Overexpression of Calreticulin Promotes Cardiac Fibroblasts Activation Via Regulating IRE1 Pathway

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Abstract

Calreticulin (CRT) is an endoplasmic reticulum (ER) chaperone involved in cardiac fibroblasts (CFs) activation. It has been reported that the expression of CRT increased in the process of CFs activation. However, the role of CRT in CFs activation and the mechanism is not yet fully elucidated. Therefore, we aimed to verify whether CRT was involved in CFs activation and the possible mechanism underlying this process. We found that CRT protein level was elevated in AngII-induced CFs activation. Knocking down CRT by its siRNA could decrease the protein expression of connective tissue growth factor (CTGF), α -smooth muscle actin (α -SMA), and transforming growth factor- β (TGF- β), and meanwhile attenuate proliferation and migration ratio of CFs. Moreover, the proliferation and migration rates of CFs were promoted and the expression of CTGF, α -SMA and TGF- β were increased when transfection with high-titer adenovirus of CRT. In AngII-induced CFs, inositol-requiring enzyme 1 (IRE-1), one of the main ER pathways, was inhibited through CRT silence and activated through CRT overexpression. Overall, this study demonstrates that CRT overexpression could promote AngII induced-CFs activation by activating IRE1 pathway, which could be a potential target for CFs activation.

Introduction

Left ventricular remodeling is the major risk factor associated with myocardial failure. Insights into the impairment in contractility of the hypertrophic myocardium have been sought in the biochemistry of cardiomyocytes contraction. Left ventricular remodeling mainly manifests as cardiomyocytes remodeling and remodeling of extracellular matrix (ECM). The remodeling of ECM is characterized by the increase of extracellular matrix secreted by fibroblasts. Cardiac fibrosis represent an important determinant of pathological left ventricular remodeling[1]. This reactive and progressive interstitial and perivascular fibrosis accounts for abnormal myocardial stiffness and ultimately ventricular dysfunction. After myocardial injury, CFs begin to activate, proliferate, and secrete collagen and other extracellular matrix proteins. Upon activation, the CFs could express the smooth muscle marker, α -smooth muscle actin (α -SMA), and meanwhile increase the expression of transforming growth factor- β (TGF- β), connective tissue growth factor (CTGF) and other pro-fibrosis cytokines [2, 3], leading to cardiac fibrosis finally. The stage is set, however, to prevent pathological left ventricular remodeling resulting from myocardial fibrosis as well as to reverse it.

Calreticulin (CRT), an intracellular chaperone protein, is crucial for the proper protein folding and the transportation of proteins through the endoplasmic reticulum (ER). CRT protein consists of N-terminal, C-terminal and the third domains between them. The N-terminal is a cleavable amino acid signal sequence which is responsible for its biological function such as chaperoning and Ca^{2+} -buffering, while the C-terminal contains endoplasmic reticulum retrieval signals.[4] Recently, CRT has been reported as a critical regulator of extracellular functions, including mediating cellular migration. It's well documented that CRT binds Ca^{2+} , which contributes to the homeostasis of Ca^{2+} . CRT has many cellular functions, including lectin-like chaperoning, Ca^{2+} storage and signaling, regulation of gene expression, etc[5]. In recent years, CRT has been demonstrated to also exist in the surface of the cell membrane, cytoplasm, nucleus, and

extracellular matrix, regulating cells proliferation, adhesion, migration, and apoptosis[6]. It is also involved in pathological processes such as wound healing, immune response, neoplasia, cardiocerebrovascular diseases, and diabetes. Previous study found that the CRT expression level increased during CFs activation[7]. Fan X et.al found that CRT over-expression was associated with the activation of the Notch pathway in Heart CF[8]. However, the underlying mechanism and significance of CRT in CFs activation remain controversial.

