Knockdown circ_0040414 inhibited inflammatory, apoptosis and promoted proliferation of cardiomyocyte via miR-186/PTEN/AKT pathway in chronic heart failure

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Research article

Keywords: Circ_0040414, CHF, miR-186, PTEN/AKT

DOI: https://doi.org/10.21203/rs.3.rs-506386/v1

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Abstract

Background

Previous studies have found that the expression of circ_0040414 is elevated in heart failure (HF) patients, but the role and mechanism of circ_0040414 in HF are not yet known. The purpose of this study is to clarify the role of circ_0040414 in chronic HF (CHF) and its potential mechanism for regulating the progression of CHF.

Methods

Peripheral blood samples of patients with chronic heart failure and normal people were collected to detect the expression difference of circ_0040414; ROC curve was made to evaluate the value of circ_0040414 in the diagnosis of CHF; CCK8 method was implemented to detect the speed of cell proliferation; flow cytometry was performed to detect the degree of cell apoptosis; ELISA assay was used to detect the inflammatory cytokines IL-6, TNF-α and IL-β secreted by cells; circinteractome and starbase databases were used to analyze the downstream target molecules of circ_0040414 and miR-186, respectively; RT-qPCR and western blot were implemented to evaluate the expression levels of RNA and protein, respectively.

Results

We found that the expression of circ_0040414 in the peripheral blood of CHF patients is significantly higher than that of normal people, and it has a high diagnostic value for CHF. Circ_0040414 promotes cardiomyocyte apoptosis and inflammation, while inhibiting the proliferation of cardiomyocytes. In terms of mechanism, circ_0040414 promotes the expression of Phosphatase and Tensin Homolog (PTEN) through sponge miR-186 and inhibits AKT signal activity.

Conclusion

We clarified that silencing circ_0040414 promotes cardiomyocyte proliferation through miR-186/PTEN/AKT signal axis, inhibits cell apoptosis and inflammation.

Background

Myocardial dysfunction is a crucial feature of cardiomyopathy caused by various reasons. Myocardial dysfunction eventually leads to heart failure (HF), which has a high incidence worldwide [1, 2]. Hypertension, diabetes and obesity can also increase heart pressure and cause heart failure. In addition, genetic factors are also a crucial factor in the onset of heart failure [3]. Chronic HF (CHF) is characterized by insufficient heart pumping ability and/or filling disorder, and the ability of heart to promote blood to
combine with oxygen is decreased, so it is also called congestive heart failure. CHF makes it difficult for patients to breathe, unable to exercise or even die [4, 5]. For CHF, the current treatment strategies carry many side effects, such as arrhythmia, and are not effective and safe, leading to a higher mortality rate [6, 7]. Therefore, there is an urgent need to find safer and more effective treatment strategies for CHF.

Circular RNA (circRNA) is a non-coding RNA with an important regulatory role, and it usually exists in organisms as a closed circular structure with a longer half-life [8]. Studies in recent years have also found that some circRNAs encode small peptides with important biological functions, and such circRNAs usually carry ORF and IRES sequences [9]. MicroRNAs (miRNAs) are non-coding RNAs with a length of 19–25 nucleotides [10], which inhibit the translation of target mRNA by binding to the 3'UTR of the target mRNA, thereby regulating a series of cell life activities [11, 12]. A large number of studies have clarified the role of miRNA in various diseases. There is evidence that miRNAs play a crucial role in tumor progression[13]. In patients with idiopathic pulmonary fibrosis (IPF), miR-186 inhibits the expression of collagen V and can be used as a potential biomarker and therapeutic target of IPF [14]. In addition, the down-regulation of microRNA-186 leads to apoptosis of rat cardiomyocytes, and microRNA-186 is a potential target of inflammation-related cardiac damage [15]. CircRNA has sponge activity, which can regulate the expression of target protein by inhibiting microRNA and play a role in various diseases. For example, in gastric cancer and breast cancer, CircRNA acts as a microRNA sponge to promote cancer progression [16, 17]. In addition, circFGFR4 and circINSR promote the differentiation and proliferation of myoblasts cells through the spongeization of miR-107 and miR-34a, respectively [18, 19]. However, there are few studies on circRNA in HF. Existing studies have found that circ_0040414 is highly expressed in patients with HF [20], but its specific role in heart failure and its molecular regulation mechanism with miR-186 have not been studied yet.

