Se alleviates homocysteine-induced fibrosis in cardiac fibroblasts via downregulation of lncRNA MEG3

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Abstract. Selenium (Se) is considered to have anti-oxidant properties, which are beneficial for heart condition. Hyperhomocysteinemia (HHCY) has been suggested to potentially lead to heart failure and is characterized by cardiac fibrosis; however, investigation on the role of Se and HHCY in cardiac fibrosis is rare. Since previous studies demonstrated the important role of the long non-coding RNA maternally expressed 3 (MEG3) in some heart diseases, the present study aimed to determine how Se and MEG3 might exert regulatory effects on HCY-induced fibrosis in cardiac fibroblasts (CFs). Mouse CFs were isolated and treated with HCY and Se. The expression of α-smooth muscle actin (α-SMA), collagen I and III was detected by western blotting to reflect CF fibrosis. Reverse transcription-quantitative PCR was performed to determine the expression levels of MEG3. Inflammation and oxidative stress responses were analyzed by measuring TNF-α, IL-1β (ELISA) and reactive oxygen species levels (using a commercial kit), respectively. Cell Counting Kit-8 was used to evaluate CF proliferation. Total and phosphorylated (p) expression of janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) was evaluated by western blotting. CFs were transfected with adenovirus expressing MEG3 short-hairpin RNA to knock down MEG3 expression. Se treatment downregulated the expression level of MEG3 in HCY-stimulated CFs, whilst inhibiting the inflammatory and oxidative stress response. Furthermore, Se inhibited the increased proliferation of CFs following HCY treatment. In addition, MEG3-knockdown in CFs could improve fibrosis caused by HCY. Furthermore, the ratios of p-JAK2/JAK2 and p-STAT3/STAT3 were decreased following treatment with Se or MEG3 silencing. Taken together, the findings from the present study suggested that Se may alleviate cardiac fibrosis by downregulating the expression of MEG3 and reducing the inflammatory and oxidative stress response in CFs. This suggests that Se may be a potential therapeutic option for treating cardiac fibrosis in the future.

Introduction

Cardiac fibrosis serves a key role in various forms of chronic heart disease, resulting in reduced tissue compliance and impaired heart function (1). At present, there is no specific therapy to treat patients with cardiac fibrosis. As closely associated to cardiac fibrosis and heart remodeling, hyper-homocysteinemia (HHCY) is generally known as a risk factor of cardiovascular diseases (2). Previous tudies have shown that HHCY can lead to myocardial interstitial and perivascular fibrosis and accelerate the progress of cardiac remodeling (2,3). However, although the unfavorable effects of HHCY on cardiovascular disease are well documented, the underlying mechanism of HHCY leading to cardiac fibrosis remains unknown.

As one of the essential trace minerals, selenium (Se) has been demonstrated to be crucial for cardiovascular health (4). Se deficiency is associated with a significantly increased risk of cardiovascular morbidity and mortality (5). Previous studies have confirmed the antioxidant properties of Se, and it has been suggested that Se protection against inflammation or oxidative stress is mainly exerted through Se-dependent glutathione peroxidases and other selenoproteins (6,7). Inflammation response is widely involved in most diseases, and to some extent, is a normal process of body defense against certain stimuli. However, excessive inflammation may

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lead to cell damage and even tissue necrosis (8). HHCY has been reported to serve an important role in pro-inflammatory processes in numerous diseases, including intestinal inflammation and aortic adventitial inflammation (9,10), although the underlying mechanisms remain unclear. The present study aimed therefore to determine whether Se could exert an anti-inflammatory protection against HHCY-induced cardiac fibrosis in mouse cardiac fibroblasts (CFs) and the potential underlying mechanisms.

Recent studies reported that some non-coding RNAs show great potential in the regulation of cardiac fibrosis (11-13). Long non-coding RNAs (lncRNAs) are a type of none-coding RNA of >200 nucleotides in length, which participate in various biological behavioral processes, including the onset and development of some cardiac diseases (12-16). The lncRNA scaffold attachment factor B interacting lncRNA (SAIL) has been shown to improve cardiac fibrosis after myocardial infarction by regulating the transcription of fibrosis-related genes, such as α-SMA, collagen I and III (12,14). The lncRNA maternally expressed 3 (MEG3) has been reported to regulate cardiomyocyte proliferation and apoptosis (15,16). Inhibiting the expression of MEG3 can reverse hypoxia-induced growth inhibition of heart progenitor cells (15). In addition, a previous study from Wu *et al* (17) demonstrated that MEG3 knockdown in cardiomyocytes can reduce cardiomyocyte apoptosis and is associated with an improvement of heart function post-myocardial infarction. Furthermore, Gong *et al* (18) attributed the beneficial effects of MEG3 silencing on suppressing cardiomyocyte apoptosis to the increased expression of microRNA-183, which mediates the inhibition of p27, by activating PI3K/AKT/FOXO3a signaling pathway in hypoxic H9C2 cells. In addition, a previous study demonstrated the protection effects of MEG3 inhibition against endoplasmic reticulum stress-mediated cardiac apoptosis by targeting p53 protein (16). However, although MEG3 was reported to be mainly enriched in CFs, study focusing on the effects of MEG3 on CFs is rare. The present study aimed therefore to determine the effect of MEG3 on HHCY-induced fibrosis in CFs and to explore the possible links between Se and MEG3.