ER plays a key role in cardiomyocytes for multifunctional organelle support. In fact, proper synthesis and correct folding of proteins in the ER are extremely important for the normal function of the heart[9]. Recent studies indicate that various cellular stresses including hypoxia, ischemia/reperfusion(I/R), pressure overload, hypertrophy, and drug-induced insults may activate ER Stress[10], leading three main ER transmembrane stress sensors, inositol-requiring enzyme 1 (IRE-1), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6), be activated and then initiating the unfolded protein response (UPR) pathways [11]. Under unstressed conditions, BiP/GRP78 directly interacts with IRE1, PERK, and ATF6, but upon an increase in misfolded protein, BiP/GRP78 is sequestered away from these sensors, and the downstream protein of ATF4, GRP94, c-ATF6 and p-IRE1 would increase, allowing activation of the UPR[12]. Targeted transport of ER proteins can cause dilated cardiomyopathy, sarcoplasmic reticulum dilation of cardiomyocytes, increased expression of CHOP in the myocardium and apoptosis. Microarray studies showed that a large number of ER Stress responsive genes such as GRP78, XBP1, and ATF4 were induced to increase within 24 hours after the occurrence of myocardial infarction in mice[13]. This suggests that ER Stress is activated in many cardiovascular diseases and plays a crucial role in the progression of the diseases.

Previous experiments in vitro and in vivo have shown that angiotensin II (AngII) can induce CFs activation through its type I receptor (AT1R)[14]. Therefore, we hypothesized that CRT might play a role in regulating AngII induced CFs activation through ER Stress. This experiment is intended to provide new insights into the pathogenesis of myocardial remodeling and to find new targets for its possible clinical treatment.

Materials And Methods

Culture of CFs

CFs were isolated from cardiac ventricles of SD rats for 0–3 days as described [15]. The minced ventricles were digested with 0.05% trypsin (Roche, USA) and 0.05% collagenase II (Roche, USA), the cells were collected and plated for 75 min at 37°C to allow fibroblasts to attach to the 10-cm cell-culture plates in 10 ml of DMEM with 10% fetal bovine serum (FBS). Then the culture medium was decanted, and the CFs were cultured in fresh medium. CFs were then subcultured, and CFs at the second passage were divided into 6-well culture plates and grown to about 70% confluence. After incubation in DMEM with 10% FBS, the culture medium was changed to serum-free DMEM, and the experiments were performed 4h later.

SiRNA Transfection.

Transient transfection was performed by use of the cationic lipid iMax(Invitrogen, USA) according to the manufacturer’s instruction. The CFs were transfected for 24h with 50 nM siRNA specific for CRT or negative control siRNA before exposure to AngII (10⁻⁵mol/L) for another 24h. The sequences of siRNAs used were showed in Table 1.

Table 1
SiRNA sequences of CRT

SiRNA names	Sequences
SiCRT-1	5'-TATCTATGCCTATGATAGT-3'
SiCRT-2	5'-GCAGACCCTGCCATCTATT-3'
SiCRT-3	5'-CCAAGAAGGTTTCATGTTCAT-3'
Negative control (NC)	Supported by RiboBioTM

Adenovirus Transfection

CRT overexpression adenovirus was designed by HanHeng Bio-Technology Limited (Shanghai). In brief, six hours post-infection, the cells were cultured in DMEM without FBS or antibiotics. After 6 hours, the culture medium was replaced with DMEM containing 10% FBS. Samples which upregulated CRT expression compared to the virus-only control were defined as positive for adenovirus (Ad)-CRT overexpression.

Western Blot

Western Blot

Total proteins were extracted from cells with radio-immunoprecipitation assay (RIPA) lysis buffer. Protein from cell extracts was quantified by Varioskan (Thermo, USA) using a BCA protein assay kit (Pierce, USA). Equivalent amounts of cells lysates (20 µg) were separated by denaturing 10% SDS-PAGE and then transferred to 0.45 µm polyvinylidene difluoride (PVDF) membrane (Millipore, USA) using a MiniProtein III system (Bio-Rad, USA). Membranes were subsequently blocked with 5% skim milk in Tris-buffered saline and Tween 20 (TBST) solution for 2 h and were incubated with primary antibodies at 4°C overnight: anti-CRT antibody diluted at 1:1000, anti-CTGF antibody at 1:500, anti-TGF-β antibody at 1:1000,anti-α-SMA antibody at 1:2000, anti-GRP78 antibody at 1:200, anti-ATF4 antibody at 1:500, anti-c-ATF6 antibody at 1:1000, anti-GRP94 antibody at 1:1000, anti-p-IRE1 antibody at 1:1000, and anti-β-actin antibody diluted at 1:3000. After washing three times for 5 minutes each time in TBST(0.05%Tween),the membrane was incubated with a diluted horseradish peroxidase-labeled secondary antibody(1:3000 anti-rabbit for CRT, CTGF, TGF-β, α-SMA, GRP78, ATF4, GRP94, p-IRE1 and 1:4000 anti-mouse for β-actin, c-ATF6 respectively)

in blotting buffer for 2h at room temperature. The membrane was washed three times, reacted with enhanced chemiluminescence (ECL) substrate kit (Thermo, USA). Specific bands were scanned and quantified by the image Lab analysis software (Bio-Rad, USA). Protein levels were standardized by comparison with those of β -actin.