PI3-K/Akt signal regulates a series of cell functions, including cell proliferation, survival and apoptosis [21]. Phosphatase and Tensin Homolog (PTEN) acts as a negative regulator of PI3-K/Akt signal by phosphorylating PIP3 to PIP2, inhibiting the activity of PI3-K/Akt signal [22]. The function of PTEN/PI3-K/Akt signal has been reported in a variety of diseases. Studies have found that the inactivation of PTEN promotes cancer progression by activating PI3-K/Akt signal [23]. Studies have also found the pharmacological effects of PTEN, and inhibition of PTEN plays an important role in preventing liver ischemia/reperfusion injury [24, 25]. Inhibition of PTEN activity relieves myocardial infarction symptoms and improves heart function [26]. However, whether and how the PTEN/PI3-K/Akt signal axis regulates CHF remains to be further studied.

In our study, we first compared the expression of circ_0040414 in peripheral blood of patients with chronic heart failure and normal people and confirmed the diagnostic potential of circ_0040414 in patients with CHF. Then, the relationship between circ_0040414 and CHF was determined and the stability difference between circ_0040414 and linear RNA was further compared. Subsequently, we clarified the effect of circ_0040414 on cardiomyocyte function, including proliferation, inflammation and apoptosis by overexpressing or silencing circ_0040414 in cardiomyocyte cell lines. Finally, we found the downstream signaling molecules of circ_0040414 through the circinteractome and starbase databases.
Significantly, we provide new insights and possible treatment reference strategies for the relief of heart failure.

**Materials And Methods**

**Tissue samples**

Peripheral blood samples of 50 patients with chronic heart failure and normal volunteers were collected from the Department of Cardiology, HeXian Memorial Hospital in Guangzhou (Guangzhou, China). All participants have been informed of the details and signed informed consent and all assays have been supported by the Medical Ethics Committee of HeXian Memorial Hospital in Guangzhou.

**Cell culture and transfection**

Human cardiomyocytes AC16 and HCM were cultured in DMEM/F12 basic medium, supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, MD, USA) and 100 U/mL penicillin and 100 µg/mL streptomycin. Both were cultured in a 37°C 5% CO2 environment. For the transfection of plasmid, RNA *interference* (RNAi), miRNA and their controls, lipofectamine 2000 (Invitrogen, California, USA) was taken to carry out, and then the old cell culture medium was replaced with fresh culture medium 6 hours after transfection.

**Quantitative PCR analysis**

The total RNA of cells and whole blood was extracted using the trizol (Thermo Fisher Scientific, Gaithersburg, MD, USA) method according to the instructions. The concentration and purity of the extracted RNA were tested by NanoDorp. Then 5ug of RNA was reverse transcribed into cDNA and the cDNA was finally diluted to 40ng/µl. SYBR premix EX TAQ II kit (Takara, Dalian, China) was used to perform amplification in the 7500 Real Time PCR System (Applied Biosystems/Life Technologies, Carlsbad, CA, USA), and the results were calculated when the assay was over. GAPDH serves as an internal reference. All primer sequences were from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China).

**CCK8**

The AC16 and HCM cells that overexpress or interfere with circ_00404141 were seeded into 96-well plates. The cells were cultured for 0h, 24h, 48h and 72h in a cell incubator, and then 10ul of CCK8 reaction solution was added to the cell culture medium at the indicated time and incubated with the cells for 4 hours. Subsequently, the light absorption values (OD value) of cells cultured for different time was measured at a wavelength of 450 nm using a microplate reader (Thermo Fisher Scientific).