Materials and methods

Isolation and culture of mice CFs. The animal experimental protocols were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (approval no. WDRM-20200608). A total of 30 male C57BL/6 mice (age, 8-10 weeks; weight, 25-29 g) were purchased from the China Three Gorges University (approval no. SCXK 2017-0012; Yichang, China). The animals were maintained in specific-pathogen-free and environmentally controlled isolation conditions with free access to food and water (temperature, 20-25˚C; humidity, 45-55%; 12-h light/dark cycle) for 1 week prior to the beginning of the study. Before collection of cardiac tissues, mice were sacrificed with pentobarbital sodium (180 mg/kg) injected intraperitoneally. Subsequently, hearts were removed quickly from the chest and washed three times in PBS containing 1% penicillin-streptomycin solution (100X) (19). Excess tissues were removed and the majority of the left ventricle was collected for further shearing into 1 mm^3

pieces with microsurgical scissors. Once broken tissue was digested by 0.05% trypsin for 3 min at 37˚C, before subsequent digestion three times at 37˚C (~3 min each time) was performed in DMEM/F12 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 0.018% collagenase II (cat. no. 2275; Biofroxx; neoFroxx GmbH). After placing at room temperature for 1 min, the supernatant was collected and neutralized in DMEM/F12 medium containing 10% FBS (TICO Europe) after each digestion. Neutralized supernatant was centrifuged at 1,000 x g for 9 min at 4˚C to collect the cell pellet, which was further resuspended in DMEM/F12 medium containing 10% FBS and seeded in a six-well plate. CFs were obtained after 1 h of adherence whereas the supernatant cells that had not adhered were discarded. CFs were cultured in DMEM/F12 medium containing 10% FBS at 37˚C for 24 h. CFs at passage two were used for subsequent experiments and were randomly divided into six groups as follows: i) Control group (Ctrl); ii) Se group, only treated with 100 nM sodium selenite (Sigma-Aldrich; Merck KGaA; cat. no. S5261); iii) HCY (200 μM)-stimulated group; iv) HCY + Se group (co-treated with HCY and Se); v) HCY + adenovirus (Ad)-shMEG3 group (HCY + Ad-shMEG3); and vi) HCY + Ad-scramble group (HCY + Ad-scr). CFs in six-well plates were transfected with MOI of 100 for 12 h at 37˚C before subsequent drug treatment. The CFs were treated with Se and HCY at the same time at 37˚C for 24 h. Adenoviruses (constructed by GV119 plasmid vector) expressing short hairpin RNA (shRNA) against MEG3 (5'-ACCCTCCTGGATTAGGCCAAA-3') and negative control shRNA (5'-TTCTCCGAACGTGTCACGT-3') were generated by Shanghai Genechem Co., Ltd.

Cell proliferation assessment. Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.; cat. no. CK04) was used to evaluate CF proliferation. Briefly, CFs were seeded in a 96-well plate at the density of 5,000 cells/well and were treated with 100 nM Se and 200 μM HCY at 37˚C for 24 h. Subsequently, 10 μl CCK-8 reagent was added to each well, and the cells were incubated at 37˚C for 90 min. The absorbance was read at a wavelength of 450 nm using a microplate spectrophotometer.

Detection of reactive oxygen species (ROS) production. Dihydroethidium (DHE; Beyotime Institute of Biotechnology; cat. no. S0063) staining was used to detect the cellular ROS production. DHE, after entering living cells, is dehydrogenated by intracellular superoxide anions to produce ethidium, which then binds to RNA or DNA to produce red fluorescence. The stronger the red fluorescence, the higher levels of intracellular superoxide production. In the present study, CFs were first seeded into 24-well plates at a density of 1x10^5 cells/well. DHE (10 μM) was then added to CFs and incubated at 37˚C for 30 min before treated being with 100 nM Se and 200 μM HCY at 37˚C for 24 h. Cells were washed with PBS and the fluorescence was observed under a fluorescence microscope using x100 magnification.

ELISA assays. Cell culture supernatant from six-well plates in each group was collected and the levels of tumor necrosis factor-α (TNF-α: ELK Biotechnology, Co., Ltd.; cat. no. ELK1395) and interleukin-1β (IL-1β; ELK Biotechnology,

Co., Ltd.; cat. no. ELK1271) were evaluated using ELISA kits according to the manufacturer's protocols.