Proliferation Assays

The proliferative activity of the cells was determined by 5-ethynyl-2-deoxyuridine (EdU) incorporation into the cells (Alexa Fluor 555 Imaging Kit, Invitrogen). The details had been described in the previous report[16]. The proliferation rate was calculated by normalizing the number of EdU positive cells to the DAPI-stained cells in 10 fields at 20 $\times$ magnification under the fluorescence microscope (Olympus).

Immunofluorescence Staining

Cardiac fibroblasts (CFs) were plated on coverlips in 24-well plates. When confluent cells reached 60–70%, the cells were transfected with siRNA, then treated with AngII (10^{-5} mol/L 24hrs). Coverslips were then fixed and blocked as described before [17], followed by exposure to the primary antibodies (anti-CRT 1:100 or anti- α -SMA 1:100, Cell Signaling, USA) at 4°C overnight. After washing with PBS, the cells were incubated with fluorescence-conjugated secondary antibodies for 2h at room temperature, away from light. The second antibody used was Alexa Fluor 488 Goat Anti-Rabbit IgG (1: 400, green fluorescence, Invitrogen, USA) or Alexa Fluor 594 Goat Anti-Rabbit IgG(1 : 400, red fluorescence, Invitrogen, USA). Cells was rinsed and incubated with diluted DAPI for 10 min, away from light. Images were collected using an Eclipse TE2000-U fluorescence microscope system (Nikon, Japan) and analyzed with Image J software (NIH, USA) to semi quantitatively determine the expression of CRT and α -SMA.

Wound Healing Assay

Wound healing assay was carried out to determine the cell protrusion and migration ability of CFs. CFs and their derivative cell lines were seeded into 6-well dishes and grew until 80%-90% confluence. Sterilized 200-microlitre pipette tip was used to generate wounding across the cell monolayer, and the debris was washed with PBS. Migration of cells into the wound was then observed at different time. Cells migrating into the wounded area or protruding from the border of the wound were visualized and photographed under the inverted microscope at different time. A total of nine areas were selected randomly in each well by a 200 $\times$ magnification and total three wells of each group were quantified in each experiment. Experiments were carried out in triplicate at least three times.

Statistical analysis

Data acquired in this study were presented in a (mean $\pm$ S.D.) manner and analyzed by SPSS software (V17.0). Comparisons were performed with ANOVA and t test. $P < 0.05$ was considered statistically significant.

Results

The CRT expression was increased in CFs activation induced by AngII.

In order to observe CFs activation, we used western blot, proliferation assays and wound healing to assess the proliferation, migration and the protein expression of TGF- β , CTGF, and α -SMA under different treatments: (1) control (without treatment); (2) stimulation with AngII (10^{-5} mol/L, 24hrs). As shown in the following images (Fig. 1), AngII treatment led to increased protein expression of TGF- β , α -SMA and CTGF (Fig. 1A) and increased proliferation (Fig. 1B) and mobility rate (Fig. 1C) ($P < 0.05$). These data suggested that AngII stimulation successfully established CFs activation.

In the meanwhile, we observed the protein of CRT was upregulated in AngII group compared with control group by western blot (Fig. 1D) ($P < 0.05$) and Immunofluorescence Staining (Fig. 1E).

Silencing of CRT attenuated CFs activation.

In order to explore the role of CRT in regulating CFs activation, we downregulated CRT protein expression by transfecting CFs with small interfering RNA (SiCRT) which specifically inhibited CRT expression. In the control group, disordered SiRNA which was transfected to CFs (SiNC group) had no effect on any mRNA. Western-blot results showed that all three SiCRT sequences could significantly reduce CRT protein expression ($P < 0.05$, Fig. 2A). SiCRT-1 was used in subsequent experiments.