**Flow cytometry**
Apoptosis detection was performed using the Apoptosis Detection Kit (PharMingen, San Jose, CA, USA), AC16 and HCM cells with indicated treatment were trypsinized, washed twice with PBS, and then resuspended in staining solution. Subsequently, Annexin V-FITC and PI were added to the cell resuspension, incubated with the cells in the dark for half an hour, then the cell suspension was centrifuged, the cells were washed twice with PBS, and BD FACSCantoTM II Flow Cytometer (BD Biosciences) was used for apoptosis detection.

**Western blot**

The cells were lysed by RIPA Lysis Buffer on ice, and the protein concentration was determined by the BCA kit (Thermo Fisher Scientific). Proteins was separated by polyacrylamide gel and transferred to PVDF membrane. The PVDF membrane loaded with protein was blocked by 5% skimmed milk for 1 hour, and then incubated with primary antibody anti-Bax (CST, #5023), anti-Cleaved Caspase-3 (CST, #9661), anti-Bcl-2 (CST, #15071), anti-PTEN (CST, #9188), anti-p-AKT (CST, #4060), anti- AKT (CST, #4685), anti-GAPDH (CST, #5174) at 4°C overnight. Subsequently, the PVDF membrane was washed 3 times with TBST for 5min each time. The washed membrane was incubated with the HRP-conjugated secondary antibody (sigma) for 1 hour, and washed 5 times with TBST for 10 minutes each time. Finally, the PVDF membrane was incubated with the enhanced chemiluminescence reagent (Thermo Fisher Scientific) in a dark room, and then the results were analyzed. ImageJ software was used to perform grayscale analysis.

**ELISA**

the supernatant of AC16 and HCM cells with indicated treatment was collected, and then the concentrations of IL-6, TNF-α and IL-β in the supernatants were detected using a sandwich ELISA kit (eBioscience, San Diego, California, USA) according to the manufacturer's instructions.

**RNA Subcellular Isolation**

The RNA Subcellular Isolation Kit (Active Motif, California, USA) was used to separate nuclear and cytoplasmic RNA according to the manufacturer's instructions. The concentration of RNA was measured by NanoDorp, and reverse transcription was performed using the SYBR premix EX TAQ II kit (Takara). The expression level of circ_00404141 was determined by RT-qPCR.

**Luciferase reporter assay**

AC16 and HCM cells were co-transfected with miR-NC/miR-186 and WT-circ_00404141/mut-circ_00404141 dual luciferase reporter plasmid using Lipofectamine 2000 reagent (Invitrogen). The dual luciferase reporter plasmid was constructed with the pmiRGLO vector (Promega, Madison, WI, USA). The cells were incubated at 37°C with 5% CO2 for 48 hours, and then taken out and washed twice with PBS. Subsequently, the luciferase activity was detected by the dual luciferase reporter system (Promega). The binding of miR-186 to PTEN mRNA was also detected by the above method.

**Statistical analysis**
SPSS 13 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of data. The data was presented as mean ± standard deviation (SD). Student’s t-test was implemented to compare the difference between the experimental group and the control group. Two-way ANOVA was performed to compare the differences between different treatment groups at different time points. \( P<0.05 \) was considered statistically significant.

