched gene number≥2 and P<0.05.

**Protein-protein interaction (PPI) network construction.** A PPI network of the DEGs was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; version 10.0; http://string-db.org) (18), with a threshold combined score of >0.9. Cytoscape (version 3.2.0) (19) was used to visualize the PPI network. In addition, the plug-in MCODE (version 1.4.2; http://apps.cytoscape.org/apps/MCODE) (20) in Cytoscape was used to identify and isolate the significantly enriched modules with scores of ≥10. In pathway enrichment analysis for significantly enriched modules was also performed based on the information provided by KEGG pathway analysis.

**Construction of TF/miRNA-target regulatory network.** Using the WEB-based Gene SeT AnaLysis Toolkit (WebGestalt; http://www.webgestalt.org/option.php) (21), the regulatory relationships between TF/miRNA and DEGs identified in the PPI network were predicted and visualized using Cytoscape software to obtain the TF/miRNA-target regulatory network with a threshold value of P<0.05.

**Patient studies.** To further validate the results of the present study, a total of 20 patients (15 males and 5 females; age range, 38-52 years; mean age 43.54±5.72 years) with HF and 20 matched healthy controls (14 males and 6 females; age range 36-58 years; mean age 44.48±6.63 years) were enrolled at Gansu Provincial Hospital in the current study from December 2017 to May 2018. Patients in this study were examined for significant symptoms of HF and had no history of myocardial infarction or revascularization. Patients were excluded if they presented with the following: Previous stroke, diabetes mellitus, blood disease, obvious disease, infectious diseases or receiving hormone treatment. Among these participants, 5 ml blood samples were collected to confirm the expression levels of G-protein coupled receptor 18 (GPR18), miR-155 and E2F transformation-specific transcription factor 2 (ETS2). This study was approved by the Ethnic Committee of Gansu Provincial Hospital and signed informed consent was obtained from all participants.

**Cell culture and transfection.** The cardiomyoblast H9c2 (2-1) cell line was purchased from the American Type Culture Collection. Cells were cultured in DMEM (Thermo Fisher Scientific, Inc.), supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 µg/ml streptomycin (Sigma-Aldrich; Merck KGaA) and 100 µg/ml penicillin (Sigma-Aldrich; Merck KGaA) and maintained in a humidified atmosphere with 5% CO2 at 37°C. For transfections, 1x10^5 H9c2 (2-1) cells/well were seeded into six-well plates and cultured overnight at 37°C with 70% confluency, and subsequently transfected with miR-155 mimic (50 nM; 5'-UUAUGCUACUGUAGUGGUGC CCUACUGCAUUAGAUAAAU-3'), miR-155 inhibitor (50 nM; 5'-ACCCCUACUGCAUAAUAA-3'), negative control (NC; 50 nM), miR-155 mimics (50 nM; 5'-UUCCGGAACGUGCAGT-3'), pcDNA3.0-ETS2 overexpression plasmid, an empty pcDNA3.0 plasmid as the NC (100 nM; pcDNA3.0), small interfering RNA (si) targeting ETS2 (si-ETS2; 100 nM; sense, 5'-GGGAACAUCCUAGGAAUAAAUTT-3' and antisense, 5'-AUUGCUCCAGAUUGUCCTTT-3') or si-NC (100 nM; sense, 5'-UUCCGGAACGUGCAGT-3'; antisense, 5'-ACCCCUACUGCAUAAUAA-3') using Lipofectamine® 2000 reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. miR-155 mimic, miR-155 inhibitor, miRNA negative control, pcDNA3.0, pcDNA3.0-ETS2, si-NC and si-ETS2 were purchased from Nanjing KeyGen Biotech Co., Ltd. Cells were transfected for 48 h prior to subsequent experimentation.

