Cathepsin B deficiency attenuates cardiac remodeling in response to pressure overload via TNF-α/ASK1/JNK pathway

Qing-Qing Wu,1,2* Man Xu,1,2* Yuan Yuan,1,2 Fang-Fang Li,1,2 Zheng Yang,1,2 Yuan Liu,1,2 Meng-Qiao Zhou,1,2 Zhou-Yan Bian,1,2 Wei Deng,1,2 Lu Gao,3 Hongliang Li,1,2 and Qi-Zhu Tang1,2

1Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China; 2Cardiovascular Research Institute of Wuhan University, Wuhan, China; and 3Department of Cardiology, Institute of Cardiovascular Disease, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People’s Republic of China

Submitted 26 August 2014; accepted in final form 2 February 2015


Heart failure (HF) is a leading cause of death in industrialized nations, especially in the aging population (19). Cardiac remodeling is the process of structural and functional changes in the left ventricle (LV) in response to internal or external cardiovascular damage or induced by pathogenic risk factors, and it is a precursor of clinical heart failure (13). The remodeling process affects not only cardiomyocytes but also interstitial tissue, fibroblasts, inflammatory cells, and endothelial cells, as well as the influence of these cells on both myocardial architecture and tissue repair (9). Divergent signaling mechanisms lead to distinct patterns of cardiac remodeling. The mitogen-activated protein kinase (MAPK) (27) and Akt (1) signaling pathways and matrix metalloproteinases (MMPs) (14) regulate the extracellular matrix and wall stiffness, as well as cardiomyocyte apoptosis pathways (40), including both the mitochondrial and endoplasmic reticulum stress pathways, leading to cell death and contributing to reduced cardiac contractility (21). Inhibition of these pathways in the heart in the setting of increased biomechanical stress remains incompletely understood.

Lysosomal cathepsins are lysosomal proteases that belong to the papain family, which contains the following 11 members in humans: cathepsin B (CTSB), C, F, H, K, L, O, S, V, X, and W, which exist at the sequence level. They were initially considered intracellular enzymes, namely, enzymes responsible for the majority of proteolysis in the acidic environment of the endosomal/lysosomal compartment, a setting in which they degrade intracellular and extracellular proteins (33). Many cathepsins are expressed in both developing and adult hearts. Previous studies have demonstrated that cathepsins are essential for both cardiomyocyte apoptosis (32) and extracellular matrix balance (22a, 29). Further studies have revealed increased cathepsin activity in both hypertrophic and failing hearts (8, 31) and showed that cathepsins are regulated under these pathological conditions.

CTSB (GenBank Accession No. AH001867.1) is both an endopeptidase and a carboxydipeptidase and is composed of a 15-residue putative signal peptide, a 75-residue propeptide, a 249-residue mature domain. A CTSB gene containing 13 exons has been mapped to chromosome 8p22 (22b). As with all other cathepsin members, CTSB is well recognized as a proteolytic enzyme, namely, an enzyme that plays an essential role in regulating innate immunity (23), extracellular matrix balance (24), inflammation, and apoptosis (11). CTSB is reportedly involved in various pathologies and oncogenic processes in humans (22, 25). Moreover, increased CTSB expression was reported in H9c2 cardiomyocytes in patients with doxorubicin-induced cardiomyopathy (2) and in patients with heart failure secondary to dilated cardiomyopathy (12). More recently, Liu et al. (17) found that a specific CTSB inhibitor, CA-074Me, significantly attenuated cardiac remodeling after myocardial infarction. This observation suggested that CTSB plays an important role in cardiovascular disease. However, whether CTSB affects chronic pressure overload-induced cardiac remodeling has never been examined. It is known that pressure overload can activate the renin-angiotensin system and induce the release of ANG II, which activates the Go (q) protein-coupled receptor signaling pathway (37). Thus ANG II was used in vitro to induce the cardiac remodeling in cardiomyocytes. In this study, we used CTSB-knockout (KO) mice and cultured CTSB-deficient and CTSB-overexpressed H9c2 cardiomyocytes to investigate the role of CTSB in cardiac remodeling in response to hypertrophic stimuli. We show that eliminating CTSB reduces cardiac hypertrophy and CTSB is dynamically regulated during cardiac hypertrophy and remodeling.
MATERIALS AND METHODS