**Results**

**circ_0040414 is highly expressed in peripheral blood of patients with chronic heart failure**

In order to explore the relationship between circ_0040414 and CHF, we conducted qRT-PCR assays to detect the difference in the expression of circ_0040414 in the peripheral blood of 50 patients with CHF and normal people and found that circ_0040414 was more highly expressed in the peripheral blood of patients with CHF than that in normal people (Fig. 1A). The subsequent ROC curve analysis of the diagnostic potential of circ_0040414 in the peripheral blood of patients with chronic heart failure and normal people confirmed that circ_0040414 is of great significance in the diagnosis of heart failure (Fig. 1B). In order to further identify circular RNA, actinomycin D was used to inhibit the synthesis of RNA in cardiomyocytes AC16, and then detected the expression of linear RNA and Circular RNA at 0, 4, 8, and 12 hours. We confirmed that linear 0040414 expression decreased, while circ_00404141 has no significant change after treatment with actinomycin D (Fig. 1C). Then we digested RNA with RNase R, and the results confirmed that RNase R digested linear 0040414 to a large extent, but could not digest circ_00404141 in AC16 (Fig. 1D). The above results indicate that circ_0040414 is directly associated with CHF and has the characteristics of circular RNA, which is not easy to be digested by RNase R.

**circ_00404141 promotes apoptosis and inflammation, inhibits proliferation of cardiomyocytes**

In order to further explore the effect of circ_00404141 on cardiomyocyte proliferation, inflammation and apoptosis, we selected human cardiomyocyte cell lines AC16 and HCM, and overexpressed and silenced circ_00404141 in both cells. We further verified the overexpression and silencing efficiency of circ_00404141 in cells (Fig. 2A and B). We further evaluated the proliferation ability of AC16 and HCM cells by the CCK8 method, and found that overexpression of circ_00404141 inhibited the proliferation of AC16 and HCM cells, while silencing circ_00404141 promoted the proliferation of both cells (Fig. 2C). We detected the apoptosis ratio of AC16 and HCM cells using flow cytometry, and found that the expression of circ_00404141 promoted cell apoptosis (Fig. 2D). We further detected the expression of pro-apoptotic proteins Bax and Cleaves-Caspase-3, and anti-apoptotic protein Bcl-2 in AC16 and HCM cells, and confirmed that overexpression of circ_00404141 promoted the expression of Bax and Cleaved-caspase 3 and reduced the expression of Bcl-2, while the silencing of circ_00404141 decreased the expression of Bax and cleaves-caspase 3, and increased the protein level of Bcl-2 (Fig. 2E). The ELISA method further detected the levels of inflammatory factors IL-6, TNF-α and IL-β in AC16 and HCM cells. Overexpression of circ_00404141 increases the levels of IL-6, TNF-α and IL-β. Knockdown of circ_00404141 reduced the levels of IL-6, TNF-α and IL-β in cells (Fig. 2F-H).
MiR-186 is the downstream target of circ_00404141

In order to further explore the mechanism by which circ_00404141 plays a crucial role in chronic heart failure, nucleocytoplasmic separation assays were performed in AC16 and HCM cells to detect the cell sub-localization of circ_00404141. circ_00404141 is mainly located in the cytoplasm, and U6 and GAPDH are internal controls for the nucleus and cytoplasm, respectively (Fig. 3A). Further analysis of the circinteractome database revealed that miR-186 is the downstream target molecule of circ_00404141 (Fig. 3B). We performed dual luciferase reporter assays in AC16 and HCM cells, inserting the fragments including binding site of circ_00404141 and miR-186 and its mutant fragments into the luciferase reporter gene plasmid to construct a dual-luciferase reporter plasmid. The results showed that overexpression of miR-186 inhibited luciferase activity in cells compared with miR-NC (Fig. 3C). After further silencing circ_00404141, the expression of miR-186 increased (Fig. 3D). Subsequently, the expression of miR-186 was detected in the peripheral blood of 50 patients with CHF and normal people, and the expression of miR-186 was low in the peripheral blood of patients with CHF (Fig. 3E). ROC curve analysis was performed to evaluate the diagnostic potential of miR-186 in the peripheral blood of patients with CHF and normal people and found that miR-186 was of great significance for the diagnosis of CHF (Fig. 3F). The above results indicate that miR-186 is a downstream target of circ_00404141 and may be used as a diagnostic indicator of CHF.

