The role and mechanism of LARP7 in regulating the STING-dependent autophagy-lysosomal negative feedback loop in diabetic cardiomyopathy

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Research Article

Keywords: Diabetic cardiomyopathy, LARP7, STING, Autophagy

Posted Date: September 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3321598/v1

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Abstract

Background

Diabetic cardiomyopathy (DCM) is an important cause of heart failure in diabetic patients, and there is no specific drug for DCM. The aim of this study was to investigate the pathogenesis of DCM and identify potential therapeutic targets.

Methods

Neonatal mouse ventricular cardiomyocytes (NMVCMs) were isolated and cultured. Type 1 diabetes mellitus (T1DM) models were established by high glucose (33 mmol/L) treatment. The knockdown/overexpression of LARP7 was achieved through adenovirus transduction, the overexpression of STING was achieved through plasmid transfection, and the inhibition of STING was achieved through C-176. Then, the expression, activation, and localization of STING and LARP7 in cardiomyocytes under different treatment conditions were observed, and the interaction between the two and the effect of this interaction on the STING-dependent autophagy-lysosomal "negative feedback loop" were explored. In addition, the fibrosis and apoptosis of cardiomyocytes were evaluated.

Results

In this study, high glucose increased the expression and activation of STING in NMVCMs, which was accompanied by increased α-SMA and caspase3, and the degradation of STING through the autophagy-lysosomal pathway was suppressed. In addition, high glucose levels caused LARP7 to translocate from the nucleus to the cytoplasm and interact with accumulated STING to inhibit STING degradation, whereas inhibiting STING or LARP7 expression significantly improved high glucose-induced myocardial injury.

Conclusion

Under high glucose conditions, LARP7 damages mouse cardiomyocytes by inhibiting the STING-dependent autophagy-lysosomal degradation pathway. Targeted inhibition of LARP7 or STING expression may be a potential therapeutic strategy for the treatment of DCM.

1. Introduction

Diabetes mellitus (DM) is a major chronic disease that endangers human health, and the cardiovascular damage caused by DM has become the main cause of death in diabetic patients[1, 2]. Diabetic cardiomyopathy (DCM) is one of the cardiac complications of DM and refers to impaired cardiac filling and decreased systolic function caused by DM, ultimately inducing heart failure[3–6]. The current treatment methods for DCM focus on controlling blood sugar, delaying ventricular remodeling, and
correcting heart failure, but there is still a lack of effective treatments for DCM. Conventional treatments place a heavy economic burden on patients and have limitations. Despite the standardized application of SGLT2 inhibitors, spironolactone, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and other drugs, there are still a large number of diabetic patients who suffer end-stage heart failure due to DCM each year\(^{7-11}\).

Stimulator of interferon gene (STING) is a signal transduction molecule closely related to the innate immune response induced by virus invasion and cell damage and activates the inflammatory response through the induction of type I interferons (IFN-I) to resist exotic pathogens\(^{12}\). Notably, pathogenic microbial DNA can stimulate STING expression and activation, and endogenous DNA release caused by various intracellular toxic stresses can play the same role\(^{13}\). Increasing evidence suggests that STING plays an important role in regulating insulin sensitivity and inducing ventricular remodeling in diabetic patients. On the one hand, the activation of the STING pathway can affect glucose metabolism homeostasis by mediating pancreatic \(\beta\) cell senescence, glucose intolerance and insulin resistance\(^{14}\). On the other hand, myocardial hypertrophy in the progression of DCM can be promoted by inducing pyroptosis and proinflammatory responses\(^{15}\). However, under physiological conditions, STING can be degraded through various pathways, such as the K48 ubiquitin–proteasome pathway and autophagy–lysosome pathway, after exerting its signal transduction function, thus avoiding tissue damage caused by its overexpression and activation\(^{16-18}\). Previously, it has been confirmed that in the course of various cardiovascular diseases, such as myocardial infarction and heart failure, pathogenic factors lead to the obstruction of STING degradation, intracellular accumulation and the overactivation of downstream signaling pathways, which play an important pathogenic role\(^{17, 19-21}\). Therefore, in this study, we aimed to explore the relationship between obstruction of the STING degradation pathway and the progression of DCM.