**Cell viability.** Cell viability was determined using a Cell Counting Kit-8 assay (CCK-8; Dojindo Molecular
Technologies, Inc.), according to the manufacturer's protocol. Briefly, 2x10^4 H9c2 (2-1) cells/well were plated into 96-well plates and incubated in a humidified atmosphere with 5% CO₂ at 37°C. Following incubation for 24, 48 and 72 h, 10 µl CCK-8 solution was added to each well and cultured for 1 h at 37°C. The optical density of each well at 450 nm was recorded using a Varioskan Flash microplate reader (Thermo Fisher Scientific, Inc.).

Flow cytometric analysis of apoptosis. Briefly, a total 1x10^5 of H9c2 (2-1) cells were seeded on a six-well plate and incubated in a humidified atmosphere with 5% CO₂ at 37°C with 70% confluency overnight. Following transfection and incubation for 48 h, the rate of apoptosis was determined using the Annexin V-FITC Apoptosis Detection kit (BioVision, Inc.), according to the manufacturer's protocol. Apoptotic cells were analyzed using a BD FACScan™ flow cytometer (BD Biosciences).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from tissue and cell lines using TRIzol™ reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. To determine mRNA expression levels, 2 µg RNA was reversed transcribed into cDNA using the PrimeScript RT Master mix (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. qPCR was subsequently performed using the SYBR® Green PCR Master mix (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The following primer pairs were used for the qPCR: GPR18 forward, 5'-CCA CCA AGA AGA GAA CCA C-3' and reverse, 5'-GAA GGG CAT AAA GCA GAC G-3'; ETS2 forward, 5'-GTG GAC CTA TTC AGC TGT GG-3' and reverse, 5'-TTC CCC GAC GTC TTG TGG AT-3'; and β-actin forward, 5'-CTG GGA CGA CAT GGA GAA AA-3' and reverse, 5'-AAG GAA GCC TGG AAG AGT GC-3'. The following thermocycling conditions were used for the qPCR: 50°C for 3 min; and 40 cycles of 95°C for 10 sec and 60°C for 30 sec. β-actin was used as the internal loading control.

For miRNA, 2 µg total RNA was reversed transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription kit according to the manufacturer's protocol (Applied Biosystems; Thermo Fisher Scientific, Inc.). qPCR was performed using SYBR® Green PCR Master mix (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The following primer pairs were used for the qPCR: miR-155 forward, 5'-GCG GTT AAT GCT AAT CGT GAT-3' and reverse, 5'-GTG CAG GGT CCG AGG T-3'; and U6 forward, 5'-CTC GCT TCG GCA GCA CA-3' and reverse, 5'-AAC GCT TCA CGA ATT TGC GT-3'. The following thermocycling conditions were used for the qPCR: 95°C for 10 min; and 40 cycles of 95°C for 15 sec, 60°C for 30 sec and 60°C for 1 min. U6 was used as the internal loading control for the miRNA. mRNA and miRNA expression levels were quantified using the 2^(-ΔΔCq) method (22).

Western blot analysis. Total protein was extracted from cells using RIPA lysis buffer containing a protease inhibitor cocktail (Roche Diagnostics GmbH). Total protein was quantified using a BCA assay kit (Pierce; Thermo Fisher Scientific, Inc.) and 25 µg protein/lane was separated using 10% SDS-PAGE. The separated proteins were subsequently transferred onto PVDF membranes (GE Healthcare) and blocked for 1 h at room temperature with 5% non-fat milk. The membranes were incubated with primary antibodies against GPR18 (1:2,000; cat. no. PA5-23218; Invitrogen; Thermo Fisher Scientific, Inc.) and β-actin (1:5,000; cat. no. sc-69879; Santa Cruz Biotechnology, Inc.) at 4°C overnight. Following the primary antibody incubation, membranes were incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (1:5,000; cat. no. sc-2030; Santa Cruz Biotechnology, Inc.) and goat anti-mouse IgG secondary antibodies (1:5,000; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) at room temperature for 1 h. Protein bands were visualized.
Figure 2. PPI network for DEG-encoded proteins and Module Analysis. (A) PPI network for Module A and B. (B) A total of 17 nodes and 136 relationship pairs with a score of 12 were identified in Module A. (C) A total of 15 nodes and 84 relationship pairs were identified in Module B. Pink circles represent upregulated DEG-encoded proteins, green squares represent downregulated DEG-encoded proteins, and the yellow area represents isolated modules in the network. PPI, protein-protein interaction; DEGs, differentially expressed genes.

