Cathepsins in heart disease—chewing on the heartache?

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CARDIAC REMODELING REFERS to the changes in cardiac structure and function that develop in response to a variety of stimuli, including inflammatory and neurohormonal mediators and increases in wall stress. While some forms of remodeling are physiologically compensatory, such as the changes in cardiac structure that occur over time with strenuous exercise, others are maladaptive and their persistence causes a progressive worsening of cardiac function that leads to heart failure. A strong association between cardiac remodeling and mortality has been noted in heart failure patients (16). Pressure overload is an important cause of remodeling in human patients as it can occur from conditions such as hypertension or chamber outflow obstruction that are common in the population. Changes that occur in the heart in response to increases in pressure include cardiomyocyte hypertrophy and enhanced deposition of extracellular matrix proteins (i.e., fibrosis). Although some of these changes help compensate for increases in wall stress, over time they prove to be maladaptive as they result in abnormalities in both systolic and diastolic function (4). Although many of the pathways involved in the development of pressure overload remodeling have been identified, a full description of all of the “players” that contribute to the process is not yet available. Identification of novel pathways may help define novel strategies to prevent or reverse remodeling.

Cathepsins are lysosomal proteases contributing to autophagic degradation of cellular substrates (30). They are composed of 11 members (cathepsin B, C, F, H, K, L, O, S, V, X, and W), most of which are ubiquitously expressed in living organisms (30). Cathepsins play a role in a number of signaling pathways and appear to be associated with several diseases, including neurological disorders (23), cancers (28), and cardiomyopathies (18). Increased cathepsin gene expression was reported in conditions of cardiac stress, remodeling, and dysfunction. Cathepsin S and K were found increased in pressure overload-induced myocardium of rodents and in humans with hypertension-induced heart failure (2). Cathepsin D was found to be elevated in the plasma of patients after myocardial infarction (MI) (21).

Validation for the important roles of cathepsins in the heart and during cardiac pathogenesis came recently from studies of cathepsin K, L, S, and D knockout mice. Loss of cathepsin K alleviates both pressure overload-induced and high-fat diet-induced cardiac hypertrophy, likely through the inhibition of mammalian target of rapamycin (mTOR) and ERK pathways (13, 14). On the contrary, several other cathepsins were found to be cardioprotective. Loss of cathepsin L promoted cardiac hypertrophy upon stress, which was associated with accumulation of protein substrates such as α-actinin, myosin, connexin-43, and H-cadherin (27). Moreover, cathepsin S seems to play a role in cardiac maintenance since its deletion exacerbated ANG II-induced cardiac inflammation (22).

While the functions of these cathepsins, specifically cathepsin L, for the heart are an area of intense investigation, little is known about the role that cathepsin B (CTSB) plays during cardiac development and disease. CTSB is a cysteine peptidase involved in numerous physiological processes such as inflammation, apoptosis, and immunity (15, 31). Generation of global CTSB knockout mice revealed its involvement in the early development of pancreatitis through premature trypsinogen activation, as well as a role in the mediation of apoptosis (25). In addition, much effort has been made to better characterize the role of this protease in the pathology of Alzheimer’s disease, due to its antiamyloidogenic and neuroprotective functions via the reduction of amyloid-β 1–42 peptide levels in the brain (20). CTSB has also been intensely investigated in the cancer field, as its overexpression was found to be associated with tumorigenesis and formation of metastases (9, 24).

Recently, CTSB expression was found to be induced in H9c2 cells, a myoblastic cell line derived from rat heart, after doxorubicin treatment (1). CTSB upregulation was closely associated with NF-κB protein levels, although an exact mechanism for the regulation and its influence on NF-κB signaling is unknown. CTSB may also play a role during post-MI remodeling (29) and in patients with dilated cardiomyopathy (7). Although the molecular mechanism remains elusive, data from the doxorubicin-induced cardiotoxicity and the MI models, as well as dilated cardiomyopathy patients, suggested a role for CTSB in the modulation of cardiomyocyte apoptosis. Also, inhibition of CTSB with the specific pharmacologic inhibitor CA-074Me was previously shown to ameliorate experimental post-MI remodeling in rats (17). Following these results, Wu et al. (34) hypothesized that CTSB could also play a prominent role in the pathology of pressure overload-induced cardiac remodeling. These experimental results, utilizing both in vivo and in vitro models of hypertrophic stimuli, are described in a recently published article in the American Journal of Physiology-Heart and Circulatory Physiology, which is highlighted below.