Animals and animal models. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (Protocol No. 00013274). All surgeries and subsequent analyses were performed in a blinded manner. Male and female CTSB+/− heterozygous mice, purchased from the Jackson Laboratory, were backcrossed for generations. After the identification of their gene type, CTSB−/− homozygous mice were used as the CTSB KO group, and wild-type (WT) mice were used as the control group. Male CTSB-KO mice, and their WT littermates, aged 8–10 wk, were subjected to either aortic banding (AB) or a sham operation, as described previously (39). The mice were euthanized by cervical dislocation 8 wk after surgery. The hearts and lungs of killed mice were harvested and weighed to compare heart weight/body weight (HW/BW, mg/g), lung weight/body weight (LW/BW, mg/g), and heart weight/tibia length (HW/TL, mg/mm) ratios between KO and WT mice.

Echocardiography and hemodynamics. Echocardiography was performed on anesthetized (1.5% isoflurane) mice using a MyLab 30C ultrasound (Biosound Esaote) with a 10-MHz linear array ultrasound transducer. The LV was assessed in both parasternal long-axis and short-axis views at a frame rate of 120 Hz. End-systole and end-diastole were defined as the phases in which the smallest and largest areas of the LV were obtained. LV end-systolic diameter and LV end-diastolic diameter were measured via LV M-mode tracing with a sweep speed of 50 mm/s at the midpapillary muscle level.

Histological analysis. Hearts were excised, placed immediately in 10% potassium chloride solution to ensure that they were stopped in diastole, washed with saline solution, placed in 10% formalin, and embedded in paraffin. Hearts were cut transversely and close to the apex to visualize the left and right ventricles. Several sections of each heart (4- to 5-μm thick) were prepared and stained with either hematoxylin and eosin for histopathology or stained with PSR (Protocol No. 00013274). All surgeries and subsequent analyses were performed in a blinded manner. Male and female CTSB+/− heterozygous mice, purchased from the Jackson Laboratory, were backcrossed for generations. After the identification of their gene type, CTSB−/− homozygous mice were used as the CTSB KO group, and wild-type (WT) mice were used as the control group. Male CTSB-KO mice, and their WT littermates, aged 8–10 wk, were subjected to either aortic banding (AB) or a sham operation, as described previously (39). The mice were euthanized by cervical dislocation 8 wk after surgery. The hearts and lungs of killed mice were harvested and weighed to compare heart weight/body weight (HW/BW, mg/g), lung weight/body weight (LW/BW, mg/g), and heart weight/tibia length (HW/TL, mg/mm) ratios between KO and WT mice.

Assessment of apoptosis. Samples were first incubated with a terminal deoxynucleotidyl transferase-mediated DUTP nick-end-labeling (TUNEL) reagent containing terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP according to the protocol of the ApopTag Plus Fluorescein In Situ Apoptosis Detection Kit (S7111; Chemicon, Temecula, CA). Paraffin-embedded sections of mouse hearts were stained with DAPI for 30 min to evaluate the cell nucleus; meanwhile, the cells were costained with an antigen. The numbers of apoptotic and total cells were counted, and the percentages of apoptotic cells were calculated by at least three independent individuals in a blinded manner.

Plasmid constructs. The pSicoR plasmid (no. 11597; Addgene), utilized for knockdown experiments in vitro, was used to construct the CTSB-specific (sh-CTSB) shRNA by cloning the annealed sense and antisense oligos to the HpaI and XhoI site of pSicoR. The secondary antibody, either goat anti-rabbit IgG (926-32211; LI-COR) or goat anti-mouse IgG (C11026-03; LI-COR), was incubated with the cell lysate for 60 min. The blots were scanned by a two-color infrared imaging system (Odyssey; LICO) to quantify protein expression. The protein expression level was normalized against GAPDH to determine the total amount of cell lysate protein.