circ_00404141 regulates cardiomyocyte proliferation, apoptosis and inflammation by targeting miR-186

In order to further verify the mediating role of miR-186 in the regulation of CHF by circ_00404141, we silenced circ_00404141 in AC16 and HCM cells and co-transfected with miR-186 inhibitor at the same time, setting them as 3 groups (si-NC, si-circ_00404141, si-circ_00404141+miR-186 inhibitor). The results showed that knocking down circ_00404141 significantly increased the expression of miR-186, while miR-186 inhibitor reduced the level of miR-186 regulated by circ_00404141 (Fig. 4A). Further CCK8 and ELISA assays showed that knocking down circ_00404141 enhanced the proliferation ability and inhibited the expression of inflammatory factors IL-6, TNF-α and IL-β of cells, while miR-186 inhibitor weakened the proliferation ability (Fig. 4B) and promoted the expression of inflammatory factors of the cells regulated by circ_00404141 (Fig. 4E-G). For the level of cell apoptosis, flow cytometry and western blot assays confirmed that knocking down circ_00404141 greatly reduced the proportion of apoptosis cells and pro-apoptotic proteins Bax and Cleaves-Caspase-3, and enhanced the level of anti-apoptotic protein Bcl-2, while miR-186 inhibitor increased the proportion of cell apoptosis (Fig. 4C) and pro-apoptotic proteins Bax and Cleaves-Caspase-3, and inhibited the level of anti-apoptotic protein Bcl-2 regulated by circ_00404141 (Fig. 4D). The above results indicate that miR-186 is the target of circ_00404141 to regulate cardiomyocyte proliferation, apoptosis and inflammation.

Circ_00404141 regulates PTEN/AKT signaling pathway in cardiomyocytes through miR-186

In order to further explore the downstream signaling pathway that circ_00404141 plays a regulatory role in cardiomyocytes through miR-186, we predicted that miR-186 targets PTEN through the starbase database (Fig. 5A). The luciferase reporter assay further confirmed that miR-186 binding to PTEN mRNA
in AC16 and HCM cells (Fig. 5B). Subsequently, western blot showed that knocking down circ_00404141 in AC16 and HCM cells reduced the protein level of PTEN and increased the expression of activated p-AKT, that is, increased the ratio of p-AKT/AKT, while the miR-186 inhibitor partly up-regulate the expression of PTEN and down-regulate p-AKT (Fig. 5C and D). The above results clarify that circ_00404141 regulates the activity of PTEN/AKT signaling pathway through miR-186.

**Discussion**

Myocardial remodeling leads to CHF [27, 28], which makes the cardiac dysfunction and inability to promote blood flow. The process of remodeling involves cardiomyocyte damage, including cell necrosis and hypertrophy. Therefore, blocking myocardial remodeling has become an effective treatment strategy for alleviating HF [29]. The regeneration potential of cardiomyocyte is poor[30], so preventing the loss of cardiomyocytes is essential for the treatment of heart-related diseases. Apoptosis is a type of programmed cell death and a major factor of cell loss. It plays a crucial role in myocardial remodeling, participates in the progression of certain diseases and leads to organ dysfunction [31]. Therefore, reducing cardiomyocyte apoptosis and enhancing its proliferation capacity are the primary treatment strategies for alleviating CHF.

Studies have found that a variety of circular RNAs regulate the progression of HF. For example, hsa_circ_0112085, hsa_circ_0062960 and hsa_circ_0053919 are highly expressed in patients with HF, and it has been proven that hsa_circ_0062960 is one of the diagnostic markers of HF [32]. It was also found that TGF-β1/Smad3 signaling pathway mediates the effect of circ_0062389 on cardiomyocyte apoptosis in HF rats, and silencing circ_0062389 inhibits cardiomyocyte apoptosis in HF rats [33]. In addition, the expression of hsa_circ_0097435 is increased in patients with HF, and its overexpression promotes apoptosis, and controls the progression of HF by inhibiting the expression of a variety of microRNAs [20]. Recent studies have found that the expression of circ_0040414 is elevated in patients with HF[20], but it is unknown whether it plays a controlling role in HF through spongy microRNA like other circular RNAs. Our study has discovered the role and regulation mechanism of circ_0040414 in HF. As a promoter of CHF, circ_0040414 regulates cardiomyocyte proliferation, inflammation and apoptosis through sponging miR-186.