SOD and MDA detection. First, CFs in six-well plate were lysed by RIPA lysis buffer (Beyotime Institute of Biotechnology; cat. no. P0013B) at 4˚C. The lysate of each group was then collected, followed by centrifugation at 2,000 x g at 4˚C for 10 min. SOD kit (cat. no. A001-3; Nanjing Jiancheng Bioengineering Institute) and MDA kit (cat. no. A003-1; Nanjing Jiancheng Bioengineering Institute) were used to evaluate the levels of SOD and MDA, respectively, according to the manufacturer's instruction.

Reverse transcription quantitative (RT-q)PCR. Total RNA was extracted from CFs using RNAiso Plus reagent (Takara Bio, Inc.; cat. no. 9108) and dissolved into diethyl pyrocarbonate-treated water. After testing the concentration of RNA, SweScript RT First Strand® cDNA Synthesis Kit (Wuhan Servicebio Technology Co., Ltd.; cat. no. G3330-50) was used to reverse transcribe RNA into cDNA according to the manufacturer's instructions. The following thermocycling conditions were used for PCR: Initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 15 sec, 60˚C for 10 sec and 72˚C for 30 sec. The reactions were conducted in an ABI VIIa7 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using a 2x SYBR Green qPCR Master Mix (Low ROX; Wuhan Servicebio Technology Co., Ltd.; cat. no. G3320-05). The sequences of the primers were as follows: MEG3 forward, 3'-GCTCATCTTATT CTGGGCACCT-5' and reverse, 3'-TCGTGGACATTCCTC TTCCG-5'; and GAPDH forward, 3'-CCTCGTCCCGTAGAC AAAATG-5' and reverse, 3'-TGAGGTCAATGAAGGGGT CGT-5'. The $2^{-\Delta\Delta Cq}$ method was used to analyze the result of qPCR (20).

Western blotting. Total protein was extracted from CFs lysed by RIPA lysis buffer (Beyotime Institute of Biotechnology; cat. no. P0013B) at 4˚C. Protein concentration was determined using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology; cat. no. P0012S). Proteins were separated by 10% SDS-PAGE (25 μg per lane) and were transferred onto PVDF membranes. Membranes were blocked in 5% non-fat milk at room temperature for 2 h, followed by incubation with primary antibodies at 4˚C overnight. The next day, membranes were washed three times with TBST (20 mM Tris, 137 mM NaCl, 0.1% Tween) and were incubated with secondary antibodies for 1 h at room temperature. Membranes were washed three times with TBST and protein bands were visualized using enhanced chemiluminescence reagent (Beyotime Institute of Biotechnology; cat. no. P0018FM). The relative protein expression levels were normalized to endogenous control GAPDH using AlphaEaseFC software (FluorChem8900; Alpha Innotech Corporation; ProteinSimple). The present study used primary antibodies against GAPDH (Abcam; cat. no. ab37168; 1:10,000), collagen I (Abcam; cat. no. ab34710; 1:1,000), collagen III (Abcam; cat. no. ab7778; 1:1,000), α-SMA (Abcam; cat. no. ab32575; 1:1,000), JAK2 (Abcam; cat. no. ab108596; 1:1,000), phosphorylated (p)-JAK2 (Cell Signaling Technology, Inc.; cat. no. 3230; 1:1,000), STAT3 (Abcam; cat. no. ab68153; 1:1,000) and p-STAT3 (Abcam;

cat. no. ab76315; 1:1,000). The secondary antibody used in the present study was the horseradish peroxidase-conjugated goat anti rabbit antibody (ASPEN; cat. no. AS1107; 1:10,000).

Statistical analysis. Statistical analysis was performed using SPSS software (version 19.0; IBM Corp). All data were presented as the means ± standard deviation. Comparisons between two groups were evaluated using unpaired two-tailed Student's t-test. Multiple comparisons were performed using one-way ANOVA followed by Bonferroni's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Se alleviates fibrosis induced by HCY. The occurrence of cardiac fibrosis is closely related to the activation and proliferation of cardiac fibroblasts. As presented in Fig. 1A, when HCY concentration was <200 μM, HCY ability to stimulate the proliferation of CFs was null, therefore the 200 μM concentration was selected for subsequent experiment. To clarify the effect of Se on cardiac fibrosis, we first examined the effect of Se on CF proliferation. Different concentrations of Se were used and the results demonstrated that 100 nM Se had a significant inhibitory effect on CF proliferation following treatment with HCY, and 200 nM Se resulted in significant cytotoxicity (Fig. 1B). Furthermore, 100 nM Se showed a significant inhibitory effect on the expression of the fibrosis related proteins α-SMA, collagen I and III following treatment with HCY (Fig. 1C). These results demonstrate that Se may effectively improve HCY-induced CF fibrosis.