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Table I. Top Kyoto Encyclopedia of Genes and Genomes pathways enriched by differentially expressed genes.
using an ECL plus kit (GE Healthcare) and analyzed with ChemiDoc XRS+ luminescent image analyzer (Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc.) and data are presented as the mean ± standard deviation of three experimental repeats. Statistical differences between 2 groups were determined using Student’s t-tests, whereas differences between >2 groups were determined using one-way ANOVA, with Tukey’s post hoc test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

### Results

**DEGs screening.** After preprocessing, a total of 419 DEGs were identified, including 366 upregulated genes and 53 downregulated genes, which suggested that DEGs could distinguish patients with HF from healthy controls. The bi-clustering of identified DEGs is presented in Fig. 1A, and the volcano plot of DEGs is presented in Fig. 1B.

**Functional enrichment of DEGs.** To further identify the molecular mechanisms that the DEGs participated within, KEGG pathway enrichment analysis for upregulated and downregulated DEGs was conducted using the DAVID online
tool. Due to the low number of downregulated DEGs identified, no KEGG pathway was indicated to be significantly enriched by the downregulated DEGs; however, 40 KEGG pathways were enriched by the upregulated DEGs, including ‘cytokine-cytokine receptor interaction’ (P=3.46x10^{-16}), ‘natural killer cell mediated cytotoxicity’ (P=6.05x10^{-14}) and ‘primary immunodeficiency’ (P=6.77x10^{-14}). The top 10 pathways are presented in Table I.

PPI network and model analysis. According to the STRING database, the PPI network for DEG-encoded proteins was constructed with 148 nodes and 482 edges (Fig. 2A). The top 10 nodes that were expressed to a higher degree were: Lymphocyte-specific protein tyrosine kinase (LCK), cluster of differentiation 3g (CD3G), cluster of differentiation 3e (CD3E), cluster of differentiation 3d (CD3D), cluster of differentiation 247 (CD247), van guanine nucleotide exchange factor 1 (VAV1), C-C motif chemokine receptor 5 (CCR5), C-X-C motif chemokine receptor 4 (CXCR4), lysophosphatidic acid receptor (LPAR2) and C-C motif chemokine receptor 2 (CCR2). Two significantly enriched modules with an enriched score ≥10 were isolated from the PPI network. Module A contained 17 nodes and 136 relationship pairs with a score of 12 (Fig. 2B), which were significantly enriched in the ‘chemokine signaling pathway’ (P=9.85x10^{-15}), ‘cytokine-cytokine receptor interaction’ (P=8.58x10^{-14}) and ‘Toll-like receptor signaling pathway’ (P=6.98x10^{-4}; Table II). A total of 15 nodes and 84 relationship pairs were included in module B with a score of 12 (Fig. 2C), and this module was significantly enriched in 15 KEGG pathways, including ‘T cell receptor signaling pathway’ (P=1.64x10^{-14}), ‘primary immunodeficiency’ (P=4.12x10^{-7}) and ‘hematopoietic cell lineage’ (P=4.59x10^{-7}; Table II).

TF/miRNA-target regulatory network. According to the WebGestalt database, among 10 nodes in this network, there were 8 TFs identified to target the DEGs identified in the current study, including ETS2, pullulanase 1 (PUL1), acute myeloid leukemia protein (AML), interferon-stimulated response elements (ISRE), polyomavirus enhancer activator 3 (PEA3), interferon 1 (IFN1), nuclear factor kappa B (NF-MAPAB) and E26 transformation-specific transcription factor (ETS). Moreover, a total of three miRNAs were predicted to target DEGs: miR-155, miR-21 and miR-519. Through combining the regulatory relationships of TF/gene and miRNA/gene, the TF/miRNA-target regulatory network was constructed (Fig. 3), which included 86 nodes (71 upregulated DEGs and 4 downregulated DEGs) and 138 relationship pairs.