LARP7, a member of the La-related protein (LARP) family, acts as an important negative transcriptional regulator of RNA polymerase II and plays an important role in the induction of ventricular hypertrophy\(^{22, 23}\). More than 50% of LARP7 in human cells forms the 7SK complex together with 7SK RNA, MePCE, HEXIM1/2 and P-TEFb. LARP7 binds to the C-terminal UUU-3’OH of 7SK RNA and maintains the stability of 7SK RNP together with MePCE\(^{24, 25}\). In myocardial tissue, pathological stimuli can lead to the dissociation of LARP7, reducing the stability of the complex, and free P-TEFb is recruited to the RNA polymerase II promoter by transcription factors such as BRD4, thus stimulating the elongation of transcription and inducing ventricular hypertrophy\(^{26}\). However, the role of free LARP7 in the cytoplasm is not clear.

In this study, a high glucose-induced DCM cell model was used to clarify the role of free LARP7 in the process of DCM, and the results indicated that high glucose inhibited the degradation of STING through the autophagy-lysosomal pathway. Additionally, LARP7 expression was upregulated, intracellular localization was altered, and STING degradation was inhibited by interacting with STING. The accumulation of STING in vivo not only caused inflammation and immune responses but also induced myocardial remodeling by inducing cardiomyocyte fibrosis and apoptosis, and the downregulation of
STING or LARP7 expression significantly inhibited the occurrence of these processes. In conclusion, this study revealed the regulatory effect of free LARP7 on the STING pathway, provided new insights for studying the pathogenesis of DCM, and revealed that targeting STING or LARP7 alone or in combination could serve as a therapeutic strategy for the treatment of DCM.

2. Methods

2.1. Culture and processing of neonatal mouse ventricular cardiomyocytes (NMVCMs)

Cardiomyocytes were obtained from the ventricles of neonatal C57BL/6 mice (1–3 days old). Neonatal mice were sterilized with 75% ethanol, and the hearts were removed and rinsed in cold phosphate-buffered saline (PBS). The myocardial samples were then cut into small pieces and digested with collagenase type 2 (Sigma–Aldrich) until the tissue pieces dissolved. The suspension was mixed with complete medium to stop digestion. The mixture was centrifuged (800 × g for 5 min), and the supernatant was retained. The cardiomyocyte-enriched fraction was resuspended in complete medium containing 20% fetal bovine serum (Gibco, California, USA). Differential adhesion was used to separate cardiomyocytes and fibroblasts. The isolated cells were placed in a culture flask at 37°C for 1 h for the fibroblasts to accumulate at the bottom; the cardiomyocytes remained suspended in the medium. Cardiomyocytes were then incubated on confocal dishes. These steps were carried out at 37°C in 95% O₂ and 5% CO₂.

LARP7 was knocked down/overexpressed in NMVCMs via adenovirus, and STING was exogenously overexpressed in NMVCMs via plasmid transfection; DCM models were established only after verifying adequate transduction/transfection efficiency after 24 h. NMVCMs were subjected to high glucose (33 mmol/L) treatment for 48 h to simulate a type 1 DCM model in vitro[27]. To determine the effect of autophagy on apoptosis and STING expression in NMVCMs, NMVCMs were treated with 3-methyladenine (3-MA) (A8353, APEXBio, Houston, TX, USA), an autophagy inhibitor, for 2 h. To evaluate the effect of STING on cardiomyocyte fibrosis and apoptosis, NMVCMs were stimulated with C-176, a potent covalent inhibitor of STING, to inhibit intracellular STING expression. Cardiomyocytes were randomly divided into the following groups: (i) control; (ii) HG; (iii) 3-MA; (iv) HG + C-176; (v) HG + LARP7-siRNA; (vi) HG + LARP7-siRNA + C-176; (vii) LARP7-siRNA; (viii) m-3*Flag-STING; (ix) m-3*Flag-STING + LARP7-siRNA; (x) HG + m-3*Flag-STING; (xi) HG + m-3*Flag-STING + LARP7-siRNA; and (xii) m-LARP7.