Se downregulates MEG3 and MEG3 knockdown decreases HCY-induced fibrosis. Since MEG3 has been reported to serve a role in cardiac fibrosis (21), the expression of MEG3 was determined in different experimental groups. As presented in Fig. 2A, HCY elevated the expression level of MEG3, whereas Se treatment reversed this increase. To verify that the regulation of Se on fibrosis was MEG3-independent, an MEG3-knockdown adenovirus (Ad-shMEG3) was used to infect CFs (Fig. 2B) and the impact of MEG3 knockdown on HCY-induced CF fibrosis was explored. Consistent with the effect of Se, MEG3 knockdown significantly inhibited the increased proliferation of CFs induced by HCY (Fig. 2C). In addition, the expression of α-SMA, collagen I and III was significantly decreased in CFs infected by Ad-shMEG3 compared with the Ad-scramble group (Fig. 2D and E). These results indicated that Se could alleviate CF fibrosis through partly downregulating MEG3.

Se or MEG3 knockdown decreases HCY-induced increase in oxidative stress. Since inflammation or oxidative stress are widely involved in progression of cardiac fibrosis (22-24), we investigated the production of ROS in the experimental groups. The results demonstrated that Se significantly inhibited the accumulation of ROS in CFs following treatment with HCY (Fig. 3A). Furthermore, Se significantly increased SOD activity and decreased MDA contents in HCY-treated CFs (Fig. 3B and C). In addition, MEG knockdown had the same anti-oxidative stress effect (Fig. 3D and F). These findings
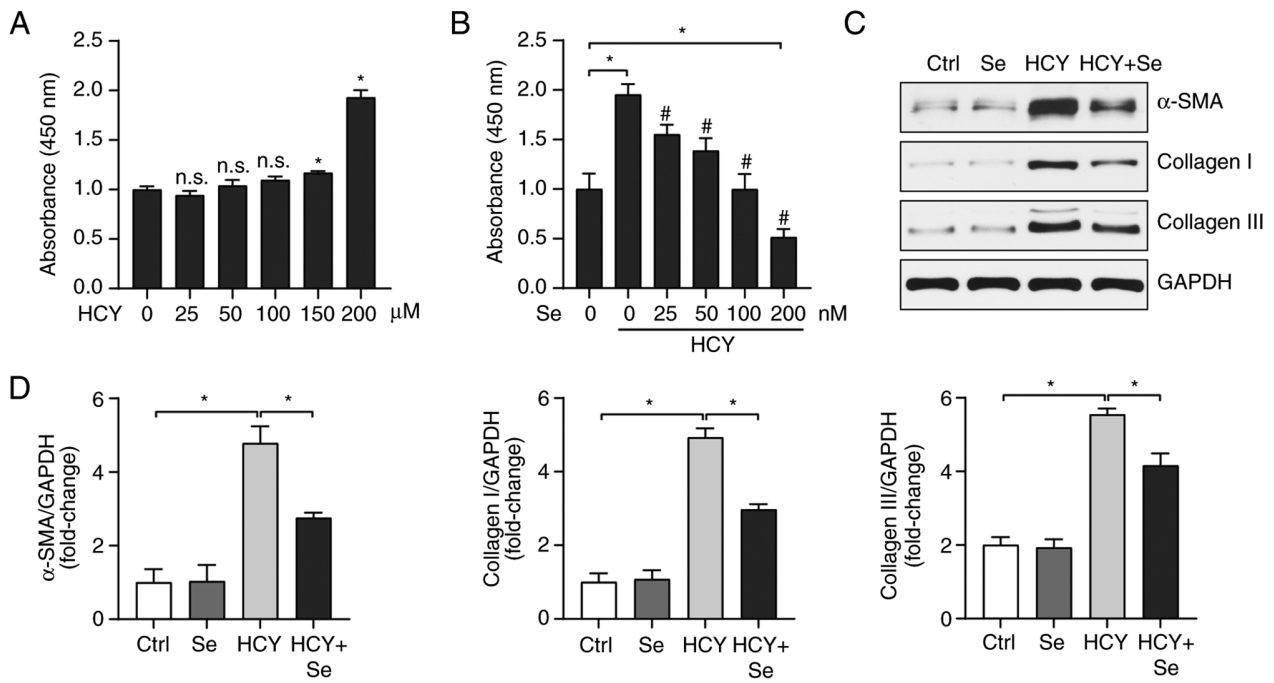
Figure 1. Se alleviates fibrosis induced by HCY. (A) Results from CCK-8 assay in CFs treated with the indicated concentrations of HCY (n=6). (B) Results from CCK-8 assay in CFs treated with HCY and the indicated concentrations of Se (n=6). (C) Expression of α-SMA, collagen I and III assessed by western blotting in CFs stimulated with the indicated treatments. (D) Relative quantitative results of protein levels (n=3). *P<0.05; #P<0.05. CCK-8, Cell Counting Kit-8; CFs, cardiac fibroblasts; HCY, homocysteine; α-SMA, α-smooth muscle actin; NS, non-significant; Se, selenium; Ctrl, control; MEG3, maternally expressed gene 3.
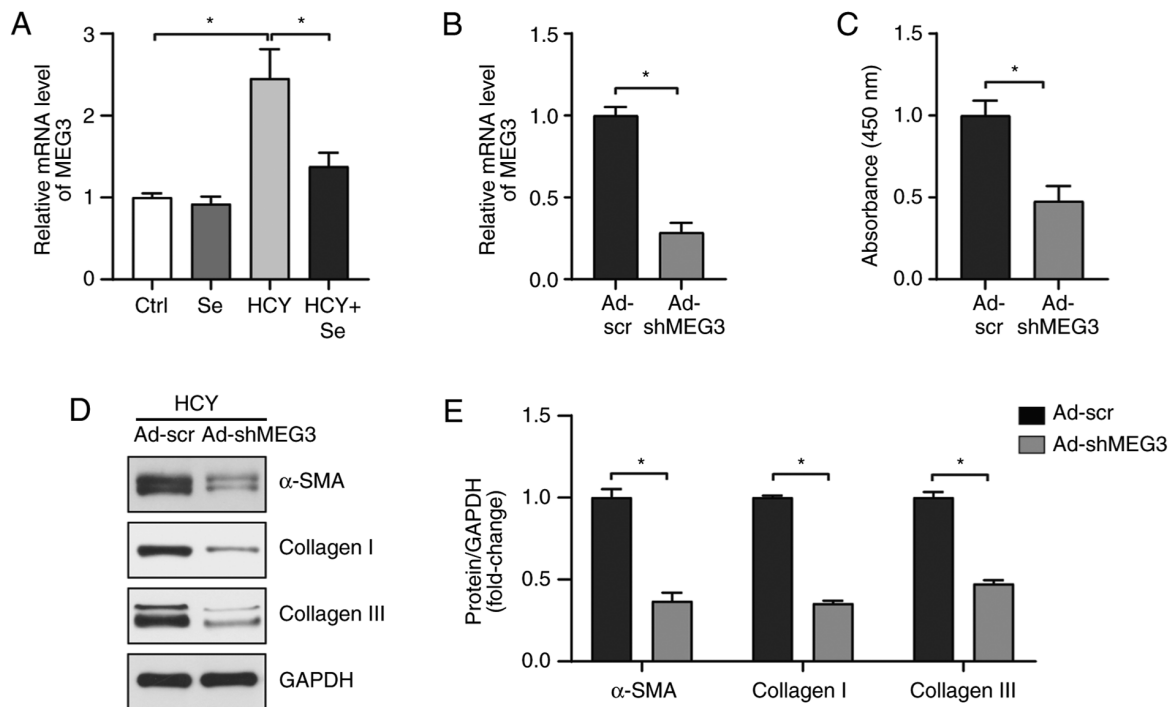