The degree of CFs activation was compared respectively in the physiological state (SiCRT and SiNC group) and AngII-stimulated conditions (SiCRT + AngII and SiNC + AngII group). The results showed that the protein levels of TGF- β , CTGF and α -SMA in the SiCRT group were respectively decreased by 8%, 24% and 41% ($P < 0.05$). Under the AngII stimulation, compared with SiNC + AngII group, TGF- β , CTGF and α -SMA protein expression reduced by 12%, 25% and 16% ($P < 0.05$) in SiCRT + AngII group (Fig. 2B). In addition, EdU (Fig. 2C) assay showed that the proliferation rate in SiCRT group decreased by 41% ($P < 0.05$) compared with SiNC, SiCRT + AngII group decreased by 28% compared with the SiNC + AngII group. Scratch experiment (Fig. 2D) showed that, compared SiCRT with SiNC group, and compared SiCRT + AngII with SiNC + AngII group, the migration rate decreased by 36% and 20% respectively ($P < 0.05$). The Immunofluorescence staining showed that SiCRT group reduced α -SMA fluorescence intensity compared with SiNC group, and the α -SMA fluorescence intensity obviously decreased in SiCRT + AngII group compared with SiNC + AngII group (Fig. 2E).

Overexpression of CRT could promote the activation of CFs.

In contrast to the alleviated activation of CFs by silencing CRT, overexpression

of CRT by adenovirus aggravating CFs activation (Fig. 3A). We upregulated CRT protein expression by specific CRT overexpression adenovirus (Ad-CRT) that specifically improved CRT expression. In the control group virus-only was transfected to the cells (Ad-NC). Western-blot results showed that CRT protein level increased in Ad-CRT group (Fig. 3A). Then the degree of CFs activation was compared respectively in the physiological state (Ad-CRT and Ad-NC group) and AngII-stimulated condition (Ad-CRT + AngII and Ad-NC + AngII group). The results showed that the protein levels of TGF- β , CTGF and α -SMA in the Ad-CRT group were 1.28, 1.62 and 1.47 folds as much as that in the Ad-NC group ($P < 0.05$). Under the AngII stimulation, compared with Ad-NC + AngII group, TGF- β , CTGF and α -SMA protein expression increased by 38%, 30% and 33% in Ad-CRT + AngII group ($P < 0.05$) respectively (Fig. 3B). In addition, EdU (Fig. 3C) assay showed that the proliferation rate in Ad-CRT group increased by 50% compared with the Ad-NC group, and Ad-CRT + AngII group increased by 60% compared with the Ad-NC + AngII group. Scratch experiment (Fig. 3D) showed that, the migration rate increased by 67% and 38% in group Ad-CRT and group Ad-CRT + AngII, compared with Ad-NC and Ad-NC + AngII respectively ($P < 0.05$). Moreover, the Immunofluorescence staining showed that α -SMA fluorescence intensity was enhanced in Ad-CRT group compared with Ad-NC group, and the α -SMA fluorescence intensity obviously increased in Ad-CRT + AngII group compared with Ad-NC + AngII group (Fig. 3E). To sum up, overexpression CRT conditions could enhance the CFs activation no matter in physiological or pathological condition.

CRT may regulate AngII-induced CFs activation via IRE1 pathway in endoplasmic reticulum stress.

In the group of AngII-treatment, levels of GRP78, ATF4, c-ATF6, GRP94 and p-IRE1, regulators in pathways of ER Stress, increased when compared with control group detected by western blot (Fig. 4A). Notably, gene silencing of CRT by specific SiRNA, the expression of GRP78 and p-IRE1 decreased (Fig. 4B), whereas the expression of ATF4, c-ATF6 had no significant change. Overexpression of CRT by adenovirus effectively upregulated GRP78 and p-IRE1 in CFs treated with AngII (Fig. 4C). It is implied that IRE1 pathway may be required for CFs activation.

Discussion

In the present study, the expression of CRT increased in the process of CFs activation induced by AngII and the underlying mechanism was investigated. Our findings are as following: [1] CRT level was elevated in CFs activation induced by AngII; [2] Silencing CRT led to the suppression of CFs activation; [3] The enhancement of CFs activation was caused by CRT overexpression; [4] CRT may improve CFs activation via mediating IRE1 pathway (Fig. 5).

CFs account for 60–70% of the cells in the heart and are a key source of components of the ECM that regulates the structure of the heart and hence mechanical, chemical, and electrical signals.[18] CFs growth and abnormally enhanced collagen synthesis contributes to myocardial stiffness and ultimately lead to ventricular dysfunction. So far, more and more attention had been paid to CFs[19–21]. As one of the pathological basis of cardiovascular diseases, it is necessary to explore the mechanism of CFs

activation. Therefore, our study used 10^{-5} mol/L AngII to stimulate CFs for 24h to explore the role of CRT and the interaction between the CRT and ER stress during CFs activation.