2.2. Western blot analysis

The cell medium was discarded, and the cells were washed with PBS. Then, the samples were homogenized in precooled PMSF-containing protein lysis buffer (1 mmol/L, pH = 7.4) in liquid nitrogen, lysed by ultrasound, and centrifuged at 12,000 rpm for 30 min to extract total protein. Protein quantification was performed using the BCA method. Twenty micrograms of total protein was electrophoresed on a 10% denaturing polyacrylamide gel, and the separated protein was
electrotransferred to a polyvinylidene fluoride (PVDF) membrane. After blocking with 5% nonfat milk, the membrane was incubated overnight at 4°C with the following primary antibodies: p-STING (Ser366), STING, α-SMA, Cl-Caspase3, and LARP7. Then, the membrane was incubated with HRP-labeled secondary antibody at room temperature for 40 min. GAPDH was used as a loading control. Blots were visualized with a chemiluminescence system (Buchinghamshire, Amersham Bioscience). Using the semiquantitative densitometry results for the developed bands, the ratio of the gray value of the target band to that of the GAPDH band was calculated to evaluate the protein level.

2.3. α-SMA and phalloidin staining of cardiomyocytes

To evaluate the degree of cardiomyocyte remodeling in different groups, α-SMA and phalloidin staining were used to analyze the degree of myocardial fibrosis and cytoskeleton morphology, respectively. Confocal dishes preseeded with NMVCMs were washed three times with precooled PBS, and the cardiomyocytes were then fixed with formaldehyde precooled to 4°C for 15–20 min, followed by 3 washes with precooled PBS. The cells were incubated in permeabilization buffer (0.1% Triton X-100 in PBS) for 15 min to rupture cell membranes and then incubated in bovine serum albumin (BSA) for 90 min to block nonspecific binding. Next, the primary antibody α-SMA (1:150, from rabbit) was added, and the cells were incubated overnight at 4°C, after which fluorescent (FITC) secondary antibody (rabbit anti-mouse, 1:200, Boster) was added, and the cells were incubated for 60 min at room temperature. DAPI was used for nuclear staining. The expression level of α-SMA in each group of cells was indicated by the intensity of green fluorescence in the field of view. Phalloidin staining was used to visually assess the morphology of the myocardial cytoskeleton in each group via laser confocal microscopy and the appropriate fluorescence channel.

2.4. Cardiomyocyte apoptosis

Cardiomyocyte apoptosis was measured using the DNA ladder assay, TUNEL staining, and western blot analysis of Cl-caspase3. TUNEL staining was used to stain myocardial tissue sections following the instructions provided with an apoptosis detection kit (Roche Germany). After mounting with an anti-fluorescence quenching agent, the sections were observed and photographed under a laser confocal microscope (Leica TCS SP8, Germany), and then, the cell apoptosis rate was determined[28].

In the DNA ladder assay, myocardial DNA was extracted using a nucleic acid extraction kit (IsoQuicks, Microprobe, USA). The extracted DNA (10 mg) was added to a 2% agarose gel containing ethidium bromide and electrophoresed in Tris-borate-EDTA (TBE) buffer at 100 V for 2 h. Then, the DNA ladders were photographed.

2.5. Immunofluorescence staining

In cardiomyocytes, the colocalization of STING and LC3 and that of STING and LARP7 were separately evaluated by immunofluorescence colocalization. NMVCMs were seeded in confocal dishes, treated and grouped. The culture medium was discarded, and the cells were washed three times with sterile PBS, incubated in permeabilization buffer (0.1% Triton X-100 in PBS) for 15 min to rupture cell membranes,
and incubated in BSA for 60 min to block nonspecific binding. The cells were incubated in diluted rabbit-derived STING antibodies or diluted mouse-derived LC3 and LARP7 antibodies overnight at 4°C. Subsequently, the cells were incubated with appropriate secondary antibodies at room temperature for 90 min. For Group 1, anti-rabbit IgG was labeled with green fluorescent FITC (to visualize LC3 localization), and anti-mouse IgG was labeled with yellow fluorescent AF647 (to visualize STING localization). For Group 2, FITC was used to visualize LARP7 localization, and CY3 was used to visualize STING localization. The distribution of different fluorescent particles in NMVCMs was evaluated using a laser confocal microscope, and the degree of merging of different groups of fluorescent particles was analyzed and calculated using Image-Pro PLUS software to evaluate the colocalization of STING and LC3 and that of STING and LARP7.