Downloaded from http://ajpheart.physiology.org/ by 10.221.0.33 on July 1, 2017
RESULTS

CTSB expression in a pressure overload-induced hypertrophic mouse model. To investigate whether CTSB is involved in cardiac hypertrophy, we first examined CTSB expression in an experimental mouse model with AB-induced cardiac hypertrophy and in an in vitro model induced by ANG II, we observed that the expression of CTSB was upregulated 1.77-fold in mouse hearts 2 wk after AB, 3.44-fold 4 wk after AB, and 4.27-fold 8 wk after AB compared with sham-operated hearts (Fig. 1A). Furthermore, using cardiomyocytes treated with ANG II (1 μM) for 24 h to induce hypertrophy, we found that the levels of CTSB were increased to 3.62-fold in these hypertrophic cardiomyocytes (Fig. 1B). We also explored the localization of CTSB by using immunofluorescence and found that CTSB could be detected in the cytoplasm of cardiomyocytes in response to pressure overload and ANG II stimuli (Fig. 1C). Together, these data indicate that CTSB is actually expressed in hearts and its protein levels are significantly increased in hypertrophic cardiomyocytes. This findings suggest that CTSB is involved in cardiac hypertrophy.

Absence of CTSB in hearts attenuates pressure overload-induced cardiac hypertrophy. We used a CTSB-KO mouse model (Fig. 2A) and subjected the mice to either AB or sham operation for 8 wk to evaluate the effect of CTSB on cardiac remodeling. After AB, CTSB-null hearts exhibited remarkable attenuation of cardiac hypertrophy, as evidenced by the following findings: gross heart shrinkage (Fig. 2B), decreased cardiomyocyte cross-sectional area, and reduced ratios of HW/
Fig. 2. Absence of CTSB attenuates pressure overload-induced hypertrophy. A: deletion of CTSB was confirmed by Western blotting (n = 4). WT represents wild-type hearts, and KO represents CTSB-knockout hearts. B: histological analyses of the gross heart and hematoxylin and eosin (H&E) and wheat germ agglutinin (WGA) staining of WT and CTSB-KO mice 8 wk after AB surgery. C: results of cell surface area (n = 100 cells) and the heart weight (HW)/body weight (BW), lung weight (LW)/BW, and HW/tibia length (TL) ratios in the indicated groups (n = 8–10). D: echocardiographic results of WT and KO mice (n = 8–10). E: real-time PCR analyses of the hypertrophic markers atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (MHC), and α-MHC induced by AB in the indicated mice (n = 6). IVSd and IVSs, interventricular septal thickness at end-diastole and end-systole.
BW, LW/BW, and HW/TL (Fig. 2C) compared with AB-treated WT hearts (CTSB+/+). Similar decreases in wall thickness were also observed, as assessed with echocardiography by measuring interventricular septal thickness at both end-diastole and end-systole. LV contraction was significantly improved in CTSB-KO hearts, as determined by measuring both fractional shortening and LV ejection fraction (Fig. 2D). Pressure-volume loop analysis further illustrated the improvements in LV hemodynamic dysfunction in CTSB-KO mice, as measured by parameters that reflect LV volume, systolic function, and diastolic function (Table 1). Moreover, the mRNA expression levels of several hypertrophic markers, including ANP, brain natriuretic peptide (BNP), and β-MHC, were much lower in CTSB-KO mice, whereas the expression of α-MHC was much higher, compared with WT mice after AB (Fig. 2E).

Absence of CTSB attenuates pressure overload-induced cardiac fibrosis. Cardiac fibrosis is an essential feature of pathological cardiac remodeling. PSR staining of cardiac tissue sections was used to quantify collagen volume. Dramatic perivascular and interstitial fibrosis was observed in WT mice in response to AB but was markedly limited in CTSB-KO mouse hearts (Fig. 3A). The mRNA levels of the fibrotic markers collagen Iα and collagen III were measured (Fig. 3B). The protein expression levels of the MMP9 were decreased in CTSB-KO hearts compared with WT hearts (CTSB+/+). The protein expression of collagen Iα and collagen III was decreased in CTSB-null hearts (Fig. 3C). Our results consistently revealed a decreased fibrotic response in CTSB-KO mice. All these data indicate that the deletion of CTSB blocks pathological cardiac hypertrophy and cardiac fibrosis induced by chronic pressure overload.