As a negative regulator of PI3K/AKT signaling, PTEN participates in the regulation of cardiomyocyte apoptosis [34, 35]. Studies have shown that the deletion of long non-coding RNA AZIN2-sv improves cardiac function and prevents heart failure. And AZIN2-sv exerts this function through the regulation of miR-214 /PTEN/AKT signal axis[36]. The above studies have discovered the regulatory role of the microRNA/PTEN/AKT pathway in heart disease. But which microRNA and how to regulate CHF have been further explored in our study. Our study found that miR-186 also has the same effect as miR-20b in heart failure. MiR-186 activates AKT signaling by binding to PTEN and inhibiting the activity of PTEN, thereby regulating a series of functions of cardiomyocytes, including promoting the proliferation and inhibiting inflammation and apoptosis of cardiomyocytes.
In summary, we clarified the role of circ_0040414 in CHF and its potential regulatory mechanism. We found that circ_0040414 is highly expressed in peripheral blood samples of patients with CHF. The low expression of circ_0040414 promotes the proliferation of cardiomyocytes, inhibits cell apoptosis and inflammation, and improves the symptoms of patients with CHF. In terms of mechanism, circ_0040414 acts as a miR-186 sponge to inhibit miR-186 and promotes the expression of PTEN, while PTEN hinders the AKT signaling pathway activity. Silence circ_0040414 activates the AKT signaling pathway and relieves the condition of patients with CHF. Our study may provide new insights and reference strategy for the treatment of patients with heart failure and enable us to have a deeper understanding of the mechanisms of heart failure.

**Abbreviations**

CHF: chronic heart failure; PTEN: Phosphatase and Tensin Homolog; CircRNA Circular RNA; miRNAs: MicroRNAs; IPF: pulmonary fibrosis.

**Declarations**

**Ethics approval and consent to participate**

All participants have been informed of the details and signed informed consent and all assays have been supported by the Medical Ethics Committee of HeXian Memorial Hospital in Guangzhou.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets generated during and/or analysed during the current study are available in the circinteractome database (https://circinteractome.nia.nih.gov/) and starbase database (http://starbase.sysu.edu.cn/index.php).

**Conflicts of interest**

The authors declare no conflict of interests.

**Funding**

Not applicable.

**Authors’ Contributions**

Y.F.Z. and Y.L.F. conceived and designed the project, Y.L.F., H.J.C., J.L.W., P.Z., D.Z., and G.X.L. acquired the data, Y.F.Z., Y.L.F., Q.X.C. and Y.X.D. analysed and interpreted the data, Y.F.Z. and Y.L.F. wrote the paper.
All authors have read and approved the manuscript and ensure that this is the case.

Acknowledgements

Not applicable.

References


Figures

**Figure 1**

Circ_0040414 is highly expressed in the peripheral blood of patients with chronic heart failure and has diagnostic value for CHF. A The expression of RNA in peripheral blood of 50 patients with chronic heart failure and normal people was evaluated by RT-qPCR. B The diagnostic potential of circ_0040414 in patients with chronic heart failure was analyzed by ROC curve. C The cardiomyocyte AC16 was treated with actinomycin D to inhibit the synthesis of total RNA, and then RT-qPCR was performed to detect the expression levels of TXNL4B mRNA and circ_0040414 at the indicated time. D AC16 cells were treated with RNase R, and then RT-qPCR was conducted to evaluate the expression levels of TXNL4B mRNA and circ_0040414. *, P < 0.05, **, P < 0.01, and ***, P < 0.001. The error bars are defined as s.d.
Figure 2