2.6. Coimmunoprecipitation (COIP) and mass spectrometry (MS)

COIP was conducted using a Proteintech immunoprecipitation (IP) kit (KIP-2). NMVCMs were preprocessed and grouped. To fully lyse cardiomyocytes, a total of 100 l of precooled IP lysis buffer (containing 1X protease inhibitor) was added to 10^6 cells for 30 min. The cells were disrupted by ultrasonication for 1 min to fully lyse and fragment the DNA and were then centrifuged at 12,000 rpm for 10 min at 4°C, after which the supernatant was collected. Then, 30 l of washed Protein A-Sepharose beads were added to each lysate tube, and the samples were incubated with slow rotation at 4°C for 120 min, followed by centrifugation at 12,000 rpm for 1 min, after which the supernatant was collected. Then, 4 µg of IP antibody and 300 l of incubation buffer were added to the supernatant; as a negative control, equal amounts of control IgG derived from the same species were added to the samples, which were then incubated overnight at 4°C. Resuspended Protein A-Sepharose beads were added to the samples, which were incubated at 4°C with slow rotation for 3 h to precipitate the immune complexes. The supernatant was discarded, and the precipitate was retained and washed 5 times. Finally, the precipitated complexes were eluted twice with 40 l of elution buffer, to which 10 l of alkaline neutralization buffer and 23 l of 5X sample buffer were added. The samples were boiled in water for 8 min and then stored at -80°C until western blot analysis.

The IP procedure for preparing samples for MS was the same as that described above. The IP samples of each group were electrophoresed to obtain gel strips, and the peptides in the gel strips were enzymatically hydrolyzed. The hydrolyzed peptides were separated by column chromatography and then injected into a tandem MS for primary and secondary MS analyses.

2.7. Transmission electron microscopy

Ventricular cardiomyocytes pretreated with high glucose and grouped were fixed with 3.0% glutaraldehyde and 1.5% paraformaldehyde, washed 3 times with PBS, fixed with osmium tetroxide, dehydrated with ethanol, and embedded in epoxy resin. The number of myocardial autophagosomes and
autophagolysosomes and the damage to mitochondria, endoplasmic reticulum and other organelles in myocardial tissue were observed under a transmission electron microscope (H-7650, Japan).

2.8. DCM mouse model

All animal experiments were performed in accordance with the guidelines of the Animal Experimentation Committee of the Second Affiliated Hospital of Xi’an Jiaotong University (Shaanxi). Streptozotocin (STZ) (50 mg/kg) was dissolved in 0.1 mol/L citrate buffer (pH = 4.5), and the STZ solution was injected intraperitoneally into mice for 5 consecutive days to construct a type 1 DCM mouse model. The control group was injected with an equal volume of citrate buffer. Blood samples were collected via the tail vein 1 week after the last injection, and mice with fasting blood glucose ≥ 16.6 mmol/L in three measurements were maintained for another 12 weeks to develop myocardial injury after the induction of DM. Mice were randomly divided into the following groups: (i) control group and (ii) T1DM group.

2.9. Immunohistochemistry and immunofluorescence

After the model was successfully constructed, the mice were sacrificed by cervical dislocation, and left ventricular specimens were obtained, fixed with 4% paraformaldehyde, and sliced into sections. The sections were heated to retrieve antigen and then incubated with anti-LC3 antibody (ab192890, Abcam, UK, 1:2000) overnight at 4°C, followed by incubation with secondary antibody (fluorescein isothiocyanate, Boster Biological Technology, BA1101) for 1 h. As a negative control, an isotype control antibody was used. Stained sections were developed with diaminobenzidine to produce a brown product. Digital scanning was performed with a digital scanner (Pannoramic MIDI, 3DHISTECH, Hungary) to analyze the immunohistochemistry results.

In the immunofluorescence assay, CY3-labeled STING, FITC-labeled LARP7, and DAPI (nuclear staining) were used, an anti-fluorescence quencher was used for mounting, and a laser confocal workstation was used for immunofluorescence photography. Semiquantitative analysis of fluorescence images was performed using ImageJ software.