CTSB regulates angiotensin II-induced H9c2 cardiomyocyte hypertrophy in vitro. Given that the deletion of CTSB in vivo may attenuate the effects of pressure overload-induced cardiac hypertrophy, we subsequently performed in vitro studies using cultured H9c2 cardiomyocytes and subsequently established H9c2 cell lines with either stable expression of sh-CTSB or overexpression of CTSB using a lentiviral vector (lenti-CTSB) (Fig. 4A). The stable cell lines were exposed to either ANG II or a PBS control for 24 h. Notably, the expression of neither sh-CTSB nor lenti-CTSB altered the size of cultured cardiomyocytes under basal PBS treatments compared with that of control cells. However, in response to ANG II-induced cell hypertrophy, the expression of sh-CTSB reduced the cell size (cross-sectional area) by 39.7% compared with shRNA group (Fig. 4B). Conversely, ANG II-induced cell hypertrophy was enhanced by 27.3% in lenti-CTSB cardiomyocytes compared with the negative control (lenti-NC) group (Fig. 4C). We also examined the mRNA and protein expression of cardiac hypertrophic markers ANP, BNP, and β-MHC. Accordingly, the expression of the hypertrophy markers (ANP, BNP, and β-MHC) was dramatically suppressed in sh-CTSB-infected cardiomyocytes in response to ANG II (Fig. 4D) but remarkably aggravated in lenti-CTSB-infected cardiomyocytes in response to ANG II (Fig. 4E), compared with the controls. These in vitro data are consistent with the in vivo results and suggest that the downregulation of CTSB mitigates pathological cardiac hypertrophy, whereas the overexpression of CTSB promotes pathological cardiac hypertrophy.

CTSB regulates apoptosis. Cardiomyocyte apoptosis plays a critical role in pathological cardiac remodeling (21, 40); therefore, it may be very intriguing to examine whether altered CTSB expression interferes with apoptosis. Mouse hearts from each group were stained for TUNEL and α-actin to determine the effect of CTSB on cardiomyocyte apoptosis. CTSB-KO hearts exhibited an attenuation of apoptosis after 8 wk of AB compared with AB-treated WT hearts (Fig. 5A). The antiapoptotic mechanisms of CTSB were assessed by Western blot analysis. We determined the expression levels of the apoptosis-related proteins Bcl-2, Bax, and Bid, as well as activated caspase signaling proteins, in both AB-induced remodeled hearts and ANG II-induced hypertrophic cardiomyocytes. We observed that CTSB-KO hearts displayed decreased expression of the proapoptotic proteins Bax and Bid, as well as increased expression of the antiapoptotic protein Bcl-2. Caspases are the primary drivers of apoptotic cell death, and our data showed that the activation of caspase 3 and caspase 9 was increased in AB WT mice but was blunted in CTSB-KO mice (Fig. 5B). To confirm these findings, we used H9c2 cells featuring stable expression of either sh-CTSB or lenti-CTSB and H9c2 control cells, followed by treatment with ANG II or PBS for 24 h. Consistent with the in vivo results, Bax, Bid, activated caspase 3, and activated caspase 9 were downregulated and Bcl-2 was upregulated in sh-CTSB H9c2 cardiomyocytes in response to ANG II, compared with control cells (Fig. 5C). In cells with lenti-CTSB, Bax, Bid, activated caspase 3, and activated caspase 9 were upregulated, and Bcl-2 was downregulated (Fig. 5D). Collectively, these studies indicate that CTSB participates in the regulation of stress-induced cardiomyocyte survival, cardiac hypertrophy, and remodeling.

Effects of CTSB on TNF-α/ASK1/JNK signaling. TNF-α is known to mediate apoptotic pathway in the myocardium after AB. Thus TNF-α provoked apoptotic signaling was examined. Consequently, our data indicated that TNF-α, ASK1, JNK, c-Jun were significantly activated in AB mice, which increased