Figure 2. Se reverses HCY-induced fibrosis by downregulating the expression of MEG3. (A) Relative mRNA levels of MEG3 in CFs treated by Se and HCY for 24 h (n=3). (B) Relative mRNA levels of MEG3 in CFs infected with Ad-scr or Ad-shMEG3 (n=3). (C) Results from Cell Counting Kit-8 assay in CFs infected with Ad-scr or Ad-shMEG3 (n=6). (D) Expression of α-SMA, collagen I and III assessed by western blotting in CFs infected with indicated adenovirus and treated with HCY. (E) Relative quantitative results of protein levels (n=3). *P<0.05. CFs, cardiac fibroblasts; HCY, homocysteine; α-SMA, α-smooth muscle actin; Se, selenium; Ctrl, control; Ad, adenovirus; sh, short hairpin; MEG3, maternally expressed gene 3.

suggested that the anti-oxidative effects of Se may be mediated by MEG3 regulation.

Se or MEG3 knockdown decreases the pro-inflammatory cytokines secretion. The effect of Se on inflammation was
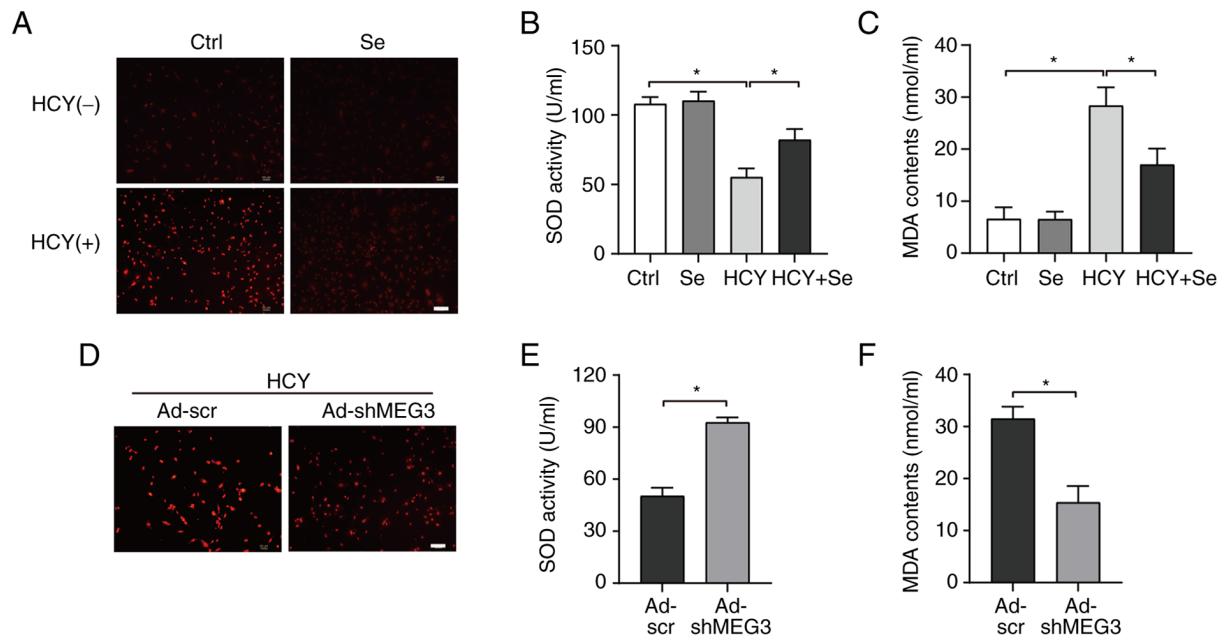
Figure 3. Se or MEG3 knockdown decreases HCY-induced oxidative stress. (A) Representative images of dihydroethidium staining in CFs stimulated with the indicated treatments. Scale bar, 100 μm. (B and C) (B) SOD activity and (C) MDA content in CFs stimulated with the indicated treatments (n=3). (D) Representative images of dihydroethidium staining in CFs infected with Ad-scr or Ad-shMEG3. Scale bar, 100 μm. (E and F) (E) SOD activity and (F) MDA content in CFs infected with Ad-scr or Ad-shMEG3 (n=3). *P<0.05. CFs, cardiac fibroblasts; HCY, homocysteine; SOD, superoxide dismutase; MDA, malondialdehyde; Se, selenium; Ctrl, control; Ad, adenovirus; sh, short hairpin; MEG3, maternally expressed gene 3.
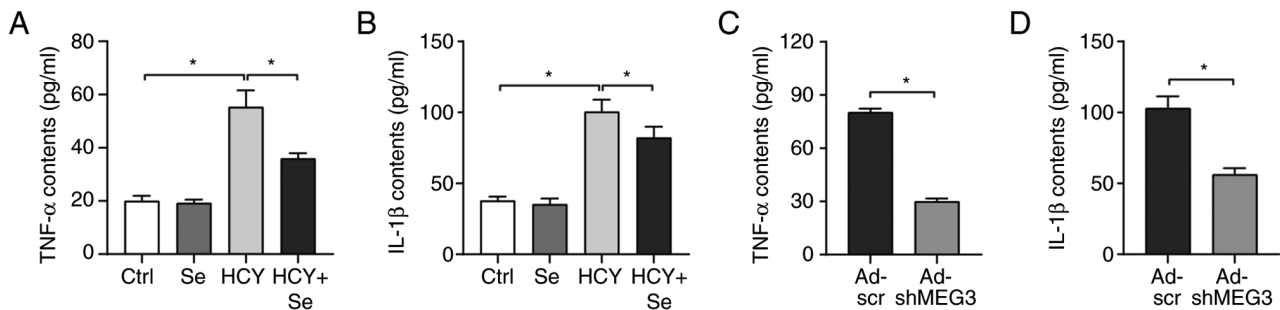

Figure 4. Se or MEG3 knockdown decreases CF pro-inflammatory cytokines secretion. (A and B) Pro-inflammatory cytokines (A) TNF-α and (B) IL-1β contents in CFs stimulated with indicated treatment were evaluated using ELISA assays (n=3). (C and D) Pro-inflammatory cytokines (C) TNF-α and (D) IL-1β contents in CFs infected with Ad-scr or Ad-shMEG3 (n=3). *P<0.05. CFs, cardiac fibroblasts; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; Se, selenium; Ctrl, control; Ad, adenovirus; sh, short hairpin; MEG3, maternally expressed gene 3.

determined in the present study. The results from ELISA demonstrated that HCY-induced increase in TNF-α and IL-1β secretion was reversed following treatment with Se compared with control group (Fig. 4A and B). Furthermore, MEG3 knockdown alleviated HCY-induced inflammation in CFs (Fig. 4C and D). These results suggested that Se may alleviate the inflammatory response caused by HCY tby regulating the expression of MEG3.