2.10. Statistical analysis

Continuous variables are presented as X ± SD. The t test was used for comparisons between two groups. After analysis of variance (ANOVA) was used for multiple comparisons, the Bonferroni-corrected t test was used for post hoc testing. Two-sided tests of significance were always used, and P < 0.05 was considered statistically significant. Data analysis was performed using SPSS 14.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. High glucose induced the expression and activation of STING in cardiomyocytes
Western blot analysis showed that the expression and activation levels of STING were significantly upregulated in the HG group, an effect that occurred in parallel with increases in α-SMA and Cl-caspase3 (Fig. 1A). The expression of α-SMA in cardiomyocytes was further evaluated by immunofluorescence, and cardiomyocyte apoptosis was evaluated using the DNA ladder assay. The results were similar to those of western blot analysis. Interestingly, the inhibition of STING using C-176 significantly ameliorated high glucose-induced cardiomyocyte fibrosis and apoptosis (Fig. 1B and C). In conclusion, high glucose caused cardiomyocyte fibrosis and apoptosis by causing the accumulation and activation of STING.

3.2. High glucose disrupted the STING-triggered autophagy “negative feedback loop”

STING contains seven regions that directly bind to the autophagy molecule LC3 (Fig. 2A). To explore the effect of high glucose on autophagy, we used immunofluorescence colocalization to detect the intracellular expression of LC3 and STING. Compared with those in the control group, LC3 expression and autophagy were lower in the HG group (Fig. 2B). COIP experiments further confirmed that high glucose weakened the binding ability of LC3 to STING (Fig. 2C). Transmission electron microscopy revealed that there were significantly fewer autophagosomes, autophagolysosomes and lysosomes in cardiomyocytes in the HG group than in the control group, in addition to many swollen, damaged, and ruptured mitochondria (Fig. 2D). Next, after the treatment of cardiomyocytes with the autophagy initiation inhibitor 3-MA, the expression of STING in the cytoplasm significantly increased, accompanied by cardiomyocyte shrinkage and poor cytoskeleton continuity. The number of apoptotic cardiomyocytes detected by the TUNEL assay also significantly increased (Fig. 2E-H). In summary, high glucose interfered with STING degradation through the autophagy-lysosomal pathway by inhibiting the binding of STING to LC3, resulting in STING accumulation, which led to structural damage and increased cardiomyocyte apoptosis.

3.3. The interaction between LARP7 and STING aggravated myocardial injury

To explore the direct role of free LARP7 in DCM, the expression of LARP7 and STING was inhibited with siRNA and C-176, respectively. The western blot results showed that STING expression was significantly upregulated in the HG group. Compared with the HG group, in the groups in which LARP7 or STING was inhibited, the activation of the STING pathway was attenuated, and cardiomyocyte apoptosis was alleviated. The inhibition of LARP7 via siRNA following the inhibition of STING by C-176 had a more obvious inhibitory effect on the STING pathway, and cardiomyocyte apoptosis was further reduced (Fig. 3A). These findings indicate that there is a regulatory mechanism between free LARP7 and STING that mediates the expression and activation of STING, thereby aggravating cardiomyocyte damage.

MS analysis of cGAS-STING-TBK1, three important molecules in the STING signaling pathway, indicated that compared with that in the negative control, there was a very high abundance of LARP7 in the final
STING IP solution (Fig. 3B). COIP experiments further confirmed that both exogenously overexpressed and endogenous STING interacted with LARP7 and that high glucose enhanced the interaction between them (Fig. 3C). LARP7 and STING were fluorescently labeled to visualize their intracellular localization. After high glucose treatment, a large amount of LARP7 translocated from the nucleus to the cytoplasm, where it colocalized with STING. Taken together, these results indicate that high glucose induced the intracellular translocation of LARP7 and that its interaction with STING accumulated in the cytoplasm to mediate cardiomyocyte apoptosis.