Table 1. Hemodynamic parameters in mice after AB surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham WT (n = 6)</th>
<th>Sham CTSB-KO (n = 6)</th>
<th>AB WT (n = 6)</th>
<th>AB CTSB-KO (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, min⁻¹</td>
<td>464.83 ± 9.52</td>
<td>402 ± 8.43</td>
<td>477.33 ± 23.45</td>
<td>442 ± 20.34</td>
</tr>
<tr>
<td>ESP, mmHg</td>
<td>103.76 ± 1.02</td>
<td>103.58 ± 0.80</td>
<td>162.95 ± 8.13*</td>
<td>147.75 ± 10.88</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>10.65 ± 0.98</td>
<td>10.63 ± 0.14</td>
<td>58.93 ± 3.33*</td>
<td>35.34 ± 0.98†</td>
</tr>
<tr>
<td>ESV, µl</td>
<td>12.67 ± 1.80</td>
<td>12.51 ± 2.45</td>
<td>31.33 ± 0.63‡</td>
<td>18.135 ± 3.10†</td>
</tr>
<tr>
<td>EDV, µl</td>
<td>29.17 ± 1.48</td>
<td>31.26 ± 2.53</td>
<td>37.78 ± 1.20*</td>
<td>29.86 ± 2.65*</td>
</tr>
<tr>
<td>dp/dt max, mmHg/s</td>
<td>10.163 ± 101</td>
<td>9.550 ± 498</td>
<td>5.705 ± 313*</td>
<td>8.343 ± 403‡</td>
</tr>
<tr>
<td>dp/dt max, mmHg/s</td>
<td>−8.196 ± 529</td>
<td>−8.441 ± 288</td>
<td>−4.584 ± 183*</td>
<td>−7.863 ± 676‡</td>
</tr>
<tr>
<td>CO, µl/min</td>
<td>8.463 ± 277</td>
<td>8.230 ± 270</td>
<td>4.422 ± 424*</td>
<td>6.060 ± 371‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. CTSB, cathepsin B; WT, wild type; KO, knockout; AB, aortic banding; HR, heart rate; ESP, end-systolic pressure; EDP, end-diastolic pressure; ESV, end-systolic volume; EDV, end-diastolic volume; dp/dt max, maximal rate of pressure development; dp/dt max, maximal rate of pressure decay; CO, cardiac output. *P < 0.05 for difference from corresponding sham group. †P < 0.05 vs. WT AB group after AB.

**AJP-Heart Circ Physiol** • doi:10.1152/ajpheart.00601.2014 • www.ajpheart.org
the release of cytochrome c. Also, CTSB deficiency inhibited the release of TNF-α/H9251, and the activation of ASK1, JNK, c-Jun, and attenuated the release of cytochrome c (Fig. 6A). Cardiac renin-angiotensin systems are known to be activated in the setting of pressure overload. In addition, ANG II can enhanced the expression of TNF-α/H9251 via AT1 and AT2 receptors in hypertrophic cardiomyocytes (41). Thus we further confirmed the effect of CTSB on the TNF-α/H9251 provoked apoptosis signaling in vitro. Notably, sh-CTSB-infected cardiomyocytes displayed decreased release of TNF-α/H9251 and activation of ASK1, JNK, and c-Jun and reduced release of cytochrome c induced by ANG II, whereas lenti-CTSB-infected cardiomyocytes revealed enhanced release of TNF-α/H9251 and phosphorylation of ASK1 and JNK c-Jun and the increased release of cytochrome c (Fig. 6, B and C). We investigated the activation of other members of MAPK in the CTSB-KO heart, a process induced by pressure overload. Our data indicated that extracellular signal-regulated kinase 1/2 (MEK-ERK1/2), p38, and Akt were significantly activated in AB mice; however, CTSB deficiency did not influence the phosphorylation of MEK-ERK1/2 or p38 compared with that in WT mice. Although Akt signaling plays a crucial role in the regulation of cardiac remodeling, we did not observe any differences in Akt activation between WT and KO mice (Figure 6D).

**Effect of JNK inhibitor and CTSB inhibitor.** To further confirm the effect of CTSB on JNK signaling, JNK inhibition...
Fig. 5. CTSB regulates apoptosis. A: representative images and quantitative results of the terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay in a section of both WT and CTSB-KO mouse hearts 8 wk after AB surgery. Top: representative blots; bottom: bar graphs represent quantitative results.