Studies have shown that CRT significantly increased during the activation of CFs [7, 22]. Although CRT are designed to stabilize and/or re-establish an ER luminal environment that is suitable for nascent ER protein folding, facilitating cell survival and recovery from acute ER stress (Survival/Recovery) [23,24], the role of CRT in cardiac fibrosis and the precise machinery has been elusive to date.

In our study, overexpression of CRT could promote the expression of α -SMA and the activation of CFs, which was consistent with the above results. The activation of CFs was involved in cardiac hypertrophy, dilated cardiomyopathy[25] and cardiac infarction[26], whose pathologic basis was myocardial remodeling. Moreover, recent study reported that CRT was the target gene of miR-455 and elevated expression of miR-455 can negatively regulate the CRT. In mouse myocardial hypertrophy early stage (2 weeks after coarctation of thoracic aorta, TAC), reducing the expression of CRT (given MiR455) may aggravate myocardial hypertrophy, but in advanced period (4 weeks after TAC), reducing the expression of CRT(given MiR455) can significantly inhibit the cardiac myocardial fibrosis, reduce myocardial remodeling level.[27]Therefore, the effect of CRT on the activation of CFs may be diverse in different stage in the cardiac hypertrophy process, and the specific mechanism needs further investigation.

Our team previously found that silencing CRT might protect cardiomyocytes from AngII induced apoptosis[28]. In this study, we observed that overexpression of CRT could increase the expressions of fibrosis initiation factors, leading to proliferation and migration of CFs, which suggested that CRT may promote AngII induced CFs activation. Furthermore, CRT silencing significantly reduced TGF- β , α -SMA and CTGF expression in activated fibroblasts. All results above supported CRT was involved the fibroblasts activation and positively regulated them. But what is the possible mechanism for that? Growing evidences from experimental and clinical research indicate that ER Stress is involved in several processes of cardiovascular disease such as atherosclerosis, ischemic heart disease and hypertrophic cardiomyopathy[29–31]. Therefore, our study assumed that ER Stress might be involved in CRT aggravating CFs activation. We tried to observe the PERK, IRE1 and ATF6 pathways related GRP78, ATF4, c-ATF6, GRP94 and p-IRE1 protein expression to evaluate the role of ER stress in process of CRT regulating CFs activation.

We observed that all UPR (unfold protein response) pathways were activated after AngII stimulation (The protein levels of GRP78, p-PRE1, c-ATF6, GRP94 and ATF4 increased in CFs activation after induced by AngII). But only p-IRE1 was significantly inhibited in AngII-treated CFs after CRT silencing and increased when overexpression CRT, suggesting that ER stress was involved in CFs activation regulated by CRT, CRT might regulate CFs activation via improving the IRE1 α -pathway.

Other studies have confirmed the ER Stress may promote myocardial fibrosis- collagen type I, the protein level of TGF- β elevated, and ER Stress inhibitors (4-phenyl butyric acid) can alleviate myocardial fibrosis [32]. Previous data also showed that after AMI, CFs expressed significantly higher levels of GRP78 [33]. In

TAC-induced hypertrophic heart tissues, Ne-treated hypertrophic cardiomyocytes and TGF- β 1-stimulated cardiac fibroblasts, the expression of markers of ER stress such as (PERK, ATF4 and ATF6) were significantly elevated.[34] This study further observed that the three pathways of ER stress were activated in AngII induced CFs activation. It was indicated that ER Stress may play a certain regulatory role in the activation of CFs, which was consistent with previous study [32].

As an intrinsic transmembrane protein of endoplasmic reticulum, IRE1 has both protein kinase and ribonuclease activity. Inhibiting IRE1 α pathway can reduce TGF- β induced hepatic fibrosis and skin tissue fibrosis, and restore fibrosis phenotype of isolated myofibroblasts in patients with systemic sclerosis. Within the lung fibroblasts, IRE1 α mediates splicing of XBP-1, leading to ER expansion that contributes to the enhanced secretory capacity needed for the secretion of extracellular matrix proteins [35]. Jody Groenendyk et. al demonstrate that early inhibition of the IRE1 α pathway of ER stress in the heart by tauroursodeoxycholic acid could prevent cardiac fibrosis and improved prognosis.[19] Accumulating evidence indicated that the IRE1 α played a nonnegligible role in cardiac fibrosis. We found that CRT can activate IRE1 pathway via upregulating p-IRE1 expression. Both CRT and IRE1 are located on the ER. There may be some sort of connection between them. The erroneous protein accumulation can activate the unfolded protein response (UPR), including the IRE1-mediated signaling pathway[36]. In addition, CRT can affect the proteins activity on the ER by affecting the concentration of Ca^{2+} [37]. Given the complex function of CRT, the underlying mechanism of its effect on p-IRE1 pathway in AngII-treated CFs remains to be clarified.