3.4. LARP7 mediates myocardial injury by inhibiting the STING-dependent autophagy-lysosomal negative feedback loop

To further explore the mechanism by which LARP7 and STING interact to induce myocardial injury, the expression of LARP7 was inhibited with siRNA, and then, STING was overexpressed via 3×Flag-STING plasmid transfection. As shown in Fig. 4A-D, the inhibition of LARP7 not only caused the expression and activation of STING to be significantly lower than those in the control group and HG group but also antagonized the activation effect caused by the exogenous overexpression of STING and alleviated apoptosis induced by the upregulation of STING. These findings indicate that LARP7 regulates the STING pathway upstream of the pathway. Next, the expression of LARP7 was upregulated, and the expression and localization of LC3 and STING in cardiomyocytes were assessed. The results showed that the upregulation of LARP7 caused a significant decrease in LC3 in cardiomyocytes, accompanied by the accumulation of intracellular STING and a reduction in its colocalization with LC3 (Fig. 4E). The above results together indicated that LARP7 blocked the degradation of STING by disrupting the STING-triggered autophagy-lysosomal "negative feedback loop", resulting in the accumulation and overactivation of STING in cardiomyocytes, eventually causing cardiomyocyte damage.

3.5. The expression of STING and LARP7 in the myocardial tissue of type 1 DCM mice increased, and autophagy was inhibited

A mouse model of type 1 DCM was constructed, and the above conclusions were verified through in vivo experiments. Compared with that in the control group, the expression of STING and LARP7 in the myocardial tissue of mice in the T1DM group was significantly higher (Fig. 5A, B). Immunohistochemistry results indicated a significantly lower amount of LC3 in the myocardial tissue of the T1DM group than in that of the control group, indicating that autophagy was inhibited (Fig. 5C). The above results serve as strong evidence to support our previous research.

4. Discussion

To our knowledge, this is the first study to reveal the role and mechanism of LARP7 in regulating the STING-dependent autophagy-lysosomal "negative feedback loop" in DCM. The main results of this study indicate that high glucose induces the enhanced expression and activation of STING in ventricular
myocytes and inhibits the degradation of STING through the autophagy-lysosomal pathway. In addition, high glucose levels cause LARP7 to dissociate from the 7SK complex and translocate to the cytoplasm to interact with STING, thereby inhibiting the degradation of STING and consequently causing STING accumulation and the activation of downstream signaling pathways, resulting in cardiomyocyte fibrosis and apoptosis. The inhibition of STING or LARP7 expression significantly ameliorated high glucose-induced myocardial injury.

As an important signal transduction molecule, STING is associated with the occurrence of various diseases[29–32]. Under the action of pathogenic factors, STING is hyperactivated and initiates a downstream signaling cascade that induces the overexpression of IFN-I and proinflammatory cytokines[31]. STING can also trigger a fibrotic cascade, resulting in increased fibrosis in multiple tissues and organs throughout the body, including the heart[33–35], and the activation of its downstream pathways is also associated with the induction of apoptosis[36]. The results of this study showed that high glucose induced the increased expression and activation of STING in mouse cardiomyocytes, accompanied by increased cardiomyocyte fibrosis and apoptosis. Myocardial fibrosis can impair diastolic and systolic function and eventually lead to refractory heart failure. Most cardiomyocytes are terminally differentiated cells with a limited proliferative capacity. An apoptosis rate of 0.1% can reduce the number of cardiomyocytes by 37% within a year[37, 38]. Based on this, the activation of the STING pathway may cause myocardial injury in the process of DCM by mediating cardiomyocyte apoptosis and fibrosis; the reduction in myocardial fibrosis and apoptosis after treating cells with C-176 supports this inference.

Under physiological conditions, STING can be degraded through various pathways after fulfilling its signal transduction function, thereby avoiding tissue damage caused by excessive activation[39, 40]. Several previous studies have demonstrated the role of impaired proteasome activity in pathological cardiac remodeling in diabetic mice; however, the aim of this study was to further explore the pathogenic mechanism of the autophagy-lysosomal "negative feedback loop" in DCM. First, this study confirmed that STING binds to LC3 in cardiomyocytes, thereby triggering the autophagy-lysosomal "negative feedback loop". However, after 3-MA was administered to inhibit autophagy, significant STING accumulation occurred in cardiomyocytes, accompanied by aggravation of cardiomyocyte injury, and the number of apoptotic cells increased significantly. The above results confirm the important role of autophagy in the degradation of STING. Compared with that in cardiomyocytes of the control group, the expression of LC3 in the cardiomyocytes of the HG group was significantly lower, and transmission electron microscopy further confirmed that high glucose inhibited autophagy, accompanied by significant cardiomyocyte damage. Collectively, these results suggest that high glucose inhibits the STING-dependent autophagy-lysosomal "negative feedback loop".