Wu et al. induced ventricular pressure overload in mice by transaortic constriction (TAC; also called aortic banding) for up to 8 wk, which caused a time-dependent increase in CTSB expression. Germline CTSB knockout mice were then compared with their wild-type (WT) littermates. Compared with WTs, CTSB knockouts demonstrated less cardiac hypertrophy post-TAC, both at the organ and cellular level. Cardiac functional parameters were also improved in the knockouts, including increased contractility (as measured by left ventricular ejection fraction, fractional shortening, and dP/dt max), improved relaxation (reduced dP/dt min), and increased cardiac output. Knockout of CTSB reduced the mRNA upregulation of the hypertrophic markers A-type natriuretic peptide (atrial natriuretic peptide or ANP), B-type natriuretic peptide (brain natriuretic peptide or BNP), and β-myosin heavy chain (β-MHC) observed post-TAC. In addition, downregulation of
α-MHC mRNA was not seen in the knockouts. Compared with WTs, CTSB knockouts demonstrated reduced interstitial and perivascular fibrosis post-TAC, which was associated with a reduction in the fibrosis-associated markers, Colla1, Col3a1, and matrix metalloproteinase-9 (MMP-9). The CTSB null mice also showed fewer terminal deoxynucleotide transferase-mediated dUTP nick end-labeling (TUNEL)-positive myocytes after aortic banding, indicating a reduction in apoptotic cell death.

As an in vitro model of myocyte hypertrophy, Wu et al. (34) used H9c2 cells stimulated with ANG II. ANG II was utilized because it (or its receptor, AT1) has been implicated in ligand- and stretch-dependent cardiomyocyte hypertrophy (33). Stimulation of the H9c2 cells with ANG II increased CTSB protein expression, increased cell size and increased expression of the hypertrophic markers ANP, BNP, and β-MHC. Knockdown of CTSB with shRNA attenuated, and overexpression of CTSB augmented, the effects of ANG II (both delivered by lentiviral vector). These data indicated that CTSB was at least partially responsible for the observed hypertrophic effects of ANG II in vitro.

Both in vivo and in vitro models demonstrated increased levels of proapoptotic proteins (Bid, Bax, cytosolic cytochrome c, and cleaved caspasas 3 and 9) and reduced levels of the antiapoptotic protein Bcl-2. These alterations in protein levels were lessened in the CTSB knockout mice post-TAC and in the CTSB knockdown H9c2 cells after ANG II stimulation. Lentiviral overexpression of CTSB in the H9c2 cells increased the ratio of proapoptotic to antiapoptotic proteins further. Therefore, CTSB was involved in the apoptosis observed in cardiomyocytes after hypertrophic stimuli, and its inhibition partially alleviated the observed cell death. This is not surprising given the known roles of cathepsins to degrade antiapoptotic proteins and induce mitochondrial cytochrome c leakage (3, 5, 26).

The authors then provided some insight into the signaling pathways that may be involved in the hypertrophic and apoptotic activity of CTSB. Aortic banding in vivo increased expression of TNF-α and phosphorylation of downstream kinases ASK1, JNK, p38, ERK1/2, and Akt, as well as phosphorylation of the transcription factor c-Jun. Knockdown of CTSB reduced the observed increases in TNF-α, p-ASK1, p-JNK, and p-c-Jun but had no effect on phosphorylation of p38, ERK1/2, and Akt. Corresponding in vitro experiments showed that ANG II stimulation of H9c2 cells increased expression of TNF-α and phosphorylation of ASK1, JNK, and c-Jun. The signaling kinases p38, ERK, and Akt were not tested in vitro. Knockdown of CTSB and lentiviral overexpression of CTSB attenuated and augmented, respectively, the ANG II-stimulated effects on TNF-α, p-ASK1, p-JNK, and p-c-Jun. Similar to what was observed with shRNA knockdown, use of the CTSB inhibitor CA-074Me attenuated the ANG II-stimulated increases in TNF-α, p-ASK1, p-JNK, and p-c-Jun in H9c2 cells. CA-074Me also attenuated the observed increases in cell size, cytochrome c release, and upregulation of the hypertrophic markers ANP, BNP, and β-MHC in H9c2 cells.