Circ_00404141 regulates the proliferation, inflammation and apoptosis of cardiomyocytes. The circ_00404141 overexpression plasmid, small interfering RNA targeting circ_00404141 and their controls were transfected into cardiomyocytes AC16 and HCM, respectively. A, B The overexpression (A) and interference efficiency (B) of circ_00404141 in AC16 and HCM cells were tested by RT-qPCR. C The proliferation ability of AC16 and HCM cells with the indicated treatment was assessed by CCK8. D The
apoptosis level of AC16 and HCM cells with indicated treatment was detected by flow cytometry. E The expression of apoptosis-related proteins Bax, Bcl-2 and cleaved-caspase 3 in AC16 and HCM cells with indicated treatment were detected by western blot. Full-length blots/gels are presented in Additional file 1. F-H The levels of IL-6 (F), TNF-α (G) and IL-β (H) secreted by AC16 and HCM cells with indicated treatment were detected by ELISA. *, P < 0.05, **, P < 0.01, and ***, P < 0.001. The error bars are defined as s.d.

Figure 3

Circ_00404141 is mainly located in the cytoplasm and targets miR-186. A Nuclear and cytoplasmic fraction separation assays were carried out in AC16 and HCM cells. Subsequently, the expression of circ_00404141 in the nucleus and cytoplasm was detected by qRT-PCR, and U6 and GAPDH were used as internal controls for the nucleus and cytoplasm, respectively. B Circ_00404141 targets miR-186 and is predicted by the circinteractome database. C The binding of circ_00404141 to miR-186 in AC16 and HCM cells with indicated treatment was evaluated by the luciferase reporter assay. D The expression level of miR-186 in AC16 and HCM cells after knocking down circ_00404141 was detected by RT-qPCR. E The expression of miR-186 in the peripheral blood of 50 patients with chronic heart failure and normal people
was assessed by RT-qPCR. F The diagnostic value of miR-186 in patients with chronic heart failure was analyzed by ROC curve. *, P < 0.05, **, P < 0.01, and ***, P < 0.001. The error bars are defined as s.d.

Figure 4

Circ_00404141 controls the proliferation, inflammation and apoptosis of cardiomyocytes through sponge miR-186. AC16 and HCM cells were transfected with si-NC, si-circ_00404141 and si-circ_00404141+miR-186 inhibitor, respectively. A The expression level of miR-186 in AC16 and HCM cells treated as above was assessed by RT-qPCR. B The light absorption value of AC16 and HCM cells treated as above at 450nm was evaluated by CCK-8. C cell apoptosis levels of AC16 and HCM cells treated as above were detected by flow cytometry. D The expression of apoptosis-related proteins Bax, Bcl-2 and cleaved-caspase 3 in AC16 and HCM cells treated as above was assessed by western blot. Full-length blots/gels are presented in Additional file 2. E-G The levels of inflammatory factors IL-6 (E), TNF-α (F) and IL-β (G)
secreted by the cells were evaluated by ELISA. *, P < 0.05, **, P < 0.01, and ***, P < 0.001. The error bars are defined as s.d.

Figure 5

MiR-186 mediates the regulation of PTEN/AKT signaling pathway by circ_00404141 in cardiomyocytes. A The downstream targets of miR-186 are predicted by starbase. B The binding of miR-186 to PTEN mRNA in AC16 and HCM cells was evaluated by the luciferase reporter assay. C, D The expression of AKT signaling pathway related proteins PTEN, p-AKT and AKT in AC16 (C) and HCM (D) cells was detected by western blot. Full-length blots/gels are presented in Additional file 3. *, P < 0.05, **, P < 0.01, and ***, P < 0.001. The error bars are defined as s.d.

Supplementary Files

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