Conclusion

Taken together, our study shows that overexpression CRT aggravates AngII-induced CFs activation via p-IRE1 pathways. Our data shows that CRT is a regulator of CFs activation, CRT and IRE1-mediated signaling pathway may be explored as a new target for CFs activation.

Declarations

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Author Contributions

Z.Y.L performed the experiments and wrote the manuscript. W.Z, J.L.L and L.X contributed to perform the statistical analysis and figure production. H.H provided oversight for the project. All authors reviewed the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request

Declarations

Conflicts of interest

The authors declare that there are no commercial or financial conflicts of interest.

Ethical approval

This study was approved by the Ethics Committee on Biomedical Research, West China Hospital of Sichuan University. Animals were treated in accordance the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

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Figures

Figure 1

CRT was up-regulated in CFs activation induced by AngII (10⁻⁵M 24hrs). (A) Western Blot assay showed TGF-β, CTGF and α-SMA increased in CFs after AngII stimulation. (B) The cell proliferation activity determined by 5-ethynyl-2-deoxyuridine (EdU) in CFs improved after AngII stimulation. (C) The migration activity of cells explored by wound healing assay in AngII-induced CFs improved. (D.E) The expression of CRT tested by western blot and Immunofluorescence Staining increased in CFs stimulated by AngII; (All data was expressed as the mean ± SD; A–D: AngII group: n = 3, control group: n = 3; P < 0.05 AngII group vs control group).

Figure 2

Down-regulation of CRT inhibited CFs activation induced by AngII. (A) The CRT protein level decreased in CFs after transfection with three specific micro SiRNA for 24h detected by Western Blot; (B) TGF- β , CTGF and α -SMA protein decreased in CFs after transfection of CRT SiRNA in the presence or absence of AngII; (C) Proliferation rate of CFs alleviated in the presence or absence of AngII with transfection of CRT SiRNA; (D) Migration rate of CFs reduced in the presence or absence of AngII after being treated with CRT SiRNA;

(E) The α -SMA fluorescence intensity decreased in the presence or absence of AngII after transfecting with CRT SiRNA; (A,B: n =3; mean $\pm$ SD. P <0.05 SiNC group vs SiCRT group, SiNC+ AngII group vs SiCRT+ AngII group)

Figure 3

Up-regulation of CRT exacerbated CFs activation induced by AngII. (A) The CRT protein level increased in CFs with specific CRT overexpression adenovirus injection (Ad-CRT) for 24h detected by Western Blot; (B)

TGF- β ,CTGF and α -SMA increased in CFs after Ad-CRT injection for 24h in the presence or absence of AngII; (C) Proliferation rate of CFs elevated in the presence or absence of AngII after Ad-CRT injection for 24h;(D) Migration rate of CFs aggravated in the presence or absence of AngII after Ad-CRT injection for 24h;(E)The α -SMA level increased by Immunofluorescence Staining in the presence or absence of AngII after specific Ad-CRT injection for 24h; (A,B: n = 3; mean $\pm$ SD. P <0.05 Ad-NC group vs Ad-CRT group, Ad-NC+ AngII group vs Ad-CRT+ AngII group);

Figure 4

The role of CRT in CFs was associated with p-IRE1 signaling activation. (A) The protein levels of ER Stress markers (GRP78, p-PRE1, c-ATF6, GRP94 and ATF4) increased in CFs after induced by AngII (n = 3, mean $\pm$ SD). (B) The protein levels of GRP78 and p-PRE1 in CFs reduced after transfection of CRT SiRNA in the presence or absence of AngII (n=3, mean $\pm$ SD). (C) The protein levels of GRP78 and p-PRE1 in CFs improved after injection of Ad-CRT in the presence or absence of AngII (n=3, mean $\pm$ SD).

Figure 5

Schematic illustration of the signaling pathways—partly—of AngII-induced cardiac fibroblasts activation