Activation of JAK2/STAT3 is mitigated following Se treatment or MEG3 knockdown. Increasing evidence suggests that JAK2/STAT3 pathway is activated during the progress of fibrosis (25,26). The phosphorylation levels of JAK2 and STAT3 were therefore determined in the present study. As presented in Fig. 5, the activation of JAK2 and STAT3 was amplified in CFs following HCY treatment. Conversely, treatment with Se or MEG3 knockdown significantly decreased

p-JAK2 and p-STAT3 expression. These findings suggested that the inhibitory effect of Se on inflammation and oxidative stress may be mediated by a negative regulation of JAK2/STAT3 pathway activation.

Discussion

Cardiovascular diseases represent the leading causes of mortality worldwide (27). Almost all cardiovascular diseases are related to the pathological myocardial remodeling process that is characterized by tissue fibrosis (28-30). It is therefore crucial to clarify the specific molecular mechanism of cardiac fibrosis and to determine some targets for the treatment of cardiovascular diseases. HHCY is characterized by an abnormally high level of homocysteine in the blood, which has been reported as an independent risk factor for several cardiovascular diseases (31,32). Previous studies have reported that HHCY
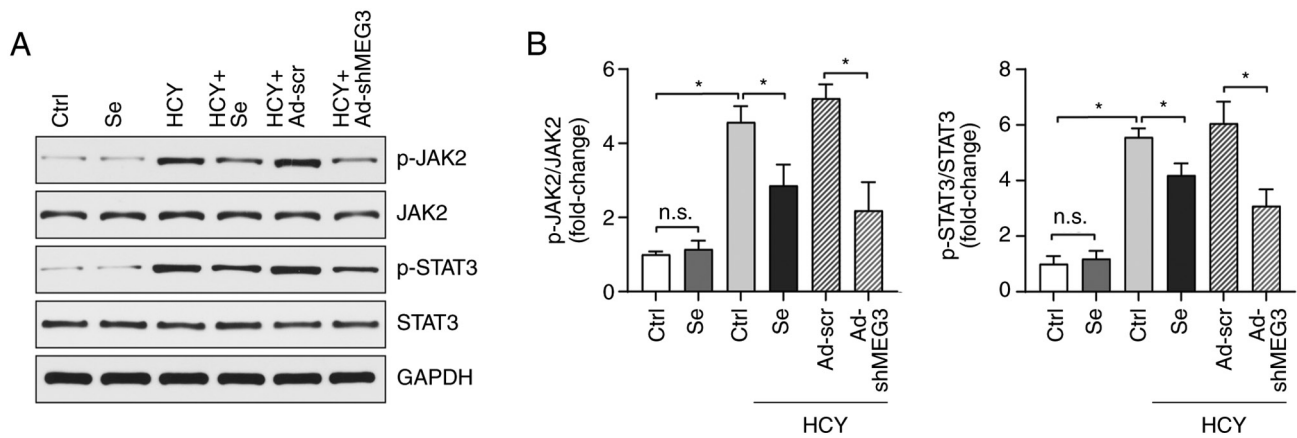
Figure 5. Activation of JAK2/STAT3 is mitigated by Se intervention or MEG3 knockdown. (A) Expression of p-JAK2, JAK2, p-STAT3 and STAT3 was evaluated by western blotting in CFs. (B) Relative quantitative results of protein levels (n=3). $^*P<0.05$. HCY, homocysteine; p, phosphorylated; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; CFs, cardiac fibroblasts; Se, selenium; Ctrl, control; Ad, adenovirus; sh, short hairpin; MEG3, maternally expressed gene 3; NS, non-significant.

accelerates the pathological cardiac remodeling by promoting myocardial interstitial and perivascular fibrosis (2,33). During pathological cardiac remodeling and cardiac fibrosis, CFs activate and secrete various matrix protein and proinflammatory cytokines including α-SMA, collagen I and III, which can promote CF migration and proliferation (34). In the present study, we demonstrated that the secretion of the pro-inflammatory cytokines TNF-α and IL-1β and the expression of the fibrosis related genes α-SMA, collagen I and III were significantly increased in HCY-treated CFs. Furthermore, HCY significantly promoted the proliferation of CFs. These results indicated that the effect of HHCY on cardiac fibrosis may be mediated by the promotion of CF proliferation and inflammation activation.