Validation of gene expression in clinical samples. To confirm the findings of the bioinformatics analysis, the expression levels of miR-155, GPR18 and ETS2 were determined in patients with HF. The expression levels of GPR18 and ETS2 were significantly increased in HF samples compared with the healthy controls (P<0.0001; Fig. 4A and C), whereas the expression levels of miR-155 were significantly decreased in HF samples compared with the healthy controls (P<0.0001; Fig. 4B). These results were consistent with the findings observed using bioinformatics.

ETS2 is a TF for GPR18. To further confirm the regulation of the upregulated expression of GPR18, the expression levels of ETS2 were verified and the expression levels of GPR18 were
determined at the cellular level following the use of ETS2 overexpression plasmids or siRNA targeting ETS2, of which their transfection was proved to be successful (Fig. S1A-D). It was demonstrated that the overexpression of ETS2 with the pcDNA3.0-ETS2 plasmid could significantly increase the mRNA and protein expression levels of GPR18 compared with the control group (P<0.001; Fig. 5A and C), whereas the genetic silencing of ETS2 with si-ETS2 could significantly decrease the expression levels of GPR18 in H9c2 (2-1) cells compared with the control group (P<0.001; Fig. 5B and C).
These findings indicated that GPR18 may be a downstream target of ETS2, and ETS2 may target GPR18 to promote the progression of HF.

miR-155 regulates the cell viability and apoptosis of H9c2 (2-1) through GPR18. The regulatory relationship between GPR18 and miR-155 was also verified at the cellular level following the use of miR-155 mimics or miR-155 inhibitors, of which their transfection was proved to be successful (Fig. S1E). The results revealed that the overexpression of miR-155 mimic decreased the expression levels of GPR18 (P<0.01), whereas the miR-155 inhibitor increased the expression levels of GPR18 in H9c2 (2-1) cells compared with the control group (P<0.001; Fig. 6A and B). The increased expression of miR-155 was also indicated to significantly increase the cell viability in H9c2 (2-1) cells (P<0.01), but attenuated apoptosis compared with the control group (P<0.001), whereas the decreased expression of miR-155 using miR-155 inhibitor demonstrated opposite effects to the miR-155 mimic-transfected cells (P<0.001; Fig. 6C-E). These findings indicated that miR-155 might inhibit the apoptosis of H9c2 (2-1) through downregulating the expression of GPR18.

**Discussion**

HF is a common clinical syndrome worldwide characterized by heart structure damage and/or heart dysfunction, resulting in fatigue and dyspnea at rest (23). HF is a multifactorial disease and its development is associated with a complex and sophisticated regulation, but the exact mechanism remains to be elucidated (24). Although numerous researches have been performed (25,26), the exact mechanism of HF remains to be elucidated. In the present study, a total of 419 DEGs were identified and their encoding proteins were used to construct a PPI network, and GPR18 was identified as a hub node in this network. Further bioinformatics and
experimental analyses showed that GPR18 could be targeted by miR-155 and the TF ETS2, suggesting that miR-155 and ETS2 might play critical role in the development of HF via targeting GPR18.