B: cardiac expression of Bax, Bid, Bcl-2, procaspase 3, and cleaved caspase 3 and caspase 9, determined by Western blot analysis. Top: representative blots; bottom: bar graphs represent quantitative results (n = 6). *P < 0.05 vs. the corresponding sham group; #P < 0.05 vs. the WT AB group. C and D: representative Western blots showing the expression of phosphorylation and total protein levels of Bax, Bid, Bcl-2, procaspase 3, and cleaved caspase 3 and caspase 9 in cardiomyocytes transfected with sh-CTSB (C) or lenti-CTSB (D) in response to ANG II (1 μM), respectively. Top: representative blots; bottom: bar graphs represent quantitative results. *P < 0.05 vs. the corresponding PBS group; #P < 0.05 vs. lenti-NC/shRNA in the ANG II group.
Fig. 6. CTSB regulates the TNF-α/ASK1/JNK signaling pathway in hypertrophic mouse heart. A: representative Western blots showing the expression levels of TNF-α, cytochrome c, and phosphorylation and total protein levels of ASK1, JNK, and c-Jun in CTSB KO mice 8 wk after AB. Top: representative blots; bottom: bar graphs represent quantitative results (n = 6). *P < 0.05 vs. the corresponding sham group; #P < 0.05 vs. the WT AB group. B and C: representative Western blots showing the expression of phosphorylation and total protein levels of ASK1, JNK, c-Jun, as well as the expression levels of TNF-α and cytochrome c in cardiomyocytes with lenti-CTSB or sh-CTSB in response to ANG II (1 μM). Top: representative blots; bottom: bar graphs represent quantitative results. *P < 0.05 vs. the corresponding PBS group; #P < 0.05 vs. lenti-NC/shRNA in the ANG II group. D: representative Western blots showing the phosphorylation and total protein levels of ERK1/2, p38, and AKT in CTSB KO mice 8 wk after AB. Top: representative blots; bottom: bar graphs represent quantitative results. (n = 6). *P < 0.05 vs. the corresponding sham group; #P < 0.05 vs. the WT AB group.
SP600125 (10 μM; S5567; Sigma) was used in lenti-CTSB-infected cardiomyocytes. CA074Me (10 μM; C5857; Sigma), a cell-permeable cathepsin B inhibitor, was also used to verify whether its pharmacological effects are similar to those of sh-CTSB knockdown. Notably, SP600125 reduced the large cell size in CTSB overexpressed cardiomyocytes in response to ANG II. Furthermore, CA074Me exhibited the similar effects with sh-CTSB knockdown by measuring the cell-surface area and the mRNA expression levels of hypertrophic markers (Fig. 7, A and B). SP600125 also reduced ANG II-induced increased phosphorylation of c-Jun and release of cytochrome c in CTSB overexpressed cardiomyocytes but did not affect the release of TNF-α, and the activation of ASK1. CA074Me, similar to cells with sh-CTSB knockdown, blocked the cardiomyocytes apoptotic signaling induced by ANG II. All these data suggest that CTSB regulates cardiac remodeling through the TNF-α/ASK1/JNK pathway (Fig. 7C).

DISCUSSION

In this study, we focused on cardiac-enriched CTSB and found that CTSB plays a critical role in cardiomyocyte hypertrophy and cardiac remodeling in response to stress. We observed that the expression level of CTSB was gradually
upregulated in mouse hearts following pressure overload and was highly expressed in the cardiomyocyte cytoplasm after pressure overload and ANG II stimulation, suggesting that CTSB may be involved in pathological cardiac hypertrophy and remodeling. We studied the effects of the loss of CTSB function via the production of CTSB-KO mice and showed that CTSB deficiency results in attenuated cardiac hypertrophy, fibrosis, and better preservation of cardiac function following pressure overload, strongly suggesting that CTSB is an essential regulator of cardiac remodeling (34). We noticed that the end-systolic pressure was significant different between sham WT and sham KO mice in Table 1, while others were not significantly different. However, whether there is a baseline difference remains uncertain since subsequent analyses failed to show a difference (data not shown). Furthermore, H9c2 cells with increased CTSB expression demonstrated accelerated progression to cardiomyocyte hypertrophy following exposure to an ANG II stimulus, suggesting that CTSB plays a key role in the regulation of hypertrophy in response to pathological stress. Liu et al. (17) found that CTSB participates in the regulation of cardiac remodeling in response to myocardial infarction, which is consistent with our study. In the future, it will be important to further define the role of CTSB in the pathophysiological stress conditions in the heart and, most importantly, to determine whether CTSB participates in the regulation of cardiac responses to stress in patients with cardiovascular disease.