In addition, in this study, there was a significant increase in LARP7 expression in high glucose-induced mouse ventricular myocytes. Immunofluorescence colocalization revealed that a large amount of LARP7 translocated from the nucleus to the cytoplasm and completely colocalized with STING, with an obvious interaction between LARP7 and STING. Previous studies have confirmed that LARP7 plays an important...
role in stabilizing the 7SK complex and regulating transcription elongation[26]. The hypothesis of this study is that LARP7, which is released into the cytoplasm, plays an important role in high glucose-induced cardiomyocyte injury. First, the results of MS and COIP experiments confirmed that LARP7 can indeed interact with STING and that high glucose strengthens the relationship between the two. Further experiments found that inhibiting LARP7 not only decreases the apoptosis induced by high glucose but also antagonizes the activation effect caused by upregulating STING. These findings indicate that LARP7 plays a key regulatory role upstream of STING. The decreased expression of LC3 molecules observed after the upregulation of LARP7 expression revealed a specific regulatory mechanism, for which in vivo animal experiments provided a strong basis. LARP7 inhibits the STING-dependent autophagy-lysosomal "negative feedback loop", causing STING accumulation and overactivation and promoting cardiomyocyte apoptosis and fibrosis, which may contribute to the development of DCM.

Taken together, our evidence indicates that LARP7 is a key molecule in high glucose-induced cardiomyocyte injury that exerts a pathogenic role by interfering with the STING-dependent autophagy-lysosomal "negative feedback loop". Inhibiting STING and LARP7 exerts a significant cardioprotective effect. Therefore, targeting STING and LARP7 may be an effective therapeutic strategy to improve DCM.

5. Conclusion

Our study demonstrated that LARP7 damages mouse cardiomyocytes under high glucose conditions by inhibiting STING-dependent autophagy-lysosomal degradation pathways. Targeted inhibition of LARP7 or STING expression may be a potential therapeutic strategy for DCM.

Declarations

Acknowledgements

Not applicable.

Author contributions

Sun Jingjing and Wang Ziming contributed to the preliminary data analysis, interpretation, and manuscript writing. Cheng Zheng, Duan Yixuan, Liu Chang and Zhao Sihai participated in the manuscript submission and editing. Deng Jie participated in the research design. All authors contributed to the final approval of the manuscript.

Funding

This study was supported by Shannxi Social Development Funding (grant No. 2017SF-134), Shannxi Science Funding (grant No. 2020JQ-553) and Xi’an Jiaotong University Funding (grant No. YXJLRH2022073).

Availability of data and materials
The data used to support the findings of this study are available from the corresponding author upon request.

**Ethics approval and consent to participate**

This research was approved by the Xi’an Jiaotong University Committee on Animal Care (Shaanxi, China).

**Consent for publication**

Not applicable.

**Animal rights**

All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, Publication No. [NIH] 86–23), and were approved by the Xi’an Jiaotong University Committee on Animal Care (Shaanxi, China).

**Competing interest**

All authors have no proprietary or commercial interest in any of materials discussed in this article.

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Figures
Figure 1

High glucose enhanced the expression and activation of STING in cardiomyocytes. A Western blot analysis results for STING, p-STING, α-SMA and Cl-caspase3 in NMVCMS treated with high glucose, *P<0.05 vs. Control group, n=6, (x±SEM). B Immunofluorescence staining results for α-SMA in cardiomyocytes treated with high glucose and C-176, a STING inhibitor, *P<0.05 vs. Control group,
#P<0.05 vs. HG group, n=8, (x±SEM). C DNA ladder assay was used to evaluate cardiomyocyte apoptosis under different treatment conditions.