GPR18 is a deorphaned lipid receptor that can be activated by behaviorally inactive atypical cannabinoid and N-arachidonoyl glycine (27). A number of tissues have reportedly demonstrated very low expression of GPR18, including the brain, heart, liver, lung and ovaries (28). A previous study identified that GPR18 serves a critical role in modulating cardiovascular function (24) and Penumarti and Abdel-Rahman (29) reported that GPR18 mediated hypertension through activating the PI3K/AKT/ERK/nNOS signaling pathway and suppressing cAMP in an animal model (30). However, the function of GPR18 in HF is relatively unreported. In the present study, GPR18 expression levels were significantly increased in patients with HF compared with healthy controls, which was further validated with clinical studies; GPR18 expression was significantly elevated in patients with HF compared with healthy controls. All of these findings indicated that GPR18 may serve a role in promoting the pathogenesis of HF.

ETS2, encoded by E26 oncogene homolog 2, serves an important role in the pathogenesis of cardiovascular disease (31). Previous studies have demonstrated that ETS2 and Mesp1 are two important TFs involved in the reprogramming of fibroblasts into cardiac progenitor-like cells (32,33). In addition, Rowell et al (34) revealed that reduced ETS2 expression in endothelial progenitor cells was a beneficial biomarker of sitagliptin efficacy in patients undergoing coronary artery-bypass grafting. The current study also revealed that ETS family members, including ETS-related transcription factor 1 (Elfi1) and ETS2, were upregulated in late-HF (34). In the present study, ETS2 expression levels were found to be significantly increased in patients with HF compared with the healthy controls. Moreover, the overexpression of ETS2 significantly increased the expression levels of GPR18, whereas the silencing of ETS2 expression significantly reduced the expression levels of GPR18 in H9c2 (2-1) cells. These results indicated that GPR18 may be a downstream target for ETS2. Considering this evidence, it was hypothesized that ETS2 may promote the pathogenesis of HF through targeting and regulating GPR18 expression.

miR-155 is an established miRNA that is involved in the regulation of a number of different cancer pathways, as well as in cardiovascular diseases (35). Bao et al (36) demonstrated that miR-155 and miR-148a could reduce cardiac injury through suppressing NF-kB expression in acute viral myocarditis. Moreover, miR-155 was demonstrated to serve as a potential marker for arrhythmic risk in patients with chronic heart failure (37) and Matsumoto et al (38) revealed that miR-155 is a risk factor for cardiac death. In the current study, miR-155 expression was significantly decreased in patients with HF, and it was observed to promote the proliferation of H9c2 (2-1) cells and reduce apoptosis in H9c2 (2-1) cells through negatively regulating the expression levels of GPR18. These findings suggested that miR-155 may serve a crucial role in regulating cell viability and apoptosis in HF through targeting and regulating GPR18 expression.

There are multiple limitations associated with the current study: For example, because no relevant clinical information was provided for the GSE84796 dataset in the GEO database, investigations between the molecular mechanisms and clinical features were not analyzed in the present study. Additionally, due to time and resource limitations, an insufficient number of matched heart tissue samples were collected from patients to confirm the results of the current study. Due to this, the study used blood samples instead to perform validation studies, but further validation in clinical samples is required in the future. Additionally, due to the limited funding for the current study, only H9c2 (2-1) cell lines were used for the following experimental validations and further validation in additional human cell lines, including HCM HJ1-I, are required to further validate the results of the current study. Finally, also due to the limited funds available, further investigations into ETS2 and subsequent pathway analysis were not performed in the present study. However, despite these limitations, the findings in the present study may provide some novel insight in understanding HF.

In conclusion, both GRP18 and ETS2 expression levels were demonstrated to be significantly increased in HF compared with healthy controls, whereas the expression levels of miR-155 were significantly reduced in HF compared with healthy controls. These findings suggested that GPR18 may be targeted by EST2 and miR-155, and miR-155 may promote cell viability and suppress apoptosis in HF through targeting and regulating GPR18.

Acknowledgements
Not applicable.

Funding
No funding was received.

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

Authors' contributions
XM and JL designed all the experiments and revised the paper; HS, YC and YZ performed the experiments; and YC and XM wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Gansu Provincial Hospital and informed consent was obtained from all participants.

Patient consent for publication
Not applicable.
Competing interests

The authors declare that they have no competing interests.

References