Given the activation of JNK by hypertrophic stimuli and its previously published roles in both hypertrophy (32) and apoptosis (35), the authors also tested the effect of the specific JNK inhibitor SP600125 on ANG II-stimulated H9c2 cells. Similar to CA-074Me, SP600125 attenuated cellular hypertrophy and the upregulation of hypertrophic markers. Although SP600125 severely reduced the phosphorylation of JNK and c-Jun and completely eliminated the release of cytochrome c, it did not inhibit upregulation of TNF-α or phosphorylation of ASK1, since these are regulated upstream of the point of inhibition. Wu and colleagues concluded that, “CTSB protein functions as a necessary modulator of hypertrophic response by regulating TNF-α/ASK1/JNK signaling pathway involved in cardiac remodeling.”

The article by Wu et al. (34) adds to the growing body of evidence that direct or indirect modulation of cathepsin activity may be beneficial for cardiac maintenance and function. The ability of cathepsins to activate apoptosis pathways has been well established in the literature, so the current finding that CTSB inhibition can ameliorate the proapoptotic effects of hypertrophic stimuli is in agreement with previous work. This could certainly explain the development of replacement fibrosis and eventual decompensated heart failure that accompanies pressure overload. However, less clear are the mechanisms involved in the blunting of cardiac hypertrophy itself by CTSB inhibition. Wu et al. have implicated the TNF-α/ASK1/JNK signaling pathway, but this pathway likely does not act alone. Knockout of NOD-like receptor family, pyrin domain-containing 3 (NLRP3) has been shown to reduce cardiac hypertrophy and fibrosis post-TAC (12). Pharmacologic inhibition of CTSB with CA-074Me has also been shown to reduce activation of the NLRP3 inflammasome postcoronary artery ligation (17). The NLRP3 system (and subsequent activation of interleukin-1β) was not tested by Wu et al. (34) and could form another important component of CTSB-activated pathway(s). However, post-TAC NLRP3 knockout mice show accelerated left ventricular dilatation and systolic dysfunction, characteristics that were not reported in the post-TAC CTSB knockouts (12, 34). Sufficient differences exist in these knockout models to warrant more study. The activation of fetal genes during hypertrophic cardiomyopathy is well-documented (e.g., increased β-MHC to α-MHC expression) (10). The finding by Wu et al. (34) that CTSB knockout mice have an elevated expression of α-MHC relative to β-MHC at baseline and post-TAC could also be an important mechanistic clue as to how this protease functions.

Whenever a particular pathway has been implicated in human disease, the question always arises regarding clinical therapeutic potential. Specific cathepsin inhibitors have been available for some time (6, 11, 17), but none has been used clinically to our knowledge. As a family, cathepsins have ubiquitous tissue expression patterns and influence many cellular processes, so the potential for side-effects and cytotoxicity is great with systemically administered therapeutics. However, localized targeting of cathepsin inhibitors, for example, localized magnetic field with promising results (19). Natural protein inhibitors of cathepsins exist (e.g., cystatins, thyropins, and serpins; Ref. 30), which could be selectively targeted to a particular tissue by gene transfer therapy. For example, adenovirus-associated virus serotype 1 (AAV1) was recently utilized to overexpress the SERCA2a sarcoplasmic reticulum calcium ATPase gene in the myocardium of heart failure patients (36). Many possibilities exist, but for now we need to focus our efforts to better understand the mechanisms involved in cathepsins...
psin-mediated cardiac remodeling, and this article by Wu et al. (34) represents a good start.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