Figure 2

High glucose levels disrupted the STING-triggered autophagy "negative feedback loop". A Molecular structure of STING. B Immunofluorescence colocalization of STING and LC3 in NMVCMS treated with...
high glucose: green fluorescent particles represent LC3, yellow fluorescent particles represent STING, and yellow fluorescent particles that overlap with green fluorescent particles suggest that STING directly interacts with LC3. C Coimmunoprecipitation (COIP) results for LC3 and STING. D TEM image of cardiomyocytes treated with high glucose. E-H NMVCMS were pretreated with an autophagy initiation inhibitor, 3-MA, and then, the expression of STING in cardiomyocytes was assessed by immunofluorescence, and the morphology of the cardiomyocyte cytoskeleton was assessed by phalloidin staining, *P<0.05 vs. Control group, n=8, (x±SEM); apoptotic cardiomyocytes were detected using the TUNEL assay, *P<0.05 vs. Control group, n=6, (x±SEM).
Figure 3

The interaction between LARP7 translocated from the nucleus and STING aggravated myocardial injury. A Western blot analysis results for LARP7, STING, p-STING, and Cl-caspase3 in NMVCMS in which the expression of LARP7 and STING was inhibited by siRNA and C-176. *P<0.05 vs. Control group, #P<0.05 vs. HG group, §P<0.05 vs. HG+LARP7-siRNA group, $P<0.05 vs. HG+NC-siRNA group, &P<0.05 vs. HG+C-176 group, n=4, (x±SEM). B Immunoprecipitation (IP) was performed for three important molecules of the
STING signaling pathway: cGAS-STING-TBK1. The final solution obtained after IP was subjected to electrophoresis, and the gel strips obtained by electrophoresis were subjected to mass spectrometry. COIP experiment results confirmed that both exogenously overexpressed STING (transfected with the 3×Flag-STING plasmid, IP: Flag) and endogenous STING (IP: STING) interacted with LARP7. After high glucose treatment, LARP7 and STING in cardiomyocytes were labeled using an immunofluorescence double-labeling colocalization method. The green fluorescent particles represent LARP7, the red fluorescent particles represent STING, and the overlapping of the two appear as yellow fluorescent particles. *P<0.05 vs. Control group, n=6, (x±SEM).
Figure 4

LARP7 mediates myocardial injury by inhibiting the autophagy-lysosomal degradation pathway. A and C Mouse cardiomyocytes were treated with siLARP7, m-3*Flag-STING, or siLARP7+m-3*Flag-STING, and then, western blot analysis of LARP7, STING, and p-STING (Ser366) was performed. *P<0.05 vs. control, #P<0.05 vs. si-LARP7, §P<0.05 vs. m-3*Flag-STING, n=6, (x±SEM). B and D A type 1 diabetes mellitus model of mouse ventricular cardiomyocytes was established with high glucose, and then, the above
procedures were repeated to assess the expression of STING, p-STING (Ser366) and Cl-caspase3. *P<0.05 vs. control group, #P<0.05 vs. HG group, §P<0.05 vs. HG+m-3*Flag-STING group, $P<0.05 vs. HG+STING control vector group, &P<0.05 vs. HG+ LARP7-siRNA group, ¶P<0.05 vs. HG+NC-siRNA group, n=4, (x±SEM). E LARP7 was upregulated, and the expression and localization of LC3 and STING in cardiomyocytes was detected using an immunofluorescence colocalization assay: green fluorescent particles represent GFP-LC3 and GFP-LC3-labeled autophagosomes, red fluorescent particles represent STING, and yellow dots are the result of the overlapping of red dots and green dots, representing the colocalization of GFP-LC3 and STING.

Figure 5

The expression of STING and LARP7 in the myocardial tissue of mice with type 1 DCM increased, accompanied by inhibition of autophagy. A mouse model of type 1 diabetic cardiomyopathy was established through the continuous intraperitoneal injection of streptozotocin (STZ), and then, the myocardial tissue of the mice was harvested and immunofluorescently labeled with STING-CY3 and LARP7-FITC. The differential expression of STING-CY3 and LARP7-FITC in the T1DM group and the control group was evaluated. A STING-CY3 emits red fluorescence, *P<0.05 vs. the control group. B LARP7-FITC emits green fluorescence, *P<0.05 vs. the control group. C Immunohistochemistry was used to visualize the expression of LC3 in mouse cardiomyocytes, *P<0.05 vs. the control group.