We found that cardiomyocyte apoptosis was significantly repressed in CTSB-null hearts. Cardiac myocyte apoptosis is a key factor in cardiac remodeling, and it may lead to cell death and contribute to reduced contractility (16, 21, 40). Previous work has demonstrated that CTSB is closely related to apoptosis (3, 4). Therefore, it is not surprising that apoptosis was attenuated in AB-treated CTSB-KO hearts. Many lysosomal proteases related to the apoptosis pathway are associated with the Bcl-2 family, which indirectly activates a mitochondrial apoptotic pathway (29a). Therefore, it is intriguing to speculate on the expression of Bcl-2 proteins in CTSB-null hearts under both normal and pathological conditions. Bcl-2 family proteins are subdivided into three groups based on their pro- or anti-apoptotic action and Bcl-2 homology (BH) domains. Proapoptotic Bcl-2-like proteins, including Bax, Bak, Bad, Bid, and Bim, may trigger a loss of integrity in the outer mitochondrial membrane, which may subsequently lead to the release of intermembrane space proteins from mitochondria, and ultimately lead to cell death. The proapoptotic Bcl-2 protein family includes Bcl-2, Bcl-xL, Bcl-w, and other similar proteins (20). In our study, as expected, the expression of the proapoptotic proteins Bax and Bid was abated and that of the antiapoptotic protein Bcl-2 was increased in CTSB-KO mouse hearts. Apoptosis-activated caspase 3 and caspase 9 were also reduced in CTSB-KO mouse hearts. Determining how CTSB regulates apoptosis and remodeling in the heart is a challenging task.

TNF-α, which has been reported to induce apoptosis in many cell types (7, 26), is known to mediate apoptosis in the myocardium after aortic banding (30). TNF-α, by binding to ligand-bound TNR receptor-1 and -2 (TNF-R1/TNF-R2), elicits its distinct signal transduction cascades including the initiation of a mitochondrial pathway of apoptosis that involves ASK1, JNK, and c-Jun (5). Internalized TNF-R2 via ASK1 can form a complex with AIP1, leading to JNK activation (15). Activated JNK signaling controls the release of cytochrome c and induces the apoptotic (38) and cardiac hypertrophic (36) programs. In our study, we found that CTSB deficiency blocked the pressure overload induced the release of TNF-α and activation of ASK1, JNK, and c-Jun (the proximal downstream product of JNK) and enhanced the release of cytochrome c. The JNK inhibitor SP600125 eliminated the activated JNK signaling provoked by TNF-α in CTSB overexpressed cardiomyocytes in response to ANG II. SP600125 also attenuated the enlarged cell size induced by ANG II in CTSB-overexpressed cardiomyocytes. Together, these data indicated that CTSB regulates cardiac apoptosis in part through the TNF-α/ASK1/JNK pathway.

However, although CTSB deficiency attenuated the increased activation of JNK and c-Jun after AB, it should be noted that this deficiency did not affect either ERK1/2 or p38 activation, which also regulate diverse cellular and physiological functions in the process of cardiac remodeling. Recent evidence suggests that CTSB inhibition blunts Akt activation in activated HSCs in response to PDGF (22). However, we observed that CTSB did not affect Akt activation in the setting of cardiac remodeling. Therefore, CTSB regulates cardiac remodeling in part through the ASK1/JNK pathway.

In conclusion, endogenous CTSB plays an essential role in mediating pathological cardiac remodeling in response to pathological conditions, partly through the TNF-α/ASK1/JNK pathway. We propose that CTSB and its downstream signaling molecules may be important targets for the future treatment of patients with heart failure.

GRANTS

This work was supported by National Natural Science Foundation of China Grants 81470516, 81270303, and 81300104, the Specialized Research Fund for the Doctoral Program of Higher Education of China Grant 20130141120042, the Natural Science Foundation of Hubei Province, China Grant 2013CFCB03, and the Fundamental Research Funds for the Central Universities of China Grant 2014302020202.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