Se is an important trace element with good antioxidant effect. Se is an essential micronutrient element found in mammals and an important antioxidant of food origin (35,36). Se exists in various selenoproteins and selenate in the form of selenocysteine, which serves an essential role in various diseases through inhibiting oxidative stress and inflammatory reaction (37). Previous studies have demonstrated that Se can protect cells against oxidative damage and endoplasmic reticulum stress-induced apoptosis, and can serve an important role in the reverse transport pathway during endoplasmic reticulum-related protein degradation (38). In addition, increased inflammation and oxidative stress are involved in the occurrence and development of most cardiovascular diseases (4). Previous studies have reported that Se alleviates the pathological progress of various cardiovascular diseases, such as heart failure and atherosclerosis, by negatively regulating inflammation and oxidative stress (39,40). However, there are only a few studies about the effect of selenium on cardiac fibrosis. The present study demonstrated that Se could effectively alleviate HCY-induced cardiac fibrosis in CFs. In addition, Se significantly inhibited the activation and proliferation of CFs, and suppressed the secretion of proinflammatory cytokines and ROS. Regarding the mechanism, Se played an anti-fibrotic effect through downregulating of MEG3. MEG3 is a lncRNA that possesses multiple biological functions and plays an important role in numerous diseases. Previous studies have demonstrated that MEG3 plays essential roles in cardiovascular diseases, such

as inducing cardiomyocyte apoptosis, contributing to myocardial ischemia-reperfusion injury and regulating cardiac remodeling in cardiac hypertrophy (41-43). In addition, it has been reported that knocking down MEG3 expression can prevent the induction of myocardial matrix metalloproteinase-2, reduce myocardial fibrosis and improve diastolic function following transverse aortic coarctation in mice (21). The present study demonstrated that MEG3 knockdown can reduce HCY-induced CF inflammation, and that Se can effectively reduce HCY-induced inflammation by regulating the expression of MEG3.

As the most conservative members of the JAK/STAT pathway family, JAK2/STAT3 are closely related to the pathogenesis, prevention and treatment of a various types of disease, especially heart diseases such as myocardial hypertrophy, myocardial ischemia, heart failure, ischemic preconditioning and ischemia-reperfusion (44-46). A previous study demonstrated that Se deficiency in cardiomyocytes can lead to a decrease in the expression of potassium channels, mitochondrial STAT3 activity and mitochondrial function, thus promoting cardiomyocyte apoptosis (47). We therefore speculated that the effect of Se on cardiac fibrosis may be mediated by the JAK2/STAT3 pathway. The present study showed that Se or MEG3 knockdown can significantly inhibit the activation of JAK2/STAT3 signal pathway induced by HCY.

JAK2/STAT3 signal pathway is one of the important pathways of cell signal transduction, which plays an important role in cell growth, activation, differentiation and apoptosis. The main substrate of JAK kinase is a cytokine receptor, which binds to ligand in the form of dimer or monomer. However, JAK kinase has been known to activate STAT family members. Once STAT factors are phosphorylated by JAKs, they become homodimers or heterodimers, which are transferred to the nucleus, where they show activation or inhibition of transcription. It has been reported that STAT1 and STAT3 members can be activated by JAK2 in the absence of new protein synthesis and hydrogen peroxide treatment (48). At present, some studies have shown that collagen synthesis in cardiac fibroblasts can be stimulated by regulating JAK/STAT pathway, which is closely related

to the pathological changes of cardiac fibrosis (49,50). In addition, a previous study reported that hydrogen sulfide can alleviate cardiac fibrosis in diabetic rats by downregulating JAK/STAT pathway to inhibit oxidative stress and ER stress, inflammatory response and apoptosis (51). The results from the present study demonstrated that the pro-inflammatory and oxidative stress responses of cardiac fibroblasts induced by HCY was activated, which were inhibited by Se. These findings suggested that Se may not only have and antioxidant effect, but may also regulate JAK/STAT signal pathway. Since JAK2/STAT3 signal pathway can affect oxidative stress and myocardial fibrosis, and because oxidative stress leads to myocardial fibrosis and myocardial remodeling in mice (23,24), we speculated that Se could inhibit cardiomyocyte apoptosis and improve myocardial fibrosis by inhibiting the activation of JAK2/STAT3 signal pathway.

In summary, the present study demonstrated that Se can inhibit the expression of MEG3 and negatively regulate the activation of JAK2/STAT3 pathway, thus effectively reducing inflammation and oxidative stress caused by HCY. These results may provide promising targets for the prevention and treatment of cardiac fibrosis.

Acknowledgements

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

WL and YL performed the experiments and participated in drafting the manuscript. SC, JL and LT performed data analysis. HX and CZ designed and supervised the studies. WL and HX confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experimental protocols were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (approval no. WDRM-20200608